FULL ARTICLE

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Use of organic acids to reduce *Salmonella* Typhimurium excretion in swine

Uso de ácidos orgânicos para reduzir a excreção de Salmonella Typhimurium em suínos

Daniela Gomes da Silva[1]; Eduarda Alexandra Gonçalves de Oliveira Moura[1]; Thainara Vitoria Carnevalli Sanches[1]; Caio Henrique Turco[1]; Beatriz Belloni Zambotti[1]; Fernando Antônio Moreira Petri[1]; Gabriel Yuri Storino[1]; Henrique Meiroz de Souza Almeida[1]; Isabela Peixoto Rabelo[1]; Marina Lopes Mechler-Dreibi[1]; Karina Sonalio[1]; Renato Ravetti[2]; Luís Guilherme de Oliveira[1]

[1] Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal – SP, Brazil
[2] Salmix Indústria e Comércio Ltda., Piedade – SP, Brazil

ABSTRACT

The use of antimicrobials as growth promoters and disease prevention is being constantly reduced in several animal production systems, including in the swine industry. Therefore, this study aimed to evaluate the effectiveness of using acidifiers to control *Salmonella* Typhimurium in 65-day-old pigs by detecting the pathogen in organs at euthanasia. For this, 24 piglets were divided into two experimental groups consisting of 12 piglets each. An untreated control group (G1) and a treatment group (G2) received a liquid organic acidifier in the drinking water for 10 days (D-5 to D5). Five days after the start of treatment (D0), all piglets were challenged with 10^6 CFU of *Salmonella* Typhimurium and assessed for 12 days (D12). Every three days (D3, D6, D9, and D12), three animals from each experimental group were euthanized and then submitted for necropsy. Samples from the intestines (ileum, cecum, mesenteric lymph nodes, and ileocolic lymph nodes), liver, spleen, and lungs were collected to isolate *Salmonella*. The results show that, numerically, *Salmonella* isolation in the organs of G2 was lower than in G1 and that the number of positive cecum samples in G1 (66.7%; 8/12) was statistically different from the number of positive models in G2 (16.7%; 2/12), with a reduction of 28.6% of the total cecum positive samples in the treated group compared to the control. Therefore, it was observed that the liquid organic acidifier product could reduce the colonization of organs by *Salmonella* Typhimurium.

Keywords: Organic acids. Swine. *Salmonella* Typhimurium. Euthanasia. Excretion.

RESUMO

O uso de antimicrobianos como promotores de crescimento e prevenção de doenças vem sendo constantemente reduzido em diversos sistemas de produção animal, inclusive na suinocultura. Portanto, o objetivo do presente estudo foi avaliar a eficácia do uso de acidificantes no controle de *Salmonella* Typhimurium em suínos de 65 dias de idade, detectando o patógeno em órgãos após a eutanásia. Para isso, 24 leitões foram divididos em dois grupos experimentais constituídos por 12 leitões cada. Um grupo controle não tratado (G1) e um grupo de tratamento (G2) que recebeu um acidificante orgânico líquido na água de beber por 10 dias (D-5 a D5). Cinco dias após o início do tratamento (D0), todos os animais foram inoculados oralmente com 10^6 UFC de *Salmonella* Typhimurium e avaliados por 12 dias (D12). A cada três dias (D3, D6, D9 e D12), três leitões de cada grupo experimental foram eutanasiados e posteriormente submetidos à necropsia. Amostras de intestino (íleo, ceco, linfonodos mesentéricos e linfonodos ileocólicos), fígado, baço e pulmões foram coletadas para o isolamento de *Salmonella*. Os resultados mostram que, numericamente, o isolamento de *Salmonella* nos órgãos do G2 foi inferior ao G1, e que o número de amostras positivas de ceco no G1 (66,7%; 8/12) foi estatisticamente diferente do número de amostras positivas no G2 (16,7%; 2/12), com redução de 28,6% do total de amostras positivas de ceco no grupo tratado em relação ao controle. Portanto, observou-se que o ácido orgânico líquido foi capaz de reduzir a colonização de órgãos por *Salmonella* Typhimurium.

