The Combination of Benzoic Acid and Essential Oils Inhibit Various Growth Parameters of 18 Common *Salmonella* Isolates

Xiaole Zhao[1,*], Mathias Spiegelhofer[2], Wen Ren[3]

[1]Key Laboratory for Deep Processing of Major Grain and Oil, Ministry of Education, College of Food Science and Engineering, Wuhan Polytechnic University, Wuhan 430023, China

[2]Dsm-firmenich, Animal Nutrition and Health, R&D Center, Tulln 3430, Austria

[3]Dsm-firmenich, Animal Nutrition and Health, R&D Center, Bazhou 065799, China

Abstract: (Background) *Salmonella*, a widespread pathogen, causes millions of infections in humans and farm animals, leading to various health issues from gastroenteritis to invasive typhoid fever. Traditional methods of controlling *Salmonella*, such as the use of antibiotics, have led to antimicrobial resistance, posing significant challenges to food safety and public health. Developing effective non-antibiotic strategies to reduce *Salmonella* infections is crucial. (Objective) This study aimed to evaluate the antimicrobial efficiency of a combination of benzoic acid and essential oils (VW) against 18 common *Salmonella* isolates using a three-way approach: minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and growth kinetic analysis. (Main Ideas) The antimicrobial effects of VW were assessed using a 96-well micro-broth dilution assay, growth curve experiments, and single-cell imaging. The results showed that VW inhibited the growth of all tested *Salmonella* strains. The MIC of VW was found to be 1.50 g/L, and the MBC ranged from 1.50 to 3.00 g/L. Growth kinetic analysis revealed that VW extended the lag time, decreased the maximum growth rate, increased the time to achieve maximum growth, and limited the maximum biomass accumulated. Single-cell imaging demonstrated that VW prolonged the interval to the first cellular division during the exponential phase of growth. (Conclusion) The study concluded that VW, at the recommended inclusion level, effectively inhibited the growth of 18 tested *Salmonella* strains. These findings highlight the potential use of VW as a dietary intervention to reduce *Salmonella* infections in farm animals, offering a viable alternative to antibiotics and contributing to the reduction of drug-resistant *Salmonella* strains. Further research is warranted to explore the application of VW in farm animal production and its impact on reducing *Salmonella* contamination in the food supply chain.

Keywords: *Salmonella*; Antimicrobial Activity; Benzoic Acid; Essential Oil

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1 Introduction

The environmentally ubiquitous nature of the > 2600 serovars of *Salmonella* was responsible for millions of infections worldwide since it affects both human and animal health [1, 2]. It is spread through contaminated feed or water and contact with infected people or animals [3]. Traditionally, adding antibiotics to feed or water has been

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*Corresponding author: Xiaole Zha, xiaole_1920@whpu.edu.cn

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the primary strategy for preventing and controlling salmonellosis in farm animal production. However, the overuse and misuse of these drugs is now recognized as a significant driver of antimicrobial resistance in *Salmonella* strains (such as *Salmonella spp.*, *Salmonella Enteritidis*), which reduced the chances of effective treatment against *Salmonella* infectious, posing threats to the food supply chain and human wellness [4-6]. Developing efficient strategies and improved methodologies to decrease *Salmonella* infection faces several challenges. *Salmonella* is hard to control due to its ability to grow and tolerate different conditions. For example, *Salmonella sp*. could grow at a wide range of temperatures (5 to 47 ℃) and pH (4.2 to 9.5) [7]. Moreover, *Salmonella* can form biofilm and thus serve as protective shells [8-10], which is associated with their resistance to antimicrobial agents and different environmental conditions [11].

Feed additive is a potential pre-harvest strategy to decrease *Salmonella* infection in farm animals, including swine and poultry [1, 12, 13]. One such possibility is to apply benzoic acid and essential oils in combination. Benzoic acid is a well-known compound with antimicrobial properties on Gram-negative bacteria such as *Escherichia coli* and *Salmonella enterica* [7]. Benzoic acid is also compensating for the limited ability of pigs to achieve a bactericidal stomach pH immediately after weaning, preventing pathobiont expansion and post-weaning diarrhea [14]. Essential oils have been reported to possess a broad spectrum of action on different microorganisms and multiple effects, such as the inhibiting quorum sensing, preventing biofilm formation, and the inhibition of the growth of resistant microorganisms [15]. Therefore, essential oils can be a powerful tool to reduce the bacterial resistance [16]. However, these studies are mainly focused on the antibacterial effects of benzoic acid and essential oil separately, the antibacterial effect of essential oil and benzoic acid combination needs to be further studied.

