Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2022 / v.24 / n.2 / 001-006

http://dx.doi.org/10.1590/1806-9061-2020-1427

Original Article

Inclusion of Organic Acids in the Drinking Water and Feed for the Control of Salmonella Heidelberg in Broilers

■Author(s)

Ferreira TS[I] https://orcid.org/0000-0003-1178-5870
Ravetti R[II] https://orcid.org/0000-0003-0064-0254
Rubio MS[I] https://orcid.org/0000-0003-1389-4130
Alves LBR[I] https://orcid.org/0000-0001-5788-5514
Saraiva MMS[I] https://orcid.org/0000-0003-1875-4495
Benevides VP[I] https://orcid.org/0000-0002-7552-1607
Lima TS[I] https://orcid.org/0000-0001-8867-6344
Lima BN[I] https://orcid.org/0000-0002-6160-097X
Almeida AM[I] https://orcid.org/0000-0002-7264-1734
Berchieri Jr A[I] https://orcid.org/0000-0003-2522-6500

[I] Faculdade de Ciências Agrárias e Veterinárias – Universidade Estadual Paulista "Júlio de Mesquita Filho" – FCAV/Unesp – Jaboticabal Campus, SP, Brazil, 14884-900.
[II] Salmix Indústria e Comércio Ltda, Piedade, SP, Brazil, 18170-000.

■Mail Address

Corresponding author e-mail address
Taisa Santiago Ferreira
Department of Pathology Theriogenology and One Health, FCAV/Unesp, Jaboticabal campus – Via de Acesso Prof. Paulo Donato Castellane s/n, Jaboticabal - SP - Brazil. ZIP code: 14884-900.
Phone: +55 16 3209 7100 (ext. 7923)
Email: taisa.santiago@unesp.br

■Keywords

Acidifying agent; Additives; Antimicrobials; Salmonellosis.

Submitted: 01/July/2021
Approved: 12/November/2021

ABSTRACT

Poultry products may be a source of foodborne human salmonellosis. The use of alternatives to antimicrobials that are not harmful to humans may reduce the presence of *Salmonella* spp. in poultry production. Among the products used, organic acids stand out. In the present study, three different organic acid (OA) blends were evaluated for the control of *Salmonella* Heidelberg (SH) in commercial broilers. Day-old chicks (n = 114) were randomly assigned to four treatments, with three replicates of 12 birds each. Birds in treatments A and B received SCFA (0.2mL/L) and SCFA + MCFA (0.2mL/L), respectively, in the drinking water, while birds in treatment C received SCFA + MCFA in the feed (2g/Kg of feed). Birds from treatment D did not receive OAs (control group). At 8 days of age, each bird was orally inoculated with SH at 10^8 CFU/mL, and cloacal swabs and SH enumeration of the cecal content were performed 24-, 48-, and 72-hours post-inoculation (hpi). The results show a reduction of both SH shedding and counts in the birds fed OAs at all pi times relative to the control birds. Fecal shedding was significantly lower in the OA-treated groups compared with the control group. As for SH presence in the cecum, significant differences were detected between groups C and D at 24 and 72 hpi, and between groups B and D at 72 hpi. The results of this study indicate that the use of feeding OAs to broilers may contribute to reduce the incidence of SH in the poultry production chain, allowing better flock health management, provided an efficient biosecurity program is employed.

INTRODUCTION

Poultry products are relevant sources of human foodborne salmonellosis. Among *Salmonella* spp. serovars associated with human foodborne infections carried by poultry products, *Salmonella* Heidelberg (SH) has been one of the most frequently isolated in the last decade (CDC, 2013; GieraltowskI *et al.*, 2016; Green *et al.*, 2018; IFSAC, 2019).

Bacteria of the genus *Salmonella* can be introduced in commercial poultry farms by infected day-old chicks or by the consumption of contaminated feed (Berchieri Junior *et al.*, 1989; Zancan *et al.*, 2000). When broilers are contaminated with *Salmonella* spp. at hatch or at an early age, their immune system is still immature, allowing their gastrointestinal tract (GIT) to be readly colonized, resulting in long periods of microorganisms fecal shedding, and may cause systemic infection (Freitas Neto *et al.*, 2020). In addition, *Salmonella* spp. may persist and spread in poultry farms carried by rodents, wild birds, and insects, such as the lesser mealworm (*Alphitobius diaperinus*) and the house fly (*Musca domestica*) (Crippen *et al.*, 2009). These factors may explain why it is difficult to eliminate them from poultry production (Andino & Hanning, 2015).