Palavras-chave: Ácidos orgânicos. Suínos. *Salmonella* Typhimurium. Eutanásia. Excreção.

Correspondence to:
Luís Guilherme de Oliveira
Universidade Estadual Paulista "Júlio de Mesquita Filho",
Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Professor Paulo Donato Castelanne, S/N, Vila Industrial
CEP: 14884-900, Jaboticabal – SP, Brazil
e-mail: luis.guilherme@unesp.br

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Introduction

Salmonella is a morphologically and biochemically homogeneous group of Gram-negative, facultatively anaerobic, mobile or immobile, and non-spore-forming microbe. It belongs to the *Enterobacteriaceae* family with wide worldwide distribution and host variety. It includes two species, divided into subspecies, and classified into more than 2,500 serotypes, according to the antigens present in the bacterial wall, flagella, and capsule (Chattaway et al., 2021; Griffith et al., 2019).

Because *Salmonella* is eliminated in the feces of infected animals, the fecal-oral route is considered the basis for the transmission of the pathogen (Fedorka-Cray et al., 2000). *Salmonella* sp. can be present at all stages of production, but the finishing phase has been identified as the most frequently involved in the infection of swine herds (Griffith et al., 2019).

The serotypes that cause subclinical disease in swine (non-adapted serotypes) are also the most critical pathogens for food safety since the carrier animal does not present clinical signs but is a permanent source of shedding for the agent from the farm to industrial processing. However, these serotypes are responsible for condemning carcasses and returning shipments destined for export, constituting significant losses for the producers and exporters (Bonardi, 2017; Campos et al., 2019).

The main control measures for *Salmonella* are linked to biosecurity and good management practices. Among these measures, the control of genetic material, the quality of food and water supplied to animals, hygiene of facilities, pest control (rodents and birds, mainly), correct disposal of waste, use of acidifiers in the water, and vaccination of animals are some of the measures commonly applied in the swine production (Denagamage et al., 2007; Desin et al., 2013; Foley et al., 2008; Kich & Malgarin, 2015).

Using acidifiers as an alternative for preventing and controlling *Salmonella* can improve zootechnical performance, facilitating the digestive process and reducing a load of pathogenic microorganisms in the intestine (Borges et al., 2015). Several studies have shown that the supply of acidified water or food throughout the production process can be an alternative for reducing the prevalence of *Salmonella* (Creus et al., 2007; Van der Wolf et al., 1999, 2001). These findings are supported by the fact that the pH is dropped to 4.2 or lower, the pH threshold below which *Enterobacteriaceae* cannot proliferate (Braz et al., 2011; Busser, 2012; Ostling & Lindgren, 1993). In addition to the ability of organic acids to enter bacterial cells in their undissociated form and after dissociating in the cell, the acids impact the bacteria's ability to synthesize proteins and DNA (Rubin et al., 1982).

The strategic use of acidified drinking water and its influence on shedding before slaughter is the subject of contentious investigations, even though the efficacy of the extended application of organic acids appears well established. Thus, this study aimed at evaluating the use of a liquid acidifier to control *Salmonella* Typhimurium in pigs. The acidifier was made available via drinking water, and the effectiveness was based on detecting *Salmonella* in the organs of experimentally infected animals.

Material and Methods

Animal selection and experimental design

This study used 24 piglets (65-day-old) from a previously selected farm with good production practices. The farm is in the northeast region of the state of São Paulo, Brazil. The animals were transported to the Swine Medicine Laboratory of the School of Agrarian and Veterinarian Sciences (FCAV/UNESP), Jaboticabal Campus, accommodated with nursery stalls and received food according to the nutritional requirements of the production phase and decent quality water ad libitum.

To evaluate if the animals were free from *Salmonella* sp., rectal swabs were collected from sows 14 days after parturition, and drag swabs were collected from the maternity floor simultaneously. Rectal swabs were collected from piglets on arrival at the Swine Medicine Laboratory before the adaptation period started.