From this perspective, a combination of benzoic acid and essential oils (VW) should be added to the farm manager's toolbox to reduce *Salmonella* infections in farm animal production. Minimum inhibitory concentration (MIC) is the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth of the bacteria [17]. The most common estimation of bactericidal activity is determining minimum bactericidal concentration (MBC), defined as the concentration killing 99.9% or more of the initial inoculums [18]. This study evaluated the antibacterial, MIC, and MBC of VW on pathogenic 18 common *Salmonella* isolates by 96-well micro-broth dilution assay. Specific inhabitation effects on various growth parameters were further conducted using a 96-well-based real-time growth kinetic assay. To investigate the time alteration to the first cellular division and provide visual evidence, a single-cell imaging study was performed with *Salmonella* enterica serovar Derby as representing *Salmonella* isolates. These data highlight a clear image of the degree of inhibition of VW against the various Salmonella isolates and support further utilization in the antibiotic replacer and feed industries.

2 Materials and Methods

2.1 Material of Benzoic Acid and Essential Oils Combination

The test product VW was the combination of pure benzoic acid (99.9% benzoic acid, DSM Limited, Shanghai, China) and a mixture of essential oils, including thymol, eugenol, piperine, and curcumin as the main ingredients (DSM Nutritional Products, Grenzach, Germany, Table 1), the recommended dosage in feed is 3.00 kg/MT. Pure benzoic acid and the essential oils were mixed at the ratio of 10: 0.4 by weight, which was established by referring to the adequate levels of 5 g/kg of pure benzoic acid and 0.2 g/kg of the essential oils when used separately in feed for pigs [14].

A working solution of VW was prepared by adding 6 g/L of VW to freshly prepared and sterilized Mueller-Hinton broth (Oxoid), followed by agitation at room temperature for 30 minutes and two consecutive filtration steps using a 40 μm and a 0.22 μm filter. Working solutions were then applied to the respective wells of a transparent 96-well flat-bottom plate, followed by two-fold serial dilution steps to achieve the desired concentration gradient of the test substances. Additionally, each plate was prepared to contain media sterility and growth control wells. The outer well of the respective plates were filled with medium to prevent excessive evaporation ("edge-effect") during the incubation period.

Table 1 Test Compounds

Type	Organic Acid	Essential oils
Compounds	Pure benzoic acid	Thymol, eugenol, piperine, curcumin

2.2 Bacterial Strains and Growth Conditions

The 18 common *Salmonella* isolates (Table 2) were provided by Biomin Research Center (Tulln, Austria). From frozen cultures (kept at -80 ℃ with 17% Glycerol), a fractured smear was performed on Mueller-Hinton agar (Oxoid) and incubated at 37 ℃ (aerobically) overnight. The colonies presented at the culture plates were checked on their macroscopic uniformity. One colony was picked and aseptically transferred to freshly prepared Mueller-Hinton broth (Oxoid), followed by an incubation at 37 ℃ (aerobically) until the culture reached mid-to-late exponential phase. Finally, the mid-to-late exponential culture was diluted to 10^6 cfu/mL with fresh media and immediately used to inoculate the prepared 96-well plates, yielding a final concentration of $5*10^5$ cfu/mL.