Despite the many measures taken to try to prevent *Salmonella* spp. gut infection of commercial poultry, its occurrence is still frequent. The use of feed additives that are not harmful to humans can help minimize its presence in farms. In particular, probiotics, prebiotics, phytogenics, and organic acids stand out (El Baaboua *et al.*, 2018; Khan & Chousalka, 2020). When associated with good management practices and biosecurity measures, feed additives can be very efficient, especially organic acids (OAs) (Oliveira *et al.*, 2000; Borsoi *et al.*, 2011a; Pickler *et al.*, 2012; Zabot *et al.*, 2018; Calaça *et al.*, 2019).

Organic acids are described as performance and intestinal health enhancers in broilers (Viola & Vieira, 2007; Calaça *et al.*, 2019). Their antimicrobial action is attributed to their capacity to reduce the pH of GIT and directly act on the cell wall of Gram-negative bacteria, resulting in bacteriostatic or bactericidal effect (Dittoe *et al.*, 2018). Although the main benefits of OAs are related to GIT pH reduction, these compounds can also prevent the spread of pathogens by penetrating their cell wall, which is sensitive to external pH variations, and changing their physiology, such as the case of enteric serovars of *Salmonella* spp. (Van Immerseel *et al.*, 2003).

Organic acids used as feed additives are classified as short-chain fatty acids (SCFA), represented by formic, acetic, propionic and butyric acids, and medium-chain fatty acids (MCFA), such as capoic, caprylic, and capric acids. In their non-dissociated form, OAs of both classes change bacterial physiology, whereas MCFAs also reduce the expression of virulence genes in bacteria, impairing their capacity to invade intestinal epithelial cells (Van Immerseel *et al.*, 2004; Rubio *et al.*, 2009). Here, we presented an *in-vivo* research using three commercial organic acid blends added to the drinking water and feed of commercial broilers, aiming to evaluate the efficacy of these compounds to control *Salmonella* Heidelberg in broilers GIT.

MATERIAL AND METHODS

The study was carried out in the Avian Pathology sector of FCAV/Unesp, Jaboticabal campus, in accordance with the Ethical Principles on Animal Experimentation developed by the Brazilian College of Animal Experimentation and approved by the internal Ethics Committee on the Use of Animals (CEUA Process 012807/19; approved on 10 October 2019).

Inoculum preparation

The inoculum was prepared using a field isolate of *Salmonella* Heidelberg resistant to nalidixic acid and spectinomycin (SH^{NalSpc}), belonging to the bacterial library of the Avian Pathology sector, FCAV/Unesp. The strain is stored at -80 °C in lysogen broth (LB; Sparks, Maryland, USA) supplemented with 30% glycerol and was seeded in 10 mL of LB broth and incubated at 37ºC for 18 h at 150 revolutions per minute (rpm).

In-vivo assay

Day-old broiler chicks were obtained from a commercial hatchery and housed, immediately after arrival, in experimental cages (36 birds per cage), equipped with trough feeders and pressure drinkers, located in an air-conditioned room. The birds were offered water and feed *ad libitum*. The feed was based on corn and soybean meal and formulated to supply the birds' nutritional requirements according to the genetic company manual with the following levels: 9,500 kcal of metabolizable energy/g of diet, 22.2% of crude protein, 1.31% of digestible lysine, 0.852% of digestible threonine and 0.94% of digestible methionine + cystine. The feed did not contain any antimicrobials, anticoccidials, or animal meals.

In order to confirm that day-old chicks were free from *Salmonella* spp. at housing, drag swabs of the chick transport crates were collected as described by Zancan *et al.* (2000). In brief, a sterile cotton gauze soaked in Buffered Peptone Water (BPW) (Oxoid®, Basingstoke, Hampshire, UK - CM0509) was dragged on the meconium present in the chick transport crate, placed in a flask containing 50 mL Selenite broth (SN) (Oxoid®, Basingstoke, Hampshire, UK - CM0395) supplemented with novobiocin (4mg/mL), and incubated at 37 °C for 24 hours. Using a bacteriological loop, the SN broth then was plated on Brilliant Green agar (BG) (Oxoid®, Basingstoke, Hampshire, UK - CM0263) and MacConkey agar (MC) (Oxoid®, Basingstoke, Hampshire, UK - CM0115). The plates were incubated at 37 °C for 24 h, and the presence of suggestive colonies of *Salmonella* spp. was evaluated.

