R. Bras. Zootec., 54:e20240221, 2025
https://doi.org/10.37496/rbz5420240221

Non-ruminants
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Evaluation of ethanolic extract from *Myrciaria cauliflora* as a feed additive in broiler chickens infected with *Salmonella* Heidelberg

Angélica Ribeiro Araújo Leonídio[1], Maria Auxiliadora Andrade[2], Eduardo de Paula Nascente[2], Amanda de Assis Carneiro[3], Beatriz Silva Gonzaga[2], Raiana Almeida Noleto Mendonça[2], Cíntia Minafra[2], José Henrique Stringhini[2], Ana Maria de Souza Almeida[2]*

[1] Instituto Federal de Educação, Ciência e Tecnologia Goiano, Urutaí, GO, Brasil.
[2] Universidade Federal de Goiás, Escola de Veterinária e Zootecnia, Goiânia, GO, Brasil.
[3] Universidade de Brasília, Brasília, DF, Brasil.

***Corresponding author:**
ana_almeida@ufg.br

Received: January 20, 2025
Accepted: June 4, 2025

How to cite: Leonídio, A. R. A.; Andrade, M. A.; Nascente, E. P.; Carneiro, A. A.; Gonzaga, B. S.; Mendonça, R. A. N.; Minafra, C.; Stringhini, J. H. and Almeida, A. M. S. 2025. Evaluation of ethanolic extract from *Myrciaria cauliflora* as a feed additive in broiler chickens infected with *Salmonella* Heidelberg. Revista Brasileira de Zootecnia 54:e20240221. https://doi.org/10.37496/rbz5420240221

Editors:
Ines Andretta
Vincenzo Tufarelli

Copyright: This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT - The objective of this study was to investigate the effects of supplementation with the ethanolic extract of *Myrciaria cauliflora* peels and seeds on the performance and intestinal health of broilers inoculated with *Salmonella* Heidelberg and reared until 28 days old. Three hundred thirty-six one-day-old male broiler chickens were distributed in a completely randomized design with four treatments and seven replications each: NC - negative control, which received a 0.85% sterilized saline solution in the crop; EJ - group that received the plant extract in the feed; SH - group that received the bacterial inoculum in the crop; and SH + EJ - group that received the bacterial inoculum in the crop and was fed the herbal extract in the diet. Supplementation with the herbal extract did not influence performance and increased *Salmonella* excretion in inoculated broiler chickens at 11 and 28 days old. *Salmonella* reduced jejunal villi height at 28 days, suggesting that the herbal extract caused a dysbiosis that favored the colonization and multiplication of *Salmonella*. The increased pathogen excretion may rise the transmission rate and the risk of foodborne illness. The use of the ethanolic extract of jabuticaba does not affect weight gain, feed conversion, average weight, histomorphometry, or intestinal biometry, but it increases *Salmonella* Heidelberg excretion.

Keywords: excretion, histomorphometry, phytogenic, salmonellosis, weight gain

1. Introduction

In recent decades, various additives have been employed in the diets of broiler chickens to enhance zootechnical indices. These supplements included the addition of low doses of antibiotics as growth promoters, which resulted in significant changes in the intestinal microbiota and, consequently, increased weight gain and feed efficiency in the broiler chickens. However, the prohibition of these additives by the European Union (Regulation EC No. 1831/2003) has adversely affected animal development and increased the incidence of enteric diseases, drawing international attention to the implications of antimicrobial use in public health (Bacanlı and Başaran, 2019; Tarradas et al., 2020; Silveira et al., 2021; Thompson et al., 2023).

In this context, the search for new additives that can satisfactorily replace antibiotic growth promoters has become essential. Among these alternatives, phytogenic additives, which are plant-derived

compounds added to animal diets, stand out. The primary benefit of supplementing animals with phytogenics involves the positive impacts they can have on animal health, such as acting on the intestinal microbiota by controlling the growth of pathogenic microorganisms, reducing ammonia production, controlling pathogen lesions, increasing mucus production in the intestines, and improving the animal digestive capacity and immune response (Ayalew et al., 2022; Abd El-Hack et al., 2022; Rahman et al., 2022; Ajmal et al., 2023; Almahallawi et al., 2024).

Myrciaria cauliflora (Mart.) O. Berg is a small tree species from the Myrtaceae family. It is widely distributed in South America, particularly in Brazil, where it is commonly known as "jabuticaba". Phytochemical analyses of the fruits have detected the presence of phenolic compounds, tannins, anthocyanins, and flavonoids (Albuquerque et al., 2020; Fidelis et al., 2020; Da Rosa et al., 2022). Ellagitannins such as strictinin, casuarinin, pedunculagin, vescalagin, and castalagin, among others, have also been identified (Pereira et al., 2016; Fidelis et al., 2020). Some studies conducted on different parts of the species have indicated antimicrobial potential against Gram-positive bacteria, Gram-negative bacteria, and fungi (Souza, 2007; Santos et al., 2019).

Ellagitannins, one of the main phytochemicals present in jabuticaba, can modulate the intestinal microbiota by increasing the population of lactobacilli and bifidobacteria, which are bacterial groups associated with various health benefits at cellular and systemic levels. Additionally, by stimulating the growth of beneficial bacteria, ellagitannins inhibit the establishment of potentially pathogenic microorganisms in the intestinal lumen (Sivamani et al., 2024).

Despite the wide variety of pharmacologically interesting compounds found in jabuticaba residues, studies on their antimicrobial activity against pathogenic microorganisms are scarce. Among the zoonotic pathogens of great importance to public health, the paratyphoid serovars belonging to the genus *Salmonella* stand out, as they are known worldwide to be the primary agents responsible for foodborne diseases in humans (Acevedo-Villanueva et al., 2021). In Brazil, *Salmonella* Heidelberg has been identified as one of the serovars frequently isolated in food poisoning outbreaks related to the consumption of eggs and poultry products. Currently, it is considered an emerging serovar, notable for its persistence in the environment (Voss-Rech et al., 2019; Soares et al., 2023).

Considering this, the objective of this study was to evaluate the inclusion of ethanolic extract from industrial jabuticaba residue in the diet on the performance and intestinal health of broilers experimentally inoculated with *Salmonella* Heidelberg.