Table 2 The 18 Test Common *Salmonella* Isolates

Species		Subspecies	Serovar	Comments
Salmonella	enterica	*enterica*	Derby	High prevalence in pigs and cattle. Ranked fourth cause of *Salmonella* outbreaks in Germany. Most common serovar isolated from infants in Children in China. 11.8% of the isolates from pig carcasses in the EU are considered multiple drug resistance (MDR).
Salmonella	enterica	*enterica*	1,4,[5],12:i-	One of the most identified serotypes in pigs, pork and humans worldwide. Often considered MDR.
Salmonella	enterica	*enterica*	Typhimurium	Well known cause if Salmonellosis in swine, poultry and humans
Salmonella	enterica	*enterica*	Choleraesuis	High prevalent serotype in swine, often associated with food-borne human infections
Salmonella	enterica	*enterica*	Choleraesuis	High prevalent serotype in swine, often associated with food-borne human infections
Salmonella	enterica	*enterica*	Enteritidis	Well known cause of Salmonellosis in swine, poultry and humans
Salmonella	enterica	*enterica*	Java	Prevalent serovar in Chicken and frequent cause of food-borne infections in human with high impact on public health due to rapidly increasing MDR.
Salmonella	enterica	*enterica*	Kentucky	One of the most common serovar in the US, often isolated from human and in poultry
Salmonella	enterica	*enterica*	Infantis	Under the top five serovars frequently causing salmonellosis in humans. Mainly poultry as reservoir
Salmonella	enterica	*enterica*	Pullorum	Connected to severe salmonellosis in Chicken and human with high MDR patterns
Salmonella	enterica	*enterica*	Gallinarium	Connected to severe salmonellosis in Chicken and human with high MDR patterns
Salmonella	enterica	*enterica*	Heidelberg	Prevalent in swine, poultry and humans
Salmonella	enterica	*enterica*	Senftenberg	Is known to be tolerant to desiccation and heat. Can persist in feed mills.
Salmonella	enterica	*enterica*	Virchow	Mostly prevalent in poultry and humans with high prevalences in Europe
Salmonella	enterica	*enterica*	Hadar	Common serovar in poultry
Salmonella	enterica	*arizonae*		Common serovar in reptiles but also increasingly reported to cause wound infections in humans
Salmonella	enterica	*diarizonae*		Common serovar in reptiles but with increasingly reported cases in children and neonates
Salmonella	*bongori*			Common serovar with reported cases of salmonellosis in children and neonates

2.3 Determination of the Antimicrobial Activity of VW on Different *Salmonella* Strains

The antimicrobial effects of VW on the 18 common *Salmonella* isolates were determined using the 96-well micro-broth dilution assay as previously described with some modifications [19]. In a 96-well microplate, 100 µL of the inoculum was mixed with 100 µL of VW dissolved in 1% (v/v) in microplates for final concentrations of 0.09, 0.19, 0.38, 0.75, 1.50 and 3.00 g/L, which were equal to the dosage in feed (0.09, 0.19, 0.38, 0.75, 1.50 and 3.00 kg/MT respectively). After the plate preparation was finished, the plates were closed with a transparent cover lid and the initial optical density (OD) at 600 nm was determined using a multi-mode plate reader (Synergy H1, Version 3.10, Biotek). The plates were subsequently incubated at 37 ℃ aerobically for 24 hours. Followed the incubation period, the terminal OD at 600 nm as benchmark for microbial growth was again measured using a multi-mode plate reader. Each experiment was conducted with three replicates, and experiments were conducted in triplicate.

MIC was calculated as the lowest concentration of the essential oil that completely inhibits bacterial growth. To evaluate MBC values, an inoculation loop was submerged into the wells exhibiting the detected MIC value and two additional concentrations higher than the observed MIC. The loops were used to create a fractured smear on Mueller Hinton agar plates. The agar plates were further incubated at the distinctive temperatures and evaluated on potential bacterial growth after 24 hours.

2.4 Growth Curve Experiments *in vitro*

For VW growth curve experiments, 6 high prevalence *Salmonella* strains (*Salmonella* enterica enterica serotype 1,4,[5],12:i-, *Salmonella* enterica enterica serotype Derby, *Salmonella* enterica enterica serotype Typhimurium, *Salmonella* enterica enterica serotype Heidelberg, *Salmonella* enterica enterica serotype Choleraesuis, *Salmonella* enterica enterica serotype Senftenberg) were selected. In a 96-well microplate, 100 μL of the inoculum was mixed with 100 μL of VW dissolved in 1% (v/v) in microplates for final concentrations of 0.09, 0.19, 0.38, 0.75, 1.50 and 3.00 g/L. After plate preparation was finished, the plate was covered with an optical film, with small holes poked at the side of each well to allow aeration. Incubation and OD measurements were performed with a multi-mode plate reader (BioTek) at 37 ℃ with continuous shaking and OD_{600} was measured at 15-min intervals for 24 h. The plate reader was contained within an anerobic chamber. Blank values were subtracted from OD measurements and the derivative of ln (OD) with respect to time was computed. The maximal growth rate (Max V) was calculated as maximum achieved growth rate in OD per hour. Lag time was defined as the time at which a culture first reached maximal growth rate. Time at Max V was defined as the time until maximum growth rate was achieved. Max OD was defined as maximum biomass accumulated was defined as the maximum optical density reached.