Experimental SH challenge and design

At 8 days of age, each bird was challenged once with 0.5 mL of the previously prepared SH^{NalSpc} inoculum containing 10^8 CFU/mL, which was administered directly into the crop with an intra-esophageal cannula. The treatments were applied between 6 hours post-inoculation (hpi) and 72 hpi. The evaluated products[1] included a blend of formic acid and propionic acid

[1](A) Axeed® Liquid; (B) Axeed® Duetto; (C) Axeed® Feed C810 (Salmix, Piedade, São Paulo, Brazil).

(SCFA) in the liquid form and a blend of formic acid and propionic acid combined with caprylic acid and capric acid (SCFA + MCFA) in liquid and powder form.

A total number of 144 broilers were randomly assigned to four treatment groups (A, B, C, or D), with three replicates of 12 birds each: in treatment A, the birds received SCFA in drinking water (0.2mL/L); in treatment B, SCFA + MCFA in the drinking water (0.2mL/L); in treatment C, SCFA + MCFA in the feed (2g/kg of feed), and birds in treatment D did not receive OAs (control treatment). The description of the treatments is shown in Table 1. Drinking water pH was measured before the beginning of the experiment and after the products were added.

Bacteriological assays

At 24, 48, and 72 hpi, four birds per replicate (12 birds per treatment) were sacrificed by neck dislocation for the collection of cecal contents. In order to determine possible SH^{NalSpc} shedding, cloacal swabs were collected immediately before euthanizing and placed in tubes containing 3 mL SN broth. After homogenization, swabs were streaked on Bright Green agar with 100μg/mL of nalidixic acid and spectinomycin (BG-Nal/Spc) and incubated at 37 °C for 24 h. In the absence of colonies after this period, the broth was again incubated at 37 °C for 24 h and plated under the same conditions.

SH^{NalSpc} in the cecal content was enumerated according to the method proposed by Barrow *et al.* (1987). In brief, the cecal content was diluted in Buffered Saline solution (PBS) at pH 7.4 at a ratio of 1:10, followed by serial decimal dilutions in tubes containing PBS at pH 7.4, which were plated on BG-Nal/Spc agar. Plates were read after incubation at 37 °C for 24 h. When the presence of SH^{NalSpc} was not detected after this period, an equal volume of SN broth at double concentration was added to the broth tubes. The tubes were incubated at 37 °C for 24 h, and the broth was then plated on BGNal/Spc agar. The number of CFU/g was transformed into $\log_{10}$ for statistical analysis and interpretation of the results.

Table 1 – Experimental groups of broilers challenged with *Salmonella* Heidelberg and fed organic acid blends in the drinking water an in the feed.

Groups	Composition	Inclusion	N. birds	Routes of administration
A	Formic acid and propionic acid*	0.2 mL/L	36 birds	Water
B	Formic acid, propionic acid, caprylic acid and capric acid**	0.2 mL/L	36 birds	Water
C	Formic acid, propionic acid, caprylic acid and capric acid***	2 g/kg	36 birds	Feed
D	Control	-	36 birds	-

*(A): inclusion of SCFA in the drinking water; (B): inclusion of SCFA + MCFA the in-drinking water; (C): inclusion of SCFA + MCFA in the feed; (D): control treatment.

Statistical analysis

Data on fecal shedding of SH^{NalSpc}, obtained from cloacal swabs of A, B, C, and D groups, were analyzed by the non-parametric Chi-Square Test at 5% of significance level (Zar, 2010). SH^{NalSpc} enumeration (CFU/g) in the cecal content were logarithmically transformed and subjected to analysis of variance (ANOVA) followed by the Tukey Test at 5% of probability level ($p<0.05$). All statistical analysis were performed using the GraphPad Prism software for Windows, version 8.00 (GraphPad Software, La Jolla, California, USA).

RESULTS

The analysis of drag swabs of the chick transport crates did not demonstrate the presence of *Salmonella* spp. Moreover, the drinking water pH immediately before OA addition was 8.31 and after the addition of treatments A and B the pH levels decreased to 3.96 and 4.2, respectively.