2. Material and methods

The project was approved by the local Ethics Committee for the Use of Animals (CEUA; approval no. 107/15) and was carried out in Goiânia, GO, Brazil (−16.67926° N, −49.25629° W).

2.1. Experimental design

For the experiment, we utilized 336 one-day-old male Cobb 500 broiler chickens, which were distributed in a completely randomized design into four treatments with seven replicates each. The broiler chickens were housed, up to 28 days old, in groups of 12 broiler chickens per experimental unit. The broiler chickens were weighed and housed according to the following design: negative control group (NC), which received a 0.85% sterilized saline solution in the crop; EJ - received only the ethanolic extract of jabuticaba in the feed; SH - received the *Salmonella* Heidelberg inoculum via crop; SH + EJ - received the *Salmonella* Heidelberg inoculum via crop and the ethanolic extract in the feed.

Inoculated and non-inoculated broiler chickens were kept in separate housing units, in four-story galvanized steel battery cages equipped with linear feeders and drinkers, as well as trays for faeces collection, maintaining the same environmental conditions between them. The battery cages were heated with 60-W incandescent lamps (one per story) until 14 days old.

2.2. Preparation of inoculum and broiler chicken inoculation

The inoculum was prepared with *Salmonella* Heidelberg isolated from samples originating from broiler chickens. These samples were provided by the Núcleo Experimental de Doenças de Aves of the Universidade Federal de Goiás where the project was carried out and typified by the FIOCRUZ laboratory (Rio de Janeiro, RJ, Brazil).

The strain was subcultured on brilliant green agar and subsequently incubated at 37 °C for 24 h. The cells were then suspended in 0.85% buffered saline solution and stored at 4 °C. The concentration of 4.6×10^7 CFU/mL was adjusted using the McFarland scale and confirmed by plating serial dilutions on MacConkey agar, followed by incubation at 37 °C for 24 h and counting the colony-forming units (CFU) of *Salmonella* Heidelberg, according to the methodology proposed by Fernández et al. (2001).

The inoculation of the broiler chickens was performed at one day old, prior to housing. Using an automatic pipettor, each bird received 0.3 mL of 0.85% buffered saline solution orally, containing approximately 4.6×10^7 CFU/mL.

2.3. Obtaining the ethanolic extract from *Myrciaria cauliflora* peels and seeds

The standardized liquid extract of phenolic compounds from jabuticaba was processed at the Laboratório de Pesquisa de Produtos Naturais of the Faculdade de Farmácia of the Universidade Federal de Goiás where the project was carried out. The residue used in this research was a byproduct of the production of fermented jabuticaba (Myrciaria cauliflora (Mart.) O. Berg), composed mainly of seeds and fruit peels, kindly provided by a winery located in Hidrolândia, GO. The residue was dried at 40 °C in an air-circulating oven. The sample was then ground in a knife mill with a 100-mesh sieve (150 µm), and the resulting powder from the plant material was properly packaged in plastic bags and stored at a temperature of −10 °C.

The hydroalcoholic extract was obtained by percolation using 45% (v/v) ethanol as the solvent. The extraction process consisted of three stages: pre-swelling: for a period of 2 h with a ratio of 1 kg of plant material to 3 L of the hydroalcoholic mixture; rest: the previously prepared mixture was left to rest for 24 h, a phase known as intermediate maceration; and packing: transferring the previous material to a 10-L percolator, completing the volume with the same hydroalcoholic mixture, and percolation with a flow rate of approximately 0.2 mL/min (List and Schmidt, 1990).

Previous analyses conducted by Moreira (2017) revealed that the ethanolic extract of jabuticaba peels and seeds used in the present study contained 14.26% total phenols, 5.12% tannins, 28.23% flavonoids, 2.10% protein, and 1.75% ether extract, in addition to saturated fatty acids myristic (0.52%), palmitic (26.65%), and stearic (2.92%); monounsaturated fatty acids palmitoleic (1.22%) and oleic (11.12%); and polyunsaturated fatty acids linoleic (39.15%) and linolenic (8.21%). The concentration of ellagic acid was determined using high-performance liquid chromatography (HPLC), which indicated 130.02±0.62 µg/mL of the extract.

2.4. Feeding program

The feeding program consisted of three different experimental diets: pre-starter (1-7 days), starter (8-21 days), and grower (22-28 days), formulated according to the composition of the feed and the nutritional requirements proposed by Rostagno et al. (2024) (Table 1).

All diets used during the experiment were based on ground corn and soybean meal, without the addition of growth promoters. The liquid ethanolic extract of jabuticaba peels and seeds was added to the feed as a substitute for the inert ingredient (starch) at a dosage of 600 mg/kg of feed. Throughout the experimental period, the broiler chickens had *ad libitum* access to feed and water.

Table 1 - Percentual composition of the experimental diets provided to the birds during the experimental period (1-28 days old)

Item	Pre-starter (1-7 days)	Starter (8-21 days)	Grower (22-28 days)
Ingredient (g/kg)			
Corn grain	554.00	574.20	623.00
Soybean meal (45%)	381.70	351.70	313.40
Soybean oil	20.80	27.40	29.90
Limestone	9.40	10.00	5.10
Dicalcium phosphate	19.00	24.60	17.10
Common salt	5.00	4.50	4.20
L-threonine	1.10	0.60	0.30
L-lysine HCL	2.90	2.00	2.00
DL-methionine	3.60	2.40	2.40
Vitamin premix[1]	1.00	1.00	1.00
Mineral premix[2]	0.50	0.50	0.50
Inert (starch)	1.00	1.00	1.00
Nutrients			
Metabolizable energy (kcal/kg)	2,950	3,000	3,100
Crude protein (%)	22.20	20.80	19.50
Calcium (%)	0.92	1.11	0.73
Available phosphorus (%)	0.47	0.40	0.34
Sodium (%)	0.22	0.21	0.20
Lysine (%)	1.31	1.28	1.08
Methionine + cystine (%)	0.94	0.81	0.79
Methionine (%)	0.65	0.54	0.52

[1] Vitamin supplement (guaranteed levels per kg of product): vit. A, 1680,000 IU; vit. D3, 400,000 IU; vit. E, 3,500 mg; vit. K, 360 mg; vit. B1, 436.50 mg; vit. B2, 1200,000 mg; vit. B6, 624 mg; vit. B12, 2400,000 mcg; folic acid, 200 mg; pantothenic acid, 3120,000 mg; niacin, 8,400 mg; biotin, 10,000 mcg.
[2] Mineral supplement (guaranteed levels per kg of product): zinc, 17,500 ppm; iron, 12,500 ppm; copper, 2,000 ppm; iodine, 187.50 ppm; selenium, 75 ppm.