The piglets underwent an adaptation period of seven days (D-12 to D-6). Afterward, they were randomly distributed into two experimental groups of 12 piglets

each and submitted to the following treatments for 10 days (D-5 to D5): G1: no treatment (control group), G2: animals treated with liquid organic acidifier in the drinking water (Axeed® Liquid, Salmix, Piedade, SP, Brazil - 200 mL/1000 liters of drinking water). This acidifier is primarily composed of organic acids like propionic acid (35%), formic acid (35%), and phosphoric acid (10-30%). Then, on D0 (5th day of treatment), the animals were orally inoculated with 10^6 CFU of *Salmonella* Typhimurium and assessed for 12 days (D12).

The study was previously approved by the Animal Use Ethics Committee (CEUA) of the School of Agrarian and Veterinarian Sciences (FCAV/UNESP), Jaboticabal Campus (Protocol no. 016527/19).

Inoculum preparation

The inoculum was prepared as recommended by Wood et al. (1991) and Oliveira et al. (2010) from a *Salmonella* Typhimurium strain isolated from porcine feces and naturally resistant to nalidixic acid.

The bacteria strain used in the inoculum preparation was submitted to the antimicrobial susceptibility test in Mueller-Hinton agar (CM0337, Oxoid, Basingstoke, Hampshire, England), according to the *Clinical and Laboratory Standards Institute* (Clinical and Laboratory Standards Institute, 2020), using commercial discs impregnated with the antibiotics ampicillin, cefotaxime, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, florfenicol, gentamicin, nalidixic acid, novobiocin, polymyxin, streptomycin, sulfamethoxazole + trimethoprim, and tetracycline.

Water pH

During the period of treatment of the animals with the acidifier (D-5 to D5), daily samples were collected from untreated drinking water (n=11) and water treated with a liquid organic acidifier (n= 11) for measuring the pH with the aid of a bench pH meter (MA-522, Marconi, Piracicaba, SP).

Euthanasia, necropsy, and harvesting of organ fragments

Every three days after inoculation (D3, D6, D9, and D12), three animals from each experimental group were euthanized following the recommendations of the National Council for the Control of Animal Experiments (CONCEA) and then subjected to the necroscopic examination. Samples from the ileum, cecum, mesenteric lymph nodes, ileocolic lymph nodes, liver, spleen, and lungs were collected aseptically, using surgical instruments and sterile gloves, placed in sample collection bags (Whirl-Pak, B01592WA, Nasco, USA), and kept refrigerated until analyzed.

Laboratory analysis

Microbiological examination

Organ fragment samples were pre-enriched in buffered peptone water (CM0509, Oxoid) at a ratio of 1:10 before the selective enrichment procedure. The samples were homogenized (Sample Homogenizer MA440, Marconi, Piracicaba, SP) and incubated for 24 h at 37° C. After this pre-enrichment step, 1 mL aliquots were transferred to tubes containing 9 mL of selenite cystine (SC) broth and incubated for 24 h to 37° C. Subsequently, 10 µL of SC broth was plated in modified-brilliant green agar (CM0329, Oxoid) with 50 µg/mL of nalidixic acid and incubated for 24 h at 37° C. Typical colonies were tested on lysine iron agar (LIA) (CM0381, Oxoid) and triple sugar iron agar (TSI) (CM0277, Oxoid) (Oliveira et al., 2010). Suspicious colonies were identified as *Salmonella* by slide agglutination with polyvalent *Salmonella* O antisera and then with *Salmonella* group B antisera (Probac of Brazil, São Paulo, SP, Brazil). Additionally, the disk diffusion test (Clinical and Laboratory Standards Institute, 2020) was used to verify whether S*almonella* isolated from organ samples after the challenge presented the same antibiotic resistance/susceptibility profile of the strain used to prepare the inoculum.