Table 2 and Figure 1 show SH fecal shedding and cecal colonization results according to the treatment, respectively. Both results demonstrated the presence of SH in the birds of all treatment groups at all evaluated times (24, 48, and 72 hpi). The number of SH-positive birds for fecal shedding was not different among A, B, and C groups ($p>0.05$); however, they were significantly lower ($p<0.05$) compared with group D (control).

Table 2 – Fecal shedding of SH^{NalSpc} in broilers treated with organic acids in drinking water and feed determined at 24-, 48-, and 72-hours post-inoculation.

Groups#	Hours post-infection*			Total
	24	48	72	
A	46.6 (7/15)	33.3 (5/15)	20 (3/15)	33.3 (15/45[a])
B	53.3 (8/15)	20 (3/15)	26.6 (4/15)	33.3 (15/45[a])
C	33.3 (5/15)	33.3 (5/15)	33.3 (5/15)	33.3 (15/45[a])
D	66.6 (10/15)	40 (6/15)	46.6 (7/15)	51.1 (23/45[b])

Means followed by different letters indicate statistical difference by the Chi-square test ($p<0.05$).

*Results are showing the percentage of collected positive swabs; in parenthesis are present as positives/total.

#(A): inclusion of SCFA in the drinking water; (B): inclusion of SCFA + MCFA the in-drinking water; (C): inclusion of SCFA + MCFA in the feed; (D): control treatment.

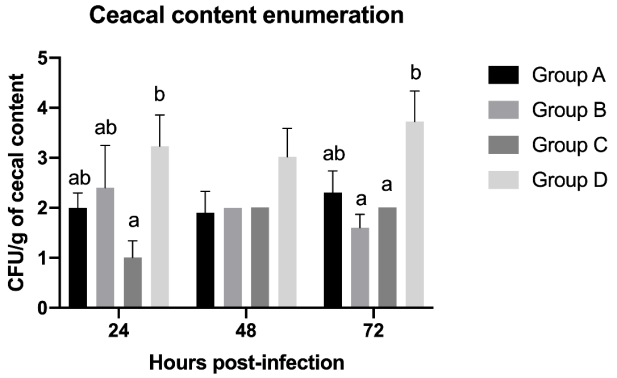

Figure 1 – *Salmonella* Heidelberg (SH) counts in the cecal of broilers challenged with SH and fed with organic acids (Group A: received SCFA in drinking water (0.2mL/L); Group B: SCFA + MCFA added to the drinking water (0.2mL/L); Group C: SCFA + MCFA in the feed (2g/kg of feed); and Group D did not receive OAs (control treatment). Different letters indicate statistical difference by the Tukey's test (p<0.05).

Likewise, the control birds (Group D) showed higher SH counts in the cecal content relative to those fed the evaluated AO blends (Figure 1). Compared with the control treatment (Group D), a significant reduction of the bacterial load was determined in Group C (p<0.05) at 24 hpi, whereas no statistical differences among treatments were detected at 48 hpi. At 72 hpi, lower SH counts were detected in Group B (p<0.01) and Group C (p<0.05) compared with Group D.

DISCUSSION

Avian salmonellosis is an important cause of concern for poultry farmers, both because the disease affects bird performance and health, and because poultry products are often associated with foodborne human salmonellosis (Chittick *et al.*, 2006; IFSAC, 2019). Controlling or minimizing the presence of paratyphoid *Salmonella* species is not an easy task, as its shedding in the feces contaminates the environment and allows its spread on poultry farms. According to Freitas Neto *et al.* (2020), measures to prevent *Salmonella* infection in young birds reduce its fecal shedding, contributing to the presence of these pathogens in the poultry production environment.

The dietary inclusion of performance-enhancing antimicrobials or antibiotic growth promoters (AGP) was of the main measures applied to mitigate the negative impacts of salmonellosis on the gastrointestinal tract of poultry for many years (Calaça *et al.*, 2019). However, the in-feed inclusion of AGPs has been banned in European countries because they may promote the emergence and selection of resistant microorganisms of public health importance, leading to consumer demands for animal products free from antimicrobial residues (COUNCIL, 2003; Seleha *et al.*, 2009). An alternative is the inclusion of organic acids (OAs) due to their antimicrobial potential (Van Immerseel *et al.*, 2007; Pickler *et al.*, 2012). The inclusion of alternative feed additives, such as probiotics and organic acids, in the drinking water or in the feed, may aid reducing *Salmonella* contamination of poultry farms as part of a comprehensive biosecurity program (Borsoi *et al.*, 2011a).