2.5. Performance

Weights of the broiler chickens and feed were recorded weekly up to 28 days old to calculate feed intake, average weight, weight gain, and feed conversion ratio. Dead broiler chickens were identified and weighed for the adjustment of feed intake and feed conversion ratio, as well as necropsied for the detection of macroscopic changes. The following parameters were observed for the performance analysis of the broiler chickens:

Average weight (AW): obtained by dividing the total weight of the broiler chickens in each plot by the total number of broiler chickens in the plot, AW = FW/TNB;

Weight gain (WG): calculated as the difference between the final and initial weights of the broiler chickens, added to the weight of the dead bird and divided by the average number of broiler chickens, WG = [(FW − IW) + Weight of the dead bird]/ANB;

Feed intake (FI): calculated as the ratio between total feed intake (supplied − leftovers) and the total number of broiler chickens;

Feed conversion ratio (FCR): calculated as the ratio: FI/AW.

2.6. Intestinal histomorphometry

At 11 and 28 days, the chickens were subjected to an 8-h feed withdrawal. One individual per plot, totaling seven per treatment, was euthanized by CO_2 inhalation followed by exsanguination via femoral

artery section. Fragments of the duodenum (collected at the pancreatic flexure) and jejunum (collected before Meckel's diverticulum) were collected and opened longitudinally, with the ends fixed with a clamp on a Styrofoam plate. Each piece was placed in pre-identified bottles containing 10% buffered formalin for the preparation of histological slides. After staining with hematoxylin and eosin (HE), histomorphometric analysis was performed by measuring villus height and crypt depth using the Image J® program version 1.45 (Rasband and Image, 2015).

Villus height was determined by measuring from the apex of the villus to the base at the junction with the crypt, and crypt depth was defined by the depth of the invagination of the crypt with the adjacent villi. Thirty readings per slide were taken for villus height and thirty consecutive readings for crypt depth per tissue fragment, always from right to left of the section, totaling 180 readings per treatment. The images were digitized using a Leica DM 4000 B optical microscope connected to a computer.

2.7. Detection of *Salmonella* in feces

At 11, 21, and 28 days old, fresh feces samples were collected from the trays of the battery cages. The samples were stored in sterilized containers and sent for bacteriological analysis. From each feces sample, 25 g were weighed and transferred to sterile Stomacher® bags containing 225 mL of 1% peptone solution and incubated at 37 °C for 18-20 h. After this period, the samples were homogenized, and 1 mL was transferred to 9 mL of Selenite Cystine broth and 0.1 mL to 10 mL of Rappaport Vassiliadis broth, followed by incubation at 37 °C for 24 h.

After this period, using a nickel-chromium loop, aliquots were streaked on the surface of Xylose-Lysine-Tergitol 4 (XLT4), Hektoen, and Brilliant Green agars and incubated again at 37 °C for 24 h. Colony-forming units with morphological characteristics of *Salmonella* were selected, and three to five CFU per plate were transferred to tubes containing Triple Sugar Iron (TSI) agar and incubated at 37 °C for 24 h. The TSI cultures with growth suggestive of *Salmonella* were subjected to urease, indole production, methyl red, motility, lysine decarboxylase, malonate, and Simmons' citrate tests. When biochemical tests were compatible with *Salmonella*, the samples were subjected to serological testing with polyvalent anti-O serum, and positive samples were sent to the Fundação Oswaldo Cruz (FIOCRUZ; RJ, Brazil) in nutrient agar for serotyping.

2.8. Goblet cell count

Samples of the duodenum and jejunum were processed and stained with Alcian Blue for the counting of goblet cells. Images of the intestinal mucosa were obtained using a Leica DM 4000 B optical microscope connected to a computer. Six fields per intestinal fragment were analyzed per slide at 10x magnification. The images were segmented using thresholding in the Image J® program version 1.45 (Rasband and Image, 2015), where the areas marked for goblet cells appeared black, and were subsequently quantified by the software.

2.9. Statistical analysis

Quantitative data on animal performance, histomorphometry, and goblet cell counts were subjected to analysis of variance (ANOVA), and means were compared using Tukey's test at the 5% significance level. The comparison of the percentage of *Salmonella* positivity in bird feces was performed using Pearson's chi-square test, with a significance level of 5%. The statistical analysis was conducted using the R software, version 3.2.2 (2015). The following model was used for the variables related to the broiler chickens:

$$Y_{ijk} = \mu + S_i + \alpha_{ij} + T_k + TS_{ik} + \beta_{ijk},$$

in which Y_{ijk} = value observed with ethanolic extract of jabuticaba with or without SH inoculation, μ = overall constant (population mean), S_i = effect of supplementation with ethanolic extract of jabuticaba (i = 600 mg/kg of feed), α_{ij} = random error associated with each observation Y_{ijk}, T_k = effect of

SH inoculation, TS_{ik} = interaction effect between the ethanolic extract of jabuticaba and SH inoculation, and β_{ijk} = random error associated with each observation Y_{ijk}.

3. Results

As observed, there was no influence of *Salmonella* inoculation or supplementation with the plant extract (P>0.05) on the performance variables in all analyzed periods (Table 2).