Detection of *Salmonella* Typhimurium by real-time polymerase chain reaction (qPCR)

The detection of *S.* Typhimurium DNA in cecum samples in SC broth was performed using the qPCR technique. The extraction of bacterial DNA was performed using the boil-centrifugation method (Freschi et al., 2005). The oligonucleotide primers (F) 5'-CGCTGGCAGAATGCTACCTC-3' and (R) 5'-AGCCCCAGTAATCCTAAAGCTTG-3' (Brunelle et al., 2011), used in the reaction, were based on the *hil*A gene from *Salmonella* Typhimurium. The amplification reaction was performed in a CFX96 Real-Time System thermocycler (BioRad®) using a final volume of 10 µL, containing a mixture of 1 µL of DNA-sample, 5 µL of SYBR PCR Master Mix (Promega®), 0.5 µL of each oligonucleotide primer and sterile ultrapure water (Nuclease-Free Water, Promega®). The thermal conditions and amplification time were 95° C for 10 min (initial denaturation), followed by 40 cycles at 95° C for 15 sec (denaturation) and 63.9° C for 30 sec (annealing and extension) in a two-step PCR protocol. As a negative control in the qPCR reactions, sterile ultrapure water

(Nuclease-Free Water, Promega®) was used. Serial dilutions of the pure culture of *Salmonella* Typhimurium were made to determine standards with different concentrations of DNA containing the target sequence. First, a 10^9 CFU/mL culture of *S.* Typhimurium was 10-fold diluted in peptone water up to 10^1 CFU/mL. Then, 2mL of each dilution (10^8 to 10^2 CFU/mL) was aliquoted and used for DNA extraction, and the DNA was used in the standard curve.

Statistical analysis

The results regarding water pH were evaluated by analysis of variance (ANOVA) and Student's t-test using the Statistical Analysis System - SAS program (SAS, 9.1.3 version, SAS Institute, Cary, NC, USA) after verification of normality by Shapiro–Wilk test. The results of feces consistency and microbiological isolation were evaluated using the chi-square test or Fisher's exact test. A value of $p<0.05$ was considered statistically significant. The kappa association coefficient determined the agreement between qPCR and microbiological isolation (Triola, 2017).

Results

Water pH

The organic acidifier product significantly decreased the pH of the drinking water in the treated group compared to the control ($p<0.05$). Detailed results are presented in Table 1.

Microbiological examination

Of the 84 organ samples collected from each experimental group on D3, D6, D9, and D12, it was possible to isolate *Salmonella* Typhimurium in 35 samples from G1 (41.7%; 35/84) and 25 samples from G2 (29.8%; 25/84) (Table 2). The animals treated with liquid organic acidifier (G2) had the lowest percentage of positive organ samples of *Salmonella* Typhimurium at times D3 (14.3%; 3/21) and D6 (33.3%; 7/21) compared with the control at D3 (57.1%; 11/21) and D6 (47.6%; 10/21), with a significant difference being observed at D3 (Figure 1). Furthermore, it was observed that the number of positive cecum samples

Table 1 – Daily pH values of untreated drinking water and water treated with liquid organic acidifier from the 1st to the 11th day of treatment (D-5 to D5)

Treatments	Moments/pH											
	D-5	D-4	D-3	D-2	D-1	D0	D1	D2	D3	D4	D5	Mean ± sd
Untreated	8.11	8.05	8.08	8.04	8.13	8.11	8.13	8.18	8.33	8.23	8.46	8.17±0.13^A
Treated	3.94	3.94	3.93	3.95	3.99	4.03	4.05	4.00	4.07	4.11	4.16	4.01±0.08^B

Means followed by the same letter in the column do not differ by Student's t-test ($p>0.05$).

Table 2 – Absolute number (n) and percentage (%) of *Salmonella* Typhimurium positive samples in the control group (untreated; G1) and the group treated with liquid organic acidifier (G2) in drinking water euthanized at 3 (D3), 6 (D6), 9 (D9) and 12 (D12) days after challenge with 10^6 CFU of *Salmonella* Typhimurium