2.5 Single Cell Imaging

For single cell imaging experiments, *Salmonella* enterica serovar Derby was selected and grown anaerobically overnight prior to spotting on either a Mueller-Hinton agar pad as a control or a pad supplemented with 3 g/L VW. Single cell imaging was conducted refer to previous study with minor modification [2]. In general, cells were imaged every 5 minutes for 140 minutes at 37 ℃. Phase-contrast images were acquired with a Nikon Ti-E inverted microscope (Nikon Instruments) using a 100X (NA 1.40) oil-immersion objective and a Neo 5.5 sCMOS camera (Andor Technology). The microscope was outfitted with an active-control environmental chamber for temperature regulation (HaisonTech, Taipei, Taiwan), and was housed in the same anaerobic chamber used to grow cultures. Images were acquired using mManager v.1.4 (http://www.micro-manager.org/) [20].

2.6 Statistical Analysis

All results are expressed as means and their respective standard deviations for each of the assays. Data were analyzed with Fit model platform of JMP (15.1.0, 2019 SAS Institute Inc., Cary, CN, USA) to determine the main effects of treatment, dosage and their interaction. Tukey's tests were used to compare the means. Significant changes are indicated by asterisks in the figures. For time to first division measurements, significance was calculated using an unpaired two-tailed t test. Significance was declared at $*p < 0.05$, $**p < 0.01$.

3 Results

3.1 The MIC and MBC for 18 *Salmonella* Strains

The results of MIC and MBC were presented in Table 3. VW presented inhibitory activity against all the 18 *Salmonella* strains under study. VW was giving a constant MIC of 1.50 g/L and an MBC between 1.50 and 3.00 g/L. The tested isolates of *Salmonella* enterica enterica Derby, Typhimurium, Heidelberg and *Salmonella* enterica diarizonae were found to be most resistant to the treatments and especially exhibiting high MBC values compared to all other tested strains, but still at the recommended inclusion level.

Table 3 Determination of the Antimicrobial Activity of VW on Different *Salmonella* Strains *in vitro*

Species		Subspecies	Serovar	MIC[1] VW[2] [g/L]	MBC[1] VW [g/L]
Salmonella	*enterica*	*enterica*	Derby	1.50	3.0
Salmonella	*enterica*	*enterica*	1,4,[5],12:i-	1.50	1.5
Salmonella	*enterica*	*enterica*	typhimurium	1.50	3.0

Species		Subspecies	Serovar	MIC[1] VW[2] [g/L]	MBC[1] VW [g/L]
Salmonella	*enterica*	*enterica*	choleraesuis	1.50	1.5
Salmonella	*enterica*	*enterica*	choleraesuis	1.50	1.5
Salmonella	*enterica*	*enterica*	enteritidis	1.50	3.0
Salmonella	*enterica*	*enterica*	java	1.50	1.5
Salmonella	*enterica*	*enterica*	kentucky	1.50	1.5
Salmonella	*enterica*	*enterica*	infantis	1.50	1.5
Salmonella	*enterica*	*enterica*	pullorum	1.50	1.5
Salmonella	*enterica*	*enterica*	gallinarium	1.50	1.5
Salmonella	*enterica*	*enterica*	heidelberg	1.50	3.0
Salmonella	*enterica*	*enterica*	Senftenberg	1.50	1.5
Salmonella	*enterica*	*enterica*	virchow	1.50	1.5
Salmonella	*enterica*	*enterica*	hadar	1.50	1.5
Salmonella	*enterica*	*arizonae*		1.50	1.5
Salmonella	*enterica*	*diarizonae*		1.50	3.0
Salmonella	*bongori*			1.50	1.5

[1]MIC and MBC values displayed as mean of three biological replicates obtained for VW against all tested isolates.
[2]VW: combination of benzoic acid and essential oils.