The results of the present study support literature findings indicating that the acidification of drinking water and of feeds using OAs contributes to the control of the spread of agents of paratyphoid infections, reduces fecal bacterial shedding, and improve the performance of broilers (Byrd *et al.*, 2001; Jarquin *et al.*, 2007; Pickler *et al.*, 2012; Machado Junior *et al.*, 2014). Other studies also report the capability of OAs to decrease *Salmonella* spp. fecal shedding and colonization levels of the internal organs of infected poultry (Thompson *et al.*, 1997; Menconi *et al.*, 2013), and suggest that investments in OAs are cost-effective in broiler production. According to Al-tarazi & Alshwabkeh (2003), the combination of formic acid with propionic acid (SCFA) can also effectively control systemic infection, as demonstrated by the reduced mortality and colonization of the crop and cecum of laying chicks challenged with *S.* Pullorum.

The most accepted antimicrobial modes of action of OAs are related to the diffusion of its undissociated form through the membrane of microorganisms and reduction of intestinal pH (Cherrington *et al.*, 1991). Upon entering the microbial cell, acids dissociate, suppressing cell enzymes and nutrient transport systems. These actions are dependent on OAs chemical formula and form, molecular weight, minimum inhibitory concentration against specific bacteria, as well as on microbial pH range and their nature (Huyghebaert *et al.*, 2011). Therefore, combinations of different organic acids may have a broader spectrum of activity, as observed in the present study.

Although treatments A (SCFA) and B (SCFA + MCFA) reduced drinking water pH from 8.31 to 3.96 and 4.2, respectively, resulting in lower cecal colonization by SH compared with the control treatment, the in-feed inclusion of SCFA + MCFA (Group C) proved to be more effective (p<0.05) in controlling SH spread in broilers. This result may be attributed to the antimicrobial activity of OAs against *Salmonella* spp. Organic acids, even at low concentrations, enhance gut acidification and remain longer in the intestinal tract of broilers when included in the feed than in the drinking water (Nakai *et al.*, 2003; Van Immerseel *et*

al., 2004). It should be noted the observed differences in cecal SH counts between the treatments including MCFA+SCFA compared with the treatment with only SCFA suggest a synergistic effect between these acids, hindering the colonization and invasion of intestinal epithelial cells by suppressing the *hilA* gene, which regulates the pathogenicity island I of bacteria of the genus *Salmonella* (Baxter & Jones, 2015).

The results of this study suggest that the in-feed inclusion of organic acids aids in the control of SH in broiler farms, particularly when applied early in the grow-out cycle, as it was shown that day-old chicks are already frequently infected at arrival on the farm (Zancan *et al.*, 2000; Freitas Neto *et al.*, 2020) and that SH is detected in the cecum as soon as 6 hours after infection (Borsoi *et al.*, 2011b). Therefore, in-feed organic acid blends may contribute to enhance the control of SH spread in broiler farms as part of an adequate biosecurity program.

ACKNOWLEDGMENTS

This study was supported by São Paulo State Research Support Foundation (FAPESP) through grants n. 2019/01809-4 (Taisa Santiago Ferreira) and 2018/03189-0 (Angelo Berchieri Junior).

REFERENCES

Al-Tarazi YH, Alshawabkeh K. Effect of dietary formic and propionic acids on *salmonella* pullorum shedding and mortality in layer chicks after experimental infection. Journal of Veterinary Medicine 2003;50 (3):112-117.

Andean A, Hanning I. *Salmonella* enterica: survival, colonization, and virulence differences among serovars. Scientific World Journal 2015;2015:1-16.

Barrow PA, Tucker JF, Simpson JM. Inhibition of colonization of the chicken alimentary tract with *Salmonella* Typhimurium gram-negative facultatively anaerobic bacteria. Epidemiology and Infection 1987;98(3):311-322.

Baxter MA, Jones BD. Two-component regulators control hila expression by controlling fimZ and hilE expression within *Salmonella* enterica Serovar Typhimurium. Infection and Immunity 2015;83(3):978-985.

Berchieri Junior A, Adachi SY, Calzada CT, Paulillo AC, Schoken-Iturrino RP, Tavechio AT. Meat meal as a source of *Salmonella* in a poultry farm. Brazilian Veterinary Research 1989;9(1):9-12.