Groups	Organs	Moments									
		D3		D6		D9		D12		Total	
		+/total	%	+/total	%	+/total	%	+/total	%	+/total	%
G1	Lung	1/3	33.3	2/3	66.7	0/3	0.00	1/3	33.3	4/12	33.3
	Liver	1/3	33.3	0/3	0.00	0/3	0.00	0/3	0.00	1/12	8.33
	Spleen	0/3	0.00	0/3	0.00	0/3	0.00	0/3	0.00	0/12	0.00
	Mesenteric Linf.	2/3	66.7	0/3	0.00	0/3	0.00	2/3	66.7	4/12	33.3
	Ileum	3/3	100	3/3	100	1/3	33.3	2/3	66.7	9/12	75.0
	Cecum	3/3	100	3/3	100	1/3	33.3	1/3	33.3	8/12	66.7
	Ileocolic Linf.	2/3	66.7	2/3	66.7	2/3	66.7	3/3	100	9/12	75.0
	Total	**12/21**	**57.1^A**	**10/21**	**47.6^A**	**4/21**	**19.0^A**	**9/21**	**42.9^A**	**35/84**	**41.7^A**
G2	Lung	0/3	0.00	1/3	33.3	1/3	33.3	2/3	66.7	4/12	33.3
	Liver	0/3	0.00	1/3	33.3	0/3	0.00	0/3	0.00	1/12	8.33
	Spleen	0/3	0.00	0/3	0.00	0/3	0.00	0/3	0.00	0/12	0.00
	Mesenteric Linf.	1/3	33.3	0/3	0.00	0/3	0.00	2/3	66.7	3/12	25.0
	Ileum	1/3	33.3	2/3	66.7	1/3	33.3	3/3	100	7/12	58.3
	Cecum	0/3	0.00	1/3	33.3	0/3	0.00	1/3	33.3	2/12	16.7
	Ileocolic Linf.	1/3	33.3	2/3	66.7	2/3	66.7	3/3	100	8/12	66.7
	Total	**3/21**	**14.3^B**	**7/21**	**33.3^A**	**4/21**	**19.0^A**	**11/21**	**52.4^A**	**25/84**	**29.8^A**

Values followed by the same letter in the column do not differ by the chi-square test ($p>0.05$).

Table 3 – Absolute number (n) and percentage (%) of cecum samples positive for *Salmonella* Typhimurium, by qPCR technique, in the control group (untreated; G1) and the group treated with the liquid organic acidifier (G2) in drinking water euthanized at 3 (D3), 6 (D6), 9 (D9) and 12 (D12) days after challenge with 10^6 CFU of *Salmonella* Typhimurium

Groups	Moments									
	D3		D6		D9		D12		Total	
	+/total	%	+/total	%	+/total	%	+/total	%	+/total	%
G1	3/3	100	3/3	100	3/3	100	2/3	66.7	11/12	91.7 [A]
G2	1/3	33.3	1/3	33.3	2/3	66.7	2/3	66.7	6/12	50.0 [B]

Values followed by the same letter in the column do not differ by the chi-square test or Fisher's exact test (p>0.05).

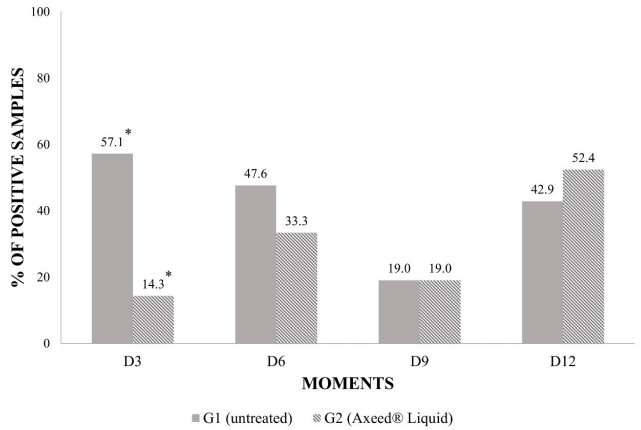

Figure 1 – Percentage (%) of *Salmonella* Typhimurium positive samples in the microbiological isolation in the control group (untreated; G1) and the group treated with liquid organic acidifier (G2) in drinking water euthanized at 3 (D3), 6 (D6), 9 (D9), and 12 (D12) days after challenge with 10^6 CFU of *Salmonella* Typhimurium. *p<0.05.

in untreated animals (G1; 66.7%; 8/12) was statistically different from the number of positive samples in treated animals (G2; 16.7%; 2/12), with a reduction of 75% of the total cecum positive samples in the treated group compared to the control. Based on the disk diffusion test, all recovered isolates showed the same antibiotic resistance/susceptibility profile as the challenge strain.