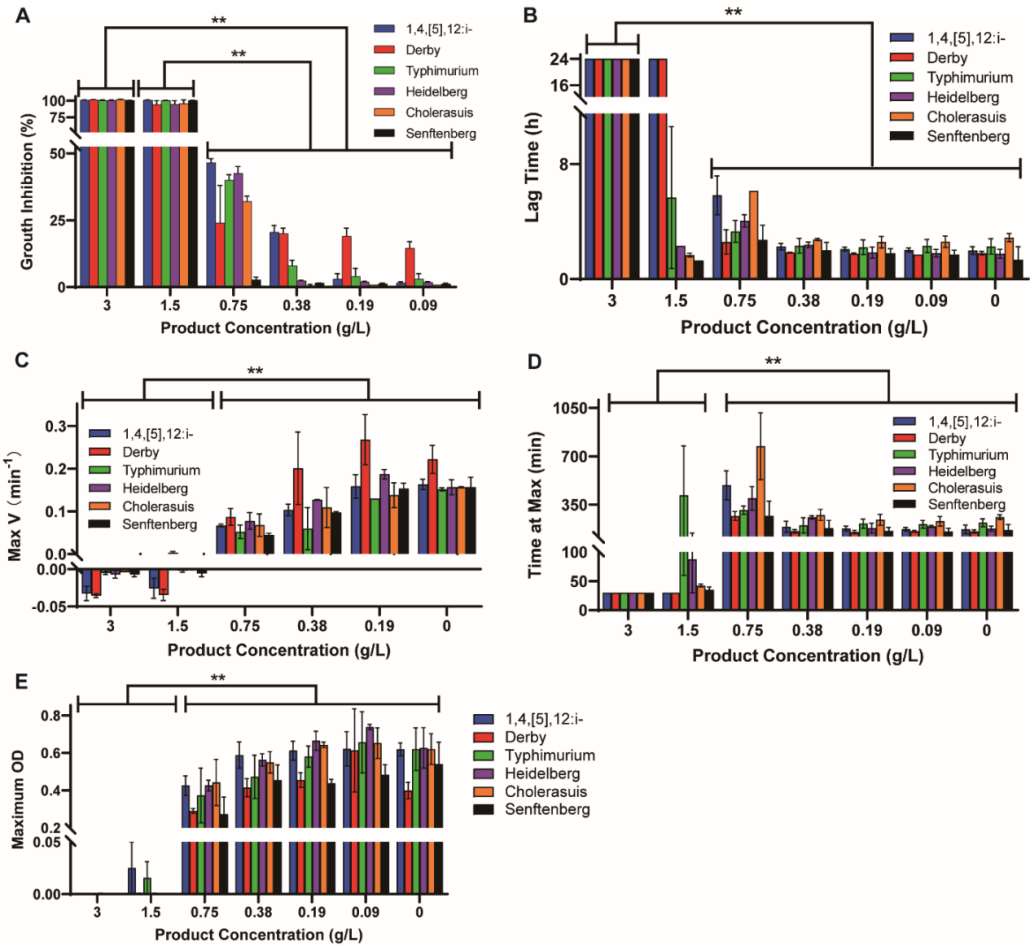

Figure 1 VW Inhibit *Salmonella* Growth.

(A) Growth inhibition values of VW against selected 6 high prevalence strains, Error bars are calculated as 2*SD for three independent measurements.
(B) Lag time: average duration of lag time in hour against applied antimicrobials.
(C) Max V = Maximum growth rate: distribution of calculated Max V among tested serotypes and product concentrations.
(D) Time at Max V = time at maximum growth rate achieved: observed time until the maximum growth rate was achieved.
(E) Max OD = Maximum biomass accumulated: calculated maximum mean of optical density reached for respective serotypes and VW concentrations.
VW: combination of benzoic acid and essential oils.