Borsoi A, Santos LR, Diniz GS, Salle CTP, Moraes HLS, Nascimento VP. Fecal *Salmonella* excretion control in broiler chickens by organic acids and essential oils blend feed added. Brazilian Journal of Poultry Science 2011a;13(1):65-69.

Borsoi A, Santos LR, Rodrigues LB, Moraes HLS, Salle CTP, Nascimento VP. Behavior of *Salmonella* heidelberg and *Salmonella* enteritidis strains following broiler chick inoculation:evaluation of cecal morphometry, liver and cecum bacterial counts and fecal shedding patterns. Brazilian Journal of Microbiology 2011b;42(1):266-273.

Byrd JA, Hargis BM, Caldwell DJ, Bailey RH, Herron KL, MCReynolds JL, *et al.* Effect of lactic acid administration in the drinking water during preslaughter feed withdrawal on *Salmonella* and Campylobacter contamination of broilers. Poultry Science 2001;80(3):278-283.

Calaça GM, Café MB, Andrade MA, Stringhini JH, Araújo ICS, Leandro NSM. Chickens experimentally challenged with *Salmonella* enterica serovar Enteritidis and Eimeria tenella and treated with organic acids. Brazilian Animal Science 2019;20:1-10.

CDC- Centers for Disease Control and Prevention. National enteric disease surveillance: *Salmonella* Annual Report; 2013. Available from: www.cdc.gov/nationalsurveillance/pdfs/*Salmonella*-annual-report-2013-508c.pdf.

Cherrington CA, Hinton M, Chopra I. Organic acids: chemistry, antibacterial activity and pratical applications. Advances in Microbial Physiology 1991;32:87-108.

Chittick P, Sulka A, Tauxe RV, Fry AM. A summary of national reports of foodborne outbreaks of *Salmonella* Heidelberg infections in the United States: clues for disease prevention. Journal of Food Protection 2006;69(5):1150-1153.

Crippen TL, Sheffield CL, Esquivel SV, Droleskey RE, Esquivel JF. The Acquisition and Internalization of *Salmonella* by the Lesser Mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Vector-Borne and Zoonotic Diseases 2009;9(1):65-72.

Council of the European Union. Council regulation on the authorization of the additive avilamycin in feedingstuffs, 2003. Available from: http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:053:0001:0003:E N:PDF.

Dittoe DK, Ricke SC, Kiess AS. Organic acids and potential for modifying the \vian gastrointestinal tract and reducing pathogens and disease. Frontiers in Veterinary Science 2018. Available from: https://www.frontiersin.org/articles/10.3389/fvets.2018.00216/full.

El Baaboua A, El Maadoudi M, Bouyahya A, Belmehdi O, Kounnoun A, Zahli R, *et al.* Evaluation of antimicrobial activity of four organic acids used in chicks feed to control *Salmonella* typhimurium: suggestion of amendment in the search standard. International Journal Microbiology 2018;2018:7351593.

Freitas Neto OC, Penha Filho RA, Berchieri Junior A. Salmoneloses aviárias. In: Andreatti Filho RL, Berchieri Junior A, Silva EN, Back A, Fábio JD, Zuanaze MAF., editors. Doenças das aves. 3rd ed. Campinas: FACTA; 2020. p.495-517.

Gieraltowski L, Higa J, Peralta V, Green A, Schwensohn C, Rosen H, *et al. Salmonella* Heidelberg Investigation Team. National outbreak of multidrug resistant *salmonella* heidelberg infections linked to a single poultry company. PLoS One 2016;11(9):e 0162369.

Green A, Defibaugh-Chávez S, Douris A, Vetter D, Atkinson R, Kissler B,*et al. Salmonella* Heidelberg investigation team from the Food Safety and Inspection Service(FSIS). Intensified sampling in response to a *salmonella* heidelberg outbreak associated with multiple establishments within a single poultry corporation. Foodborne Pathogens and Disease 2018;15(3):153–160.

Huyghebaert G, Ducatelle R, Van Immerseel. An update on alternatives to antimicrobial growth promoters for broilers. The Veterinary Journal 2011;187(2):182-188.

IFSAC - Interagency Food Safety Analytics Collaboration Project. Foodborne illness source attribution estimates for 2017 for *Salmonella*, Escherichia coli O157, Listeria monocytogenes, and Campylobacter using multi-year outbreak surveillance data, United States.2019. Available from: https://www.cdc.gov/foodsafety/ifsac/pdf/P19-2017-report-TriAgency-508.pdf.