Detection of Salmonella Typhimurium in cecal samples by qPCR

Using the qPCR technique, it was possible to identify an increase of cecum samples positive for *Salmonella* Typhimurium (34 total positive samples) compared to microbiological isolation (21 total positive samples). All positive samples on microbiological examination were also positive on qPCR. The agreement between qPCR and bacteriological examination was moderate (kappa=0.49).

The number of positive samples for *Salmonella* Typhimurium from animals treated with liquid organic acidifier (G2) was significantly lower (50%; 6/12) than that observed in the control group (91.7%; 11/12) (Table 3), which characterizes a reduction of 45.4% in the number of positive cecum samples in the treated group compared to the control. Although the bacterial load estimated by qPCR was not statistically evaluated, it was noted that animals treated with liquid organic acidifier (G2) had lower counts of *Salmonella* Typhimurium/µL of SC broth (4.45±1.46 $\log_{10}$ copies) than that observed in the control group (5.10±2.02 $\log_{10}$ copies), which represents a difference of 11.8% in the total number of copies between groups.

Necroscopic examination

The main macroscopic findings in piglets after challenge with 10^6 CFU of *Salmonella* Typhimurium were: i) intestinal button-shaped ulcers (ileocecal region) in 66.7% of the animals from G1 (8/12) and G2 (8/12); ii) and lymphoid tissue hyperplasia in the colon region in 8.33% (1/12) animals from G1 and 25.0% (3/12) in animals from G2.

Discussion

It is known that long-period administration of organic acids to pigs via feed or drinking water can effectively reduce *Salmonella*. However, the strategic use of organic acids for a brief period after weaning needs to be clarified. Thus, the present study evaluated using a liquid organic acidifier via drinking water to control *Salmonella* Typhimurium in pigs. The bacterial isolation in the organs and the lesions found in necroscopic evaluation indicate the success of the infection challenge. Likewise, the acidifier maintained the pH of drinking water at around 4, providing an ideal acidity for consumption. Using drinking water pH lower than 4 is not attractive, as it would reduce water consumption by the piglets (Busser et al., 2008).

In the microbiological examination, animals treated with a liquid organic acidifier had the lowest percentage of *Salmonella* Typhimurium isolation at moments D3 and D6, with a statistical difference observed three days after the challenge (D3). Interestingly, it was noted that the number of positive cecum samples from animals treated with the acidifier via water was significantly lower (16.7%) than in the control group (66.7%). Due to its high sensitivity, the analysis of cecum samples by the qPCR technique detected a more

significant number of positive samples than the microbiological exam. The positive samples in the microbiological exam were confirmed in the qPCR. The untreated group had 91.7% positivity, while the treated group showed 50% positivity, which characterizes a reduction of 45.4% in the total cecum samples positive throughout the experimental period. Thus, it can be inferred that organic acids may be an interesting alternative for antibiotics used in swine production, as they can be used for decontamination of food, but especially in drinking water, where it decreases the pH and consequently reduces colonization of specific pathogens, like *Salmonella* (Berge & Wierup, 2012; Boyen et al., 2008).

Salmonella can proliferate and contaminate pig carcasses for a long period. Researchers have reported that *Salmonella* serotypes present in the slaughterhouse waiting area could be found in the cecum and lymph nodes after only a few hours of exposure (Hurd et al., 2001, 2002). Sporadic violations of regular slaughter hygiene, such as laceration of the intestine during evisceration, can also increase the overall contamination level of a single day's production (Berends et al., 1997). According to Pesciaroli et al. (2017), the presence of piglets with high *Salmonella* load in the cecum influenced the proportion of carcasses contaminated by the bacteria on the same day, a fact observed by a correlation, albeit weak, between carcass contamination and *Salmonella* load in the cecum. It was then suggested that the intestinal content of pigs with high bacterial loads could be the source of *Salmonella* present in their carcasses. This high bacterial load in the cecum can be attributed to re-infection or recent infection during transport, as *Salmonella* numbers in the intestines and feces of carrier pigs may rise during stress. In addition, a European Food Safety Authority study estimated that 90-100% of human infections caused by pork are attributable to pigs excreting more than 10^4 CFU of *Salmonella*/g in feces. Therefore, it was proposed that reducing the count of *Salmonella* in the intestine would be an effective strategy to reduce the human risk of *Salmonella* infection (Snary et al., 2016).