Table 2 - Initial average weight (IAW), final average weight (FAW), weight gain (WG), feed intake (FI), feed conversion ratio (FCR), significance level (P), and coefficient of variation (CV) observed in the experimental treatments for the performance results evaluation

Variable	Treatment[1]				CV (%)	P-value
	NC	EJ	SH	SH + EJ		
			1-7 days			
IAW (g)	41.8	41.8	41.9	42.0	0.53	0.4573
FAW (g)	129.4	127.9	134.7	133.9	4.67	0.1486
WG (g)	87.6	86.2	92.9	91.8	6.86	0.1649
FI (g)	85.2	86.0	89.9	85.2	7.83	0.6242
FCR (g/g)	1.00	1.00	0.96	0.93	5.93	0.2100
			1-14 days			
IAW (g)	364.8	363.5	375.1	385.6	4.54	0.0855
FAW (g)	322.9	321.7	333.1	343.4	5.13	0.0938
WG (g)	428.8	422.2	432.3	436.8	4.04	0.4824
FI (g)	1.32	1.33	1.29	1.30	4.77	0.5400
			1-21 days			
IAW (g)	769.6	757.6	763.9	733.4	4.41	0.2163
FAW (g)	727.8	715.8	725.8	691.2	4.53	0.1599
WG (g)	1018.6	1012.1	993.4	970.4	4.30	0.1785
FI (g)	1.39	1.38	1.39	1.40	3.87	0.9100
			1-28 days			
IAW (g)	1172.3	1178.4	1162.4	1171.0	4.76	0.9608
FAW (g)	1130.5	1136.6	1120.5	1128.9	4.93	0.9598
WG (g)	1747.1	1732.9	1734.5	1756.9	4.01	0.9066
FI (g)	1.51	1.52	1.54	1.56	4.57	0.5900

[1] NC - negative control; EJ - received the plant extract in the feed; SH - received the *Salmonella* Heidelberg inoculum via crop; SH + EJ - received the *Salmonella* Heidelberg inoculum via crop and the plant extract in the feed.

Even though it did not interfere with the performance of the broiler chickens, *Salmonella* was detected in the feces of inoculated broiler chickens at all evaluated ages (Table 3). *Salmonella* was not detected in samples from the negative control group and the group that received only the extract, confirming that there was no cross-contamination.

The frequency of *Salmonella* isolation in the feces showed a significant difference between the groups (P = 0.0179) at 28 days old, being higher in the inoculated group that received the ethanolic extract in the feed (SH + EJ) (Table 3). This result indicates that the plant product used intensified the intestinal colonization by the inoculated bacteria.

No influence of the plant product (P>0.05) was observed on the intestinal histomorphometry of the broiler chickens at the evaluated ages (Table 4).

It was observed that at 11 days old, inoculation had no effect (P>0.05) on villus height, crypt depth, and villus:crypt ratio in the analyzed intestinal segments. However, at 28 days old, the presence of *Salmonella*, regardless of supplementation with the plant extract, reduced jejunal villus height (P<0.05), demonstrating that the agent was capable of inducing cellular destruction.

Table 3 - Frequency of *Salmonella* isolation in feces samples from broiler chickens in each of the experimental treatments

Treatment[1]	Feces					
	11 days old		21 days old		28 days old	
	(+)	%	(+)	%	(+)	%
NC	0/7	0	0/7	0	0/7	0
EJ	0/7	0	0/7	0	0/7	0
SH	1/7	14.3	3/7	42.8	1/7	14.3
SH + EJ	3/7	42.8	3/7	4.8	6/7	85.7
P-value[2]	0.0719		0.0542		0.0179	

[1] NC - negative control; EJ - received the plant extract in the feed; SH - received the *Salmonella* Heidelberg inoculum via crop; SH + EJ - received the *Salmonella* Heidelberg inoculum via crop and the plant extract in the feed.
[2] Pearson's chi-square test.

Table 4 - Average villus heights (AV), crypt depths (CD), and villus:crypt ratio (V:C) in the duodenum and jejunum of broilers at 11 and 28 days old observed in each of the experimental treatments

Treatment[1]	Duodenum			Jejunum		
	AV (µm)	CD (µm)	V:C	AV (µm)	CD (µm)	V:C
			11 days old			
NC	1627.74	319.51	5.28	736.02	211.92	3.41
EJ	1412.21	273.37	5.49	745.55	234.69	3.34
SH	1524.61	277.22	5.52	872.94	250.54	3.51
SH + EJ	1434.84	317.32	4.53	749.78	217.67	3.43
CV (%)	16.96	13.88	21.76	14.69	20.4	18.23
P>F	0.6302	0.3118	0.6041	0.3413	0.6834	0.985
			28 days old			
CN	1704.68	246.39	5.94	1308.51a	255.35	5.13
EJ	1657.7	259.81	6.59	1308.38a	227.76	5.39
SH	1673.07	317.77	5.32	1080.96b	204.79	5.23
SH + EJ	1613.14	342.99	4.81	1029.82b	261.31	3.98
CV (%)	15.49	16.44	26.86	9.03	13.95	15.01
P>F	0.9706	0.0609	0.4226	0.004	0.1566	0.0899

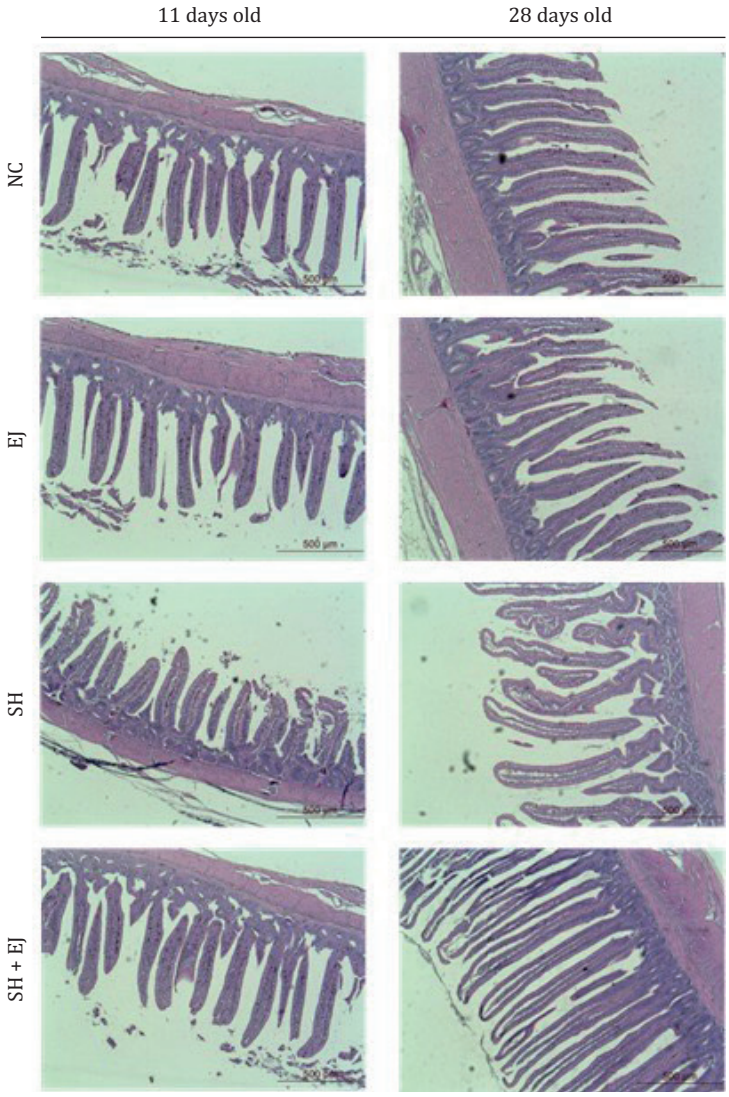
[1] NC - negative control; EJ - received the plant extract in the feed; SH - received the *Salmonella* Heidelberg inoculum via crop; SH + EJ - received the *Salmonella* Heidelberg inoculum via crop and the plant extract in the feed.
Means followed by different letters in the same column indicate significant differences using Tukey's test at 5%.