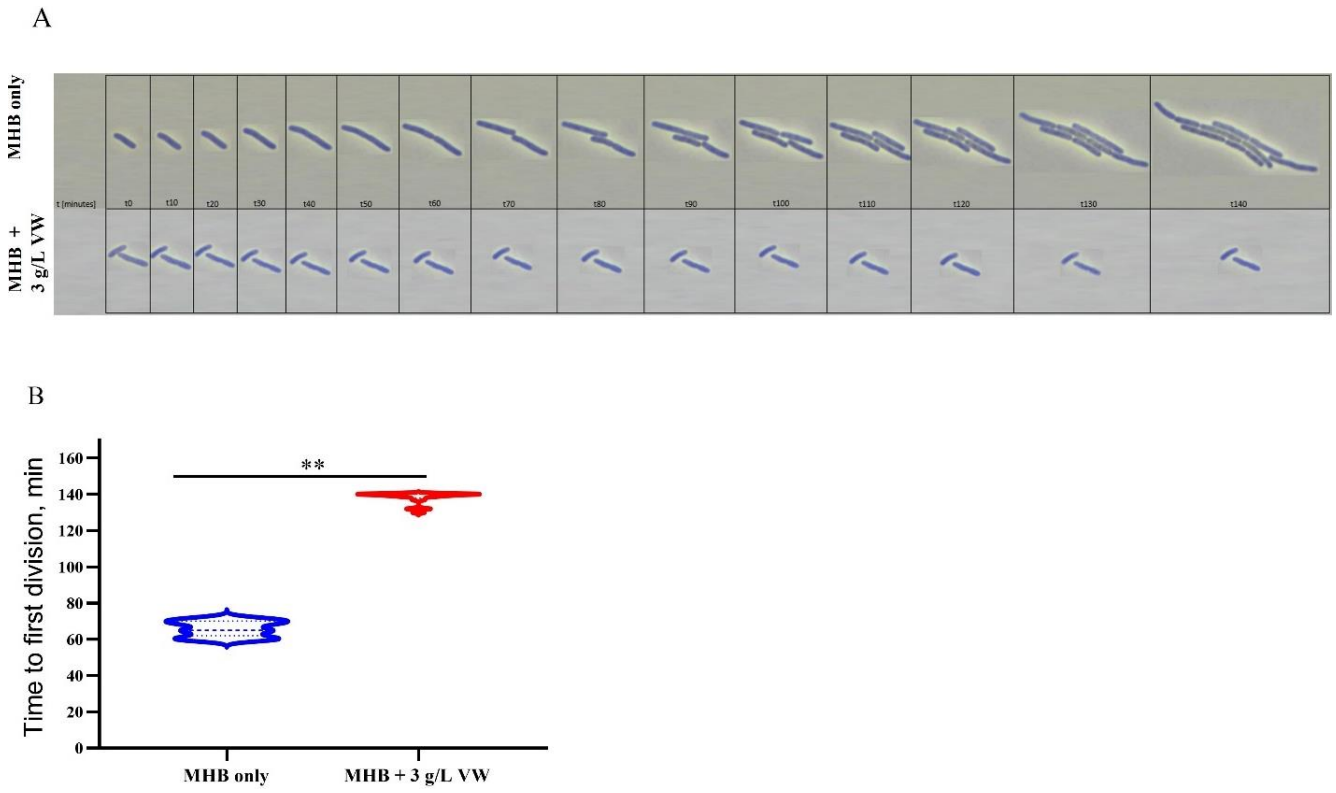

Figure 2 Single Cell Imaging.

(A) Time-lapse imaging of anaerobic *Salmonella* grown on either a Mueller-Hinton agar (MHB) pad as a control or a pad supplemented with 3 g/L VW.
(B) Time to the first cellular division.
VW: combination of benzoic acid and essential oils.