Jarquin RL, Nava GM, Wolfenden AD, Donoghue A M, Hanning I, Higgins SE, *et al.* The Evaluation of organic acids and probiotic cultures to reduce *salmonella* enteriditis horizontal transmission and crop infection in broiler chickens. International Journal of Poultry Science 2007;6(3):182-186.

Khan S, Chousalka KK. *Salmonella* Typhimurium infection disrupts but continuous feeding of Bacillus based probiotic restores gut microbiota in infected hens. Journal of Animal Science and Biotechnology 2020;11(29).

Machado Junior P, Beirão BC, Fernandes Filho T, Lourenço MC, Joineau ML, Santin E, *et al.* Use of blends of organic acids and oregano extracts in feed and water of broiler chickens to control *Salmonella* Enteritidis persistence in the crop and ceca of experimentally infected birds. The Journal of Applied Poultry Research 2014;23(4):671-682.

Menconi A, Reginatto AR, Londero A, Pumford NR, Morgan M, Hargis BM, *et al.* Effect of organic acids on *Salmonella* Typhimurium infection in Broiler Chickens. International Journal of Poultry Science 2013;12(2):72-75.

Nakai SA, Siebert KJ. Validation of bacterial growth inhibition models based on molecular properties of organic acids. International Journal of Food Microbiology 2003;86(3):249-255.

Oliveira GH, Berchieri Junior A, Barrow PA. Prevention of *Salmonella* infection by contact using intestinal flora of adult birds and/or a mixture of organic acids. Brazilian Journal of Microbiology 2000;31(2):116-120.

Pickler L, Hayashi RM, Lourenço MC, Miglino BL, Caron LF, Beirão BCB, *et al.* Avaliação microbiológica, histológica e imunológica de frangos de corte desafiados com *Salmonella* Enteritidis e Minnesota e tratados com ácidos orgânicos. Pesquisa Veterinária Brasileira 2012;32(1):27-36.

Rubio CF, Ordóñez C, González JA, Gallego AG, Honrubia MP, Mallo JJ, *et al.* Food additives based on butyric acid helps protect broilers from infection by *Salmonella* enteritidis. Poultry Science 2009;88(5):943-948.

Saleha AA, Myaing TT, Ganapathy KK, Zulkifli I, Raha R, Arifah k. Possible effect of antibiotic-supplemented feed and environment on the occurrence of multiple antibiotic resistant Escherichia coli in chickens. International Journal of Poultry Science 2009;8(1):28-31.

Thompson JL, Hinton M. Antibacterial activity of formic and propionic acids in the diet of hens on *Salmonella*s in the crop. British Poultry Science 1997;38(1):59-65.

Van Immerseel F, De Buck J, Pasmans F, Velge P, Bottreau E, Fievez V, *et al.* Invasion of *Salmonella* enteritidis in avian intestinal epithelial cells in vitro is influenced by short-chain fatty acids. International Journal of Food Microbiology 2003;85(3):237-248.

Van Immerseel F, De Buck J, Boyen F, Bohez L, Pasmans F, Volf J, *et al.* Medium-chain fatty acids decrease colonization and invasion through hilA suppression shortly after infection of chickens with *Salmonella* enterica serovar Enteritidis. Applied and Environmental Microbiology 2004;70(6):3582–3587.

Van Immerseel F, Russel JB, Flythe MD, Gantois I, Timbermont L, Pasmans F, *et al.* The use of organic acids to combat *Salmonella* in poultry:a mechanistic explanation of the efficacy. Avian Pathology 2007;35(3):182-188.

Viola EP, Vieira SL. Suplementação de acidificantes orgânicos e inorgânicos em dietas para frangos de corte:desempenho zootécnico e morfologia intestinal. Revista Brasileira de Zootecnia 2007;36(4):1097-1104.

Zabot S, Ruschel J, Marchesi CM, Alfaro AT, Oliveira TCRM, Hashimoto EH. Ácidos orgânicos e compostos clorados para controle de *Salmonella* spp. em frangos. Segurança Alimentar e Nutricional 2018;25(1):76-84.

Zancan FT, Berchieri Junior A, Fernandes SA, Gama NMSQ. *Salmonella* sp investigation in transport boxes of day-old birds. Brazilian Journal of Microbiology 2000;31(3):230-232.

Zar JH. Biostatistical analysis. 5th ed. Upper Saddle River: Prentice-Hall/ Pearson; 2010.