The broiler chickens that received only the *Salmonella* inoculum had a lower number of goblet cells in the jejunum at 11 days old (P<0.05) (Table 5; Figure 1).

Table 5 - Count of intestinal goblet cells at 11 and 28 days old recorded in each of the experimental treatments

Treatment[1]	11 days old		28 days old	
	Duodenum	Jejunum	Duodenum	Jejunum
NC	2226.3	2104.9a	1615.2	1769.0
EJ	1967.6	2383.7a	1787.8	2085.0
SH	1622.0	1292.9b	1727.7	2192.1
SH + EJ	2063.4	1910.4ab	1930.0	2362.3
CV (%)	28.4	19.7	15.6	17.7
P>F	0.5818	0.01	0.4692	0.1999

[1] NC - negative control; EJ - received the plant extract in the feed; SH - received the *Salmonella* Heidelberg inoculum via crop; SH + EJ - received the *Salmonella* Heidelberg inoculum via crop and the plant extract in the feed.
Means followed by different letters in the same column indicate significant differences using Tukey's test at 5%.



HE, 4x.
NC - negative control.

Figure 1 - Histomorphometry of villi height and crypt depth in the jejunum of 11- and 28-day-old broiler chickens non-inoculated and inoculated with *Salmonella* Heidelberg (SH) fed diet supplemented with ethanolic extract of jabuticaba (EJ).

4. Discussion

The results obtained in the present study are consistent with those found by Moreira (2017), who used the same amount of ethanolic extract of jabuticaba (600 mg/kg of feed) and also did not detect any effects of the product on the performance of broilers up to 21 days old.

Inoculation with *Salmonella* Heidelberg also did not affect the performance of the broiler chickens at the evaluated ages (P>0.05). A similar result was reported by Amerah et al. (2012) and Martins (2015), who also did not observe effects of *Salmonella* Heidelberg inoculation on the performance of broilers. There is evidence that paratyphoid *Salmonella* infections do not significantly interfere with the zootechnical performance of animals, as these microorganisms are capable of coexisting in balance with the host (Vellano et al., 2022).

Although it was isolated in the feces at all ages studied, inoculation with *Salmonella* Heidelberg did not produce clinical signs or mortality in the broiler chickens, characterizing a state of subclinical carrier. This excretion of *Salmonella* Heidelberg is alarming because it means a higher risk of transmission, due to the increase in bacteria in the environment and also a higher risk to food safety, since meat from asymptomatic carrier chickens may end up being sold (Collineau et al., 2020). Thus, the findings of the present study highlight the importance of monitoring the presence of *Salmonella* Heidelberg in poultry farms to prevent further spread of the agent on farms and in chicken meat.

This characteristic, inherent to paratyphoid *Salmonella*e, establishes an important source of contamination for the entire production chain. Infection of newly hatched chickens with *Salmonella* through oral inoculation or contact with excretions from diseased avian can lead to the establishment of intestinal colonization that persists into adulthood (Walker et al., 2021; Shanmugasundaram et al., 2021; Rana et al., 2021), promoting the dissemination of the agent during stressful periods (Berchieri Júnior et al., 2011).

Given its high phenolic content, the jabuticaba extract was expected to reduce *Salmonella* growth. These phytochemicals exhibit antimicrobial activity through various mechanisms such as inhibition of microbial enzymes, deprivation of substrates necessary for microbial growth, direct action on metabolism through inhibition of oxidative phosphorylation, deprivation of iron ions, promotion of bacterial aggregation—preventing cell separation during binary fission—, and damage to the cell membrane, among others (Chen et al., 2024; Choi and Kim, 2020; Choi et al., 2022; Górniak et al., 2019; Zhang et al., 2023; Tan et al., 2022).

The antimicrobial activity of *Myrciaria cauliflora* against *Staphylococcus aureus, Listeria monocytogenes, Salmonella enteritidis*, and *Escherichia coli* showed zones of inhibition for all bacteria tested, except from *Salmonella*, since none of the samples showed zone of inhibition for these bacteria (Silva et al., 2014).

A possibility for the extract having no effect on *Salmonella* may be attributed that Gram-negative bacteria such as *Salmonella* has a system of lipid bilayers that makes it difficult for antimicrobial agents to penetrate (Schved et al., 1994). The antibacterial action on other species of bacteria, such as Gram-positive bacteria, may have caused a dysbiosis that favored the colonization of *Salmonella* in the intestine of broiler chicken of the SH+EJ group (Silva et al., 2014).

This result suggests that supplementation with the extract promoted an imbalance in the intestinal microbiota which, combined with the constant reinfection facilitated by the close contact of the broiler chickens within the cage, likely allowed the colonization of enterocytes by *Salmonella*. This assertion is supported by Paz (2006) and Rocha (2008), who highlighted that a healthy intestinal microbiota contributes to host protection through competitive exclusion, which prevents the establishment of enteric pathogens.