3.2 VW Inhibited the Growth of *Salmonella in vitro*

VW was sufficient to limit *Salmonella* growth *in vitro* (Figure 1). In Figure 1A, growth inhabitation values displayed as percentage inhibition compared to a growth control for VW against the selected 6 high-prevalence strains are plotted. Growth inhibition values aligned with observed MIC values and showed a clear cut-off between 1.5 and 0.75 g/L. The lag time differed slightly among serotypes with a mean value of approximately two hours (Figure 1B). It was observed that the application of VW increased the lag time dramatically if applied in higher dosages (between 1.50 and 3.00 g/L) and the effect was reduced with decreasing concentration of VW. For VW concentrations of 3.00 g/L, it was high enough to inhibit the growth of the tested serotypes completely, no lag time is displayed. The time at Max V was measured and could be a significant indicator of microbial adaptation to existing antimicrobial challenges. As seen in Figure 1C and 1D, the time at Max V was related to VW concentration and increased VW concentrations (≥1.50 g/L) led to a significant time increase. Even in lower VW concentrations (< 1.50 g/L), it was possible to increase this time by several hours compared to the control group. As expected, the Max OD value reached was proportional to the VW concentration and significantly decreased with increasing VW concentration (Figure 1E). In general, VW with 1.50 and 3.00 g/L was able to retard all tested *Salmonella* growth by extending the lag time, decreasing Max V, increasing time at Max V and limit the Max OD accumulated (OD value) during the experiment.

3.3 VW Prolonged the Interval to the Initial Cellular Division During the Exponential Phase of Growth

To gain more insight into how VW limits *Salmonella* growth, we performed microscopy in an anaerobic cham-

ber on *Salmonella* enterica serovar Derby cells grown on Mueller-Hinton agar pads with and without 3 g/L VW. Time-lapse imaging provided directly visual evidence that VW inhibited growth of *Salmonella* (Figure 2A) when compared to the control treatment. At single bacteria level, VW extended the time to the first cellular division during exponential growth (Figure 2B).

4 Discussion

Since chickens serves as the reservoir of *Salmonella* and pigs are an essential non-typhoidal *Salmonella* infection source for humans, innovative on-farm non-antibiotics strategies for reducing *Salmonella* infection are critical for preventing food safety and human health [12, 13]. Benzoic acid or essential oils, used as potential antibiotics alternatives have received significant attentions, given its potential antimicrobial properties in chickens and pigs. Essential oils can exert their biological functions by affecting bacterial biofilms and destroying ion gradients through hydrophobicity [21], which enables them to partition with the lipids present in the cell membrane of bacteria and mitochondria, rendering them more permeable by disturbing the cell structures. This eventually results in the death of bacterial cells due to leakage of critical molecules and ions from the bacterial cell to a great extent [16, 22]. Additionally, organic acids have gained wide application in livestock production due to their possessing a variety of functions such as antibacterial (such as *Salmonella*, *Campylobacter*, *Clostridium perfringens* and other pathogens), immune-regulation, barrier-protection, health-promotion and/or growth promoters in livestock and poultry [23-25]. However, the inhibitory efficiency of benzoic acid and essential oils (thymol, eugenol, piperine and curcumin) combination have not been formally investigated. Herein, the present study assessed the efficacy of the combination of benzoic acid and essential oils (VW) on antimicrobial properties of 18 common *Salmonella* isolates.

The current study highlights the antibacterial activity of VW against all 18 tested *Salmonella* isolates with an MIC of 1.50 g/L. These results are consistent with those obtained by poultry trials, which found that some essential oils, such as carvacrol, thymol, trans-cinnamaldehyde and eugenol, have antibacterial effects against *Salmonella* in chickens [26-28]. VW was able fully inhibit the growth of all tested *Salmonella* strains in concentrations at or below the expected dosing (3.00 g/L). Therefore, it can be concluded that VW was highly effective against the tested strains and was able to exhibit not only a growth retarding effect but also a bactericidal activity at the recommended inclusion levels and below. In this aspect, VW constitutes a viable antibiotic alternative due to its vigorous antibacterial activity that affect multiple *Salmonella* strains targets at the same MIC. Interestingly, previous studies have indicated that dietary essential oils combined with supplementation of organic acids showed synergistic beneficial effects on higher efficacy in controlling harmful intestinal bacterial infection such as *Salmonella spp.* [29-31] compared with individual addition. Similarly, several studies have also reported an inhabitation on the growth of *Salmonella* after supplementation different organic acid and essential oil combination products. For example, Hu et al. showed that the commercial blend product of coated essential oils and organic acids (contain thymol, carvacrol, cinnamaldehyde, caprylic acid, benzoic acid and butyric acid) exhibited vigorous antibacterial activity against *Salmonella*, and the MIC and MBC values against *Salmonella enterica* of this product was 2.35 and 4.69 g/L, respectively [12]. VW showed lower MIC (1.50 *vs.* 2.35 g/L) and MBC (1.50-3.00 *vs.* 4.69 g/L) values when compared with the commercial blend product in Hu's study, which indicated a higher efficiency on antimicrobial activity of VW. Some studies also confirmed the antimicrobial activity of organic acid and essential oils combination *in vivo*, which was the limitation of current and pending for further investigate. Dietary supplementation with coated essential oils and organic acids mixture at 500 and 800 mg/kg could reduce *Salmonella* load in the liver, spleen and cecum of chickens [12]. Another study used a specific blend of essential oils based on a mixture of cinnamaldehyde and thymol alone or in combination with sodium butyrate, which also significantly reduced *Salmonella* colonization in the cecum of chickens [30].