Several studies have shown the advantage of lowering *Salmonella* prevalence over a lengthy period (from roughly 25 kg live weight to slaughter age) by acidifying feed and drinking water (Busser, 2012; Van der Wolf et al., 1999, 2001). On the other hand, by using a treatment with an acidifier for 14 days before slaughter, Busser et al. (2009) did not observe a significant effect of a mix of organic acids (containing formic acid, propionic acid, acetic acid, sorbic acid, and a liquid carrier) in preventing contamination of carcasses by *Salmonella*. According to the authors, the acidification period may have needed to be longer, the amount supplied may have needed to be more adequate, and cross-contamination and infection may have happened while being transported and waiting (Busser, 2012).

The longer supply of acidified water with a mixture of acids (lactic, formic, propionic, and acetic) at a concentration of 0.035% during the fattening period (6-7 weeks of treatment) showed a reduction in the number of seropositive animals when compared to the control, as well as a decrease in the excretion of the bacteria in the feces of the treated group (Argüello et al., 2013). These results are crucial to demonstrate that this intervention can be a valuable strategy to reduce the prevalence of *Salmonella* on the farm. Likewise, Michiels et al. (2012) used a mix of formic acid, acetic acid, propionic acid, sorbic acid, and natural extracts as active compounds. They observed a significant reduction in the amount and duration of excretion of *Salmonella* after the challenge.

As reported by Braz et al. (2011), the use of acidifiers as growth promoters in pigs during the nursery phase did not differ from the use of antibiotics, suggesting that it might be used as a substitute to cut down on the use of antibiotics as growth promoters in the swine industry. Additionally, a recent review of organic acids used in pig production reinforced the idea of supplying organic acids in experimental conditions to increase growth performance and reduce the use of antimicrobials, especially during the weaning period (Busser, 2012; Tugnoli et al., 2020). It is also known that organic acids can inhibit the growth of pathogenic bacteria while not affecting beneficial microorganisms such as *Lactobacilli* (Tugnoli et al., 2020). Likewise, Ahmed et al. (2014) showed that using acidifiers significantly decreased fecal counts of *Salmonella* Typhimurium and *E. coli* and increased beneficial bacteria such as *Lactobacillus* spp. and *Bacillus* spp. counts compared to the results of the group that received only basal diet (control group).

Although the quantification of *Salmonella* Typhimurium in the cecum samples was performed, the results were considered qualitative only once these samples were pre-enriched immediately after euthanasia, and the methodological bias regarding the initial concentration of bacteria cannot be ruled out.

According to the information above, it is thought that acidifying feed and water at critical points in a pig's early life and periods of more significant stress will continue to be a viable choice for reducing the risk of S*almonella* in swine populations, as it can also reduce the contamination of carcasses in slaughterhouses, as demonstrated previously. The slaughter process is a critical step in the production line to reduce and minimize the entry of *Salmonella* into the food chain and protect consumer health. Therefore, it is essential to maintain a correct self-control system based

on hazard analysis and critical control points, as well as a high level of hygiene. When there is contamination by *Salmonella* in the processing line, this pathogen can quickly spread through the slaughter line, contaminating machines, knives, carcasses, and even workers (Migura, 2021). Thus, all parties interested in the production process must take responsibility for their share in the ongoing cooperative effort to control *Salmonella*.

Conclusion

Using the liquid organic acidifier in the drinking water of nursery piglets was an effective strategy for reducing the pH of drinking water and reducing the number of positive organ samples for *Salmonella* at euthanasia.

Conflict of Interest

This study was co-funded by Salmix Industry and Trade Ltd., the developer of the tested product Axeed® Liquid.

Ethics Statement

The study was approved by the Animal Use Ethics Committee (CEUA) of the School of Agrarian and Veterinarian Sciences (FCAV/UNESP), Jaboticabal Campus (Protocol no. 016527/19).

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