The imbalance in the intestinal microbiota promoted by the plant extract also explains the lower recovery of *Salmonella* from the feces of the group that received only the bacterial inoculum (SH) at most of the evaluated ages. This is because the acquisition of a diversified intestinal microbiota from the first week of life reduces the intensity and duration of *Salmonella* colonization (Kogut and Miyakawa, 2023; Robinson et al., 2022; Litvak et al., 2019).

Further studies would also be needed to determine whether the inhibitory action of *Myrciaria cauliflora* against arginase (Silva et al., 2014), could have influenced the intestine colonization of SH + EJ group by *Salmonella*, since the competition between the inducible nitric oxide synthase (iNOS) and arginase for arginine contributes to the outcome of several parasitic and bacterial infections (Eriksson et al., 2000; Lahiri et al., 2008).

The evaluation of the influence of extracts or organic acids on the histomorphometry of chickens supplemented with these products and also challenged with *Salmonella* is essential to understand whether the possible intestinal lesion was caused only by the pathogen or also by the feed additive (Yang et al., 2019; Leonídio et al., 2024). The extract did not influence intestinal histomorphometry

of the broiler chickens at the evaluated ages (Table 4), as observed in the study by Moreira (2017), who did not observe any alteration in villus length or crypt depth in broilers supplemented with the ethanolic extract of jabuticaba peels and seeds.

The intestinal impacts of the *Salmonella* challenge, regardless of supplementation with plant extract, can be seen in broiler chickens at 28 days of age. There was a reduction in the height of the jejunal villi ($P<0.05$), demonstrating that the bacteria were capable of inducing cell destruction. Given the results, it is also possible to infer that the *Salmonella* Heidelberg strain used in our study was not capable of causing early intestinal lesions, since no changes were observed in the intestinal histomorphometry of broiler chickens at 11 days of age. In other studies with broiler chickens also challenged with *Salmonella* Heidelberg, it was also not possible to observe an early influence on the height of the jejunal villi, only after 21 days of life Previato do Amaral et al. (2016).

The reduction in jejunal villi did not interfere with the performance of the inoculated broiler chickens (Table 2). According to Previato do Amaral et al. (2016), this indicates that the evaluation of a single parameter is not sufficient to demonstrate the absorptive potential of the intestine. Villus measurements are commonly used to investigate the effects of nutrients on gastrointestinal physiology, but there is evidence that the correlation between bird performance and intestinal histomorphometry may not necessarily be related to villus size (Belote et al., 2023).

The groups of chickens challenged with *Salmonella* at 11 days of age had a reduction in goblet cells compared with the other groups ($P<0.05$) (Figure 1). This finding is expected in cases of intestinal infection, since the reduction in goblet cells minimizes the production of intestinal mucus and weakens the host's protective barriers in loco (Belote et al., 2023), since the reduction in mucus production provides better conditions for the multiplication of enteropathogens (Amit-Romach et al., 2009).

The reduction in goblet cells is one of the initial lesions in intestinal damage processes (Belote et al., 2023), which may elucidate this finding in the groups of birds in this study since no significant differences were found in *Salmonella* excretion or intestinal histomorphometry at this age (Tables 3 and 4).

Given the findings observed in this manuscript, it is believed that further studies are needed to evaluate the potential applications of jabuticaba extract in commercial poultry production.

5. Conclusions

Supplementation of broiler chickens with the ethanolic extract of industrial residue from *Myrciaria cauliflora* does not affect weight gain, feed intake, feed conversion ratio, average weight, or intestinal histomorphometry; however, it increases the excretion of *Salmonella* Heidelberg in inoculated broiler chickens. *Salmonella* Heidelberg demonstrated the ability to reduce the number of goblet cells in the jejunum of chickens at 11 days of age.

Data availability

The contents underlying the research text are included in the manuscript itself.

Author contributions

Conceptualization: Leonídio, A. R. A.; Andrade, M. A.; Carneiro, A. A.; Mendonça, R. A. N. and Minafra, C. **Data curation:** Leonídio, A. R. A.; Andrade, M. A. and Nascente, E. P. **Formal analysis:** Leonídio, A. R. A.; Andrade, M. A. and Almeida, A. M. S. **Funding acquisition:** Leonídio, A. R. A. **Investigation:** Leonídio, A. R. A. **Methodology:** Leonídio, A. R. A.; Andrade, M. A. and Minafra, C. **Project administration:** Andrade, M. A. and Stringhini, J. H. **Resources:** Leonídio, A. R. A. **Software:** Leonídio, A. R. A. and Mendonça, R. A. N. **Supervision:** Andrade, M. A. and Stringhini, J. H. **Validation:** Leonídio, A. R. A. and Almeida, A. M. S. **Visualization:** Leonídio, A. R. A. **Writing – original draft:** Leonídio, A. R. A. **Writing – review & editing:** Leonídio, A. R. A.; Andrade, M. A.; Nascente, E. P.; Gonzaga, B. S.; Stringhini, J. H. and Almeida, A. M. S.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors would like to express their gratitude to the farms, the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Laboratório de Enterobactérias of the Fundação Oswaldo Cruz, PhD Dâmaris Silveira from the Faculdade de Ciências da Saúde of the Universidade de Brasília, and the Escola de Veterinária e Zootecnia of the Universidade Federal de Goiás.

References

Abd El-Hack, M. E.; El-Saadony, M. T.; Salem, H. M.; El-Tahan, A. M.; Soliman, M. M.; Youssef, G. B. A.; Taha, A. E.; Soliman, S. M.; Ahmed, A. E.; El-kott, A. F.; Al Syaad, K. M. and Swelum, A. A. 2022. Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. Poultry Science 101:101696. https://doi.org/10.1016/j.psj.2022.101696

Acevedo-Villanueva, K. Y.; Akerele, G. O.; Hakeem, W. G. A.; Renu, S.; Shanmugasundaram, R. and Selvaraj, R. K. 2021. A novel approach against *Salmonella*: A review of polymeric nanoparticle vaccines for broilers and layers. Vaccines 9:1041. https://doi.org/10.3390/vaccines9091041