The growth kinetic values obtained from the current study were shown to directly correlate with the applied VW concentrations and differed slightly among the tested serotypes of *Salmonella* enterica. Those benchmarks could be used to evaluate the effect of VW on specific *Salmonella* serotypes in detail and allow for a closer look into microbial growth behavior under challenging conditions. Evaluating such parameters from kinetic studies on

18 common *Salmonella* isolates is time-consuming, and a unilateral approach is not advised. Therefore, in current study 6 high prevalence *Salmonella* strains (*Salmonella* enterica enterica serotype 1,4,[5],12:i-, *Salmonella* enterica enterica serotype Derby, *Salmonella* enterica enterica serotype Typhimurium, *Salmonella* enterica enterica serotype Heidelberg, *Salmonella* enterica enterica serotype Choleraesuis, *Salmonella* enterica enterica serotype Senftenberg) were selected. Here, the current study proved VW has significant effects on various microbial growth parameters such as “lag time”, “Max V”, “Max OD” and “time at Max V”. At the single bacteria level, VW extended the time to the first cellular division during exponential growth of *Salmonella* enterica serovar Derby. All the tested serotypes presented minor differences for the calculated parameters, but a general trend was visible, indicating a ubiquitous effect rather than serotype specific response. The findings from current study suggest that when the concentration of VW is at the recommend level, all tested *Salmonella* isolates could not facilitate cellular division required for growth, leading to an extended lag time.

Overall, the results demonstrate that the VW supplementation could inhibit *Salmonella* growth and provided insights into the mechanisms underlying the antimicrobial activity. We acknowledge some limitations with this study This study was conducted on an in-vitro level, therefore, the results may not represent the general animal population level. The inoculated dose and selected *Salmonella* isolates might not represent farm animal production situations. The inhibition effects of VW should be confirmed with a bioassay study with the inhibited or non-inhibited vectors fed to live farm animals.

5 Conclusion

In summary, this study demonstrated that the combination of benzoic acid and essential oils (VW) effectively inhibited the growth of 18 common *Salmonella* strains, with an MIC of 1.50 g/L and an MBC ranging from 1.50 to 3.00 g/L. The findings highlight the potential of VW as a dietary intervention to reduce *Salmonella* infections in farm animals, offering a viable alternative to antibiotics. Future studies should focus on in vivo trials to evaluate the efficacy of VW in live animals, particularly in poultry and swine, which are major reservoirs of *Salmonella*. Research could also explore the potential of combining VW with other non-antibiotic strategies, such as probiotics or prebiotics, to enhance its antimicrobial effects. Additionally, the economic viability of using VW in large-scale farming operations should be assessed to determine its feasibility as a practical solution for reducing *Salmonella* contamination in the food supply chain.

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Institutional Review Board Statement

Not applicable.

Disclosure Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors Statement

Xiaole Zhao: Writing-original Draft Preparation, Writing-review and Editing, Methodology, Formal analysis, Data curation. Mathias Spiegelhofer: Methodology, Software, Validation, Investigation, Resources, Project Administration. Wen Ren: Conceptualization, Validation, Formal Analysis, Investigation, Data Curation, Writing-original Draft Preparation, Writing-review and Editing, Funding Acquisition.

Data Availability

The data presented in this study are available on request from the corresponding author.

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