Albuquerque, B. R.; Pereira, C.; Calhelha, R. C.; Alves, M. J.; Abreu, R. M. V.; Barros, L.; Oliveira, M. B. P. P. and Ferreira, I. C. F. R. 2020. Jabuticaba residues (*Myrciaria jaboticaba* (Vell.) Berg) are rich sources of valuable compounds with bioactive properties. Food Chemistry 309:125735. https://doi.org/10.1016/j.foodchem.2019.125735

Almahallawi, R.; Al-Hoshani, N.; Al-Nabati, E. A.; Althubyani, S. A.; Negm, S.; El-lkott, A. F.; Bajaber, M. A.; Albohiri, H. H.; Khan, S. A.; Mahjoub, H. A.; Nazish, N.; Alsolami, A. M. and Ahmed, A. Z. 2024. Exploring the anticoccidial, growth-promoting, hematological and serological potential activities of *Linum usitatissimum* essential oil in broiler birds. Pakistan Veterinary Journal 44:117-122. https://doi.org/10.29261/pakvetj/2024.137

Ajmal, A. S.; Hussain, Z.; Jalees, M. M.; Shafi, J.; Manzoor, S. and Haq, A. U. 2023. Performance of broiler birds on feeding natural anti stressors in summer during heat stress. Asian Journal of Agriculture and Biology 2023(2). https://doi.org/10.35495/ajab.2022.024

Amerah, A. M.; Mathis, G. and Hofacre, C. L. 2012. Effect of xylanase and a blend of essential oils on performance and *Salmonella* colonization of broiler chickens challenged with *Salmonella* Heidelberg. Poultry Science 91:943-947. https://doi.org/10.3382/ps.2011-01922

Amit-Romach, E.; Uni, Z.; Cheled, S.; Berkovich, Z. and Reifen, R. 2009. Bacterial population and innate immunity-related genes in rat gastrointestinal tract are altered by vitamin A-deficient diet. The Journal of Nutritional Biochemistry 20:70-77. https://doi.org/10.1016/j.jnutbio.2008.01.002

Ayalew, H.; Zhang, H.; Wang, J.; Wu, S.; Qiu, K.; Qi, G.; Tekeste, A.; Wassie, T. and Chanie, D. 2022. Potential feed additives as antibiotic alternatives in broiler production. Frontiers in Veterinary Science 9:916473. https://doi.org/10.3389/fvets.2022.916473

Bacanlı, M. and Başaran, N. 2019. Importance of antibiotic residues in animal food. Food and Chemical Toxicology 125:462-466. https://doi.org/10.1016/j.fct.2019.01.033

Belote, B. L.; Soares, I.; Sanches, A. W. D.; Souza, C.; Scott-Delaunay, R.; Lahaye, L. Kogut, M. H. and Santin, E. 2023. Applying different morphometric intestinal mucosa methods and the correlation with broilers performance under *Eimeria* challenge. Poultry Science 102:102849. https://doi.org/10.1016/j.psj.2023.102849

Berchieri Júnior, A.; Silva, E. N.; Di Fábio, J.; Sesti, L. and Zuanaze, M. A. F. 2011. Doenças das aves. 2.ed. FACTA, Campinas.

Chen, X.; Lan, W. and Xie, J. 2024. Natural phenolic compounds: Antimicrobial properties, antimicrobial mechanisms, and potential utilization in the preservation of aquatic products. Food Chemistry 440:138198. https://doi.org/10.1016/j.foodchem.2023.138198

Choi, J. and Kim, W. K. 2020. Dietary application of tannins as a potential mitigation strategy for current challenges in poultry production: A review. Animals 10:2389. https://doi.org/10.3390/ani10122389

Choi, J.; Marshall, B.; Ko, H.; Shi, H.; Singh, A. K.; Thippareddi, H.; Holladay, S.; Gogal Jr., R. M. and Kim, W. K. 2022. Antimicrobial and immunomodulatory effects of tannic acid supplementation in broilers infected with *Salmonella* Typhimurium. Poultry Science 101:102111. https://doi.org/10.1016/j.psj.2022.102111

Collineau, L.; Phillips, C.; Chapman, B.; Agunos, A.; Carson, C.; Fazil, A.; Reid-Smith, R. J. and Smith, B. A. 2020. A within-flock model of *Salmonella* Heidelberg transmission in broiler chickens. Preventive Veterinary Medicine 174:104823. https://doi.org/10.1016/j.prevetmed.2019.104823

Da Rosa, R. G.; Sganzerla, W. G.; Barroso, T. L. C. T.; Buller, L. S.; Berni, M. D. and Forster-Carneiro, T. 2022. Sustainable production of bioactive compounds from jabuticaba (*Myrciaria cauliflora*): A bibliometric analysis of scientific research over the last 21 years. Sustainable Chemistry and Pharmacy 27:100656. https://doi.org/10.1016/j.scp.2022.100656

Eriksson, S.; Björkman, J.; Borg, S.; Syk, A.; Pettersson, S.; Andersson, D. I. and Rhen, M. 2000. *Salmonella* typhimurium mutants that downregulate phagocyte nitric oxide production. Cell Microbiol 2:239-250. https://doi.org/10.1046/j.1462-5822.2000.00051.x

Fernández, A.; Lara, C.; Loste, A.; Calvo, S. and Marca, M. C. 2001. Control of *Salmonella enteritidis* phage type 4 experimental infection by fosfomycin in newly hatched chicks. Comparative Immunology, Microbiology and Infectious Diseases 24:207-216. https://doi.org/10.1016/S0147-9571(00)00028-X

Fidelis, M.; Vieira do Carmo, M. A.; Azevedo, L.; Cruz, T. M.; Marques, M. B.; Myoda, T.; Sant'Ana, A. S.; Furtado, M, M.; Wen, M.; Zhang, L.; Rosso, N. D.; Genovese, M. I.; Oh, W. Y.; Shahidi, F.; Pap, N. and Granato, D. 2020. Response surface optimization of phenolic compounds from jabuticaba (*Myrciaria cauliflora* [Mart.] O.Berg) seeds: Antioxidant, antimicrobial, antihyperglycemic, antihypertensive and cytotoxic assessments. Food and Chemical Toxicology 142:111439. https://doi.org/10.1016/j.fct.2020.111439

Górniak, I.; Bartoszewski, R. and Króliczewski, J. 2019. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochemistry Reviews 18:241-272. https://doi.org/10.1007/s11101-018-9591-z

Kogut, M. H. and Miyakawa, M. E. F. 2023. Phenotype alterations in the cecal ecosystem involved in the asymptomatic intestinal persistence of paratyphoid *Salmonella* in chickens. Animals 13:2824. https://doi.org/10.3390/ani13182824

Lahiri, A.; Das, P. and Chakravortty, D. 2008. Arginase modulates *Salmonella* induced nitric oxide production in RAW264.7 macrophages and is required for *Salmonella* pathogenesis in mice model of infection. Microbes and Infection 10:1166-1174. https://doi.org/10.1016/j.micinf.2008.06.008

Leonídio, A. R. A.; Minafra, C.; Andrade, M. A.; Nascente, E. P.; Nascimento, G. M.; Santos, J. B.; Stringhini, J. H. and Almeida, A. M. S. 2024. Effects of encapsulated butyric acid on general performance, intestinal health, and colonization in organs of poultry infected with *Salmonella* Enteritidis. Revista Brasileira de Zootecnia 53:e20220150. https://doi.org/10.37496/rbz5320220150

List, P. H. and Schmidt, P. C. 1990. Phytopharmaceutical technology. CRC Press, Florida.

Litvak, Y.; Mon, K. K. Z.; Nguyen, H.; Chanthavixay, G.; Liou, M.; Velazquez, E. M.; Kutter, L.; Alcantara, M. A.; Byndloss, M. X.; Tiffany, C. R.; Walker, G. T.; Faber, F.; Zhu, Y.; Bronner, D. N.; Byndloss, A. J.; Tsolis, R. M.; Zhou, H. and Bäumler, A. J. 2019. Commensal *Enterobacteriaceae* protect against *Salmonella* colonization through oxygen competition. Cell Host & Microbe 25:128-139. https://doi.org/10.1016/j.chom.2018.12.003

Martins, J. C. G. 2015. Ácidos orgânicos e probiótico na redução da colonização de *Salmonella enterica* Heidelberg em frangos de corte. Dissertação (M.Sc.). Universidade Tecnológica Federal do Paraná, Londrina.

Moreira, J. S. 2017. Extrato padronizado de jaboticaba na alimentação de frangos e poedeiras comerciais. Tese (D.Sc.). Universidade Federal de Goiás, Goiânia.

Paz, A. S. 2006. Utilização de diferentes aditivos promotores de crescimento na alimentação de frangos de corte. Dissertação (M.Sc.). Universidade Federal da Bahia, Cruz das Almas.

Pereira, E. P. R.; Faria, J. A. F.; Cavalcanti, R. N.; Garcia, R. K. A.; Silva, R.; Esmerino, E. A.; Cappato, L. P.; Arellano, D. B.; Raices, R. S. L.; Silva, M. C.; Padilha, M. C.; Meireles, M. A.; Bolini, H. M. A. and Cruz, A. G. 2016. Oxidative stress in probiotic Petit Suisse: Is the jabuticaba skin extract a potential option? Food Research International 81:149-156. https://doi.org/10.1016/j.foodres.2015.12.034

Previato do Amaral, P. F. G.; Otutumi, L. K.; Rodrigues, G. V.; Lima, E. T.; Fernandes, J. I. M.; Vendrame, A.; Mezalira, T. S.; Suenaga, S. S.; Sestari, D. A. O.; Cestari, I. E. D. and Martins, L. A. 2016. Assessment of benzophenanthridne and protopine alkaloids in broiler challenged and not by *Salmonella* Heidelberg. Brazilian Journal of Poultry Science 18:525-534. https://doi.org/10.1590/1806-9061-2015-0045

R Core Team. 2015. The R Foundation for Statistical Computing, Vienna, Austria.

Rahman, M. R. T.; Fliss, I. and Biron, E. 2022. Insights in the development and uses of alternatives to antibiotic growth promoters in poultry and swine production. Antibiotics 11:766. https://doi.org/10.3390/antibiotics11060766

Rana, S.; Maurya, S.; Mohapatra, G.; Singh, S.; Babar, R.; Chandrasekhar, H.; Chamoli, G.; Rathore, D.; Kshetrapal, P. and Srikanth, C. V. 2021. Activation of epigenetic regulator KDM6B by *Salmonella* Typhimurium enables chronic infections. Gut Microbes 13:1986665. https://doi.org/10.1080/19490976.2021.1986665

Robinson, K.; Assumpcao, A. L. F. V.; Arsi, K.; Erf, G. F.; Donoghue, A. and Jesudhasan, P. R. R. 2022. Effect of *Salmonella* Typhimurium colonization on microbiota maturation and blood leukocyte populations in broiler chickens. Animals 12:2867. https://doi.org/10.3390/ani12202867

Rocha, T. M. 2008. Controle de *Salmonella* Typhimurium em frangos de corte utilizando composto com ácido benzóico, fumárico e 2-hidróxi-metiltio-butanóico. Dissertação (M.Sc.). Universidade Federal de Goiás, Goiânia.

Rostagno, H. S.; Albino, L. F. T.; Calderano, A. A.; Hannas, M. I.; Sakomura, N. K.; Perazzo, F. G.; Rocha, G. C.; Saraiva, A.; Abreu, M. L. T.; Genova, J. L. and Tavernari, F. C. 2024. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 5.ed. UFV, Viçosa, MG.

Santos, D. P.; Lopes, D. P. S.; Calado, S. P. M.; Gonçalves, C. V.; Muniz, I. P. R.; Ribeiro, I. S.; Galantini, M. P. L. and Silva, R. A. A. 2019. Efficacy of photoactivated *Myrciaria cauliflora* extract against *Staphylococcus aureus* infection – A pilot study. Journal of Photochemistry and Photobiology B: Biology 191:107-115. https://doi.org/10.1016/j.jphotobiol.2018.12.011

Schved, F.; Henis, Y. and Juven, B. J. 1994. Response of spheroplasts and chelator-permeabilized cells of gram-negative bacteria to the action of the bacteriocins pediocin SJ-1 and nisin. International Journal of Food Microbiology 21:305-314. https://doi.org/10.1016/0168-1605(94)90060-4

Shanmugasundaram, R.; Acevedo, K.; Mortada, M.; Akerele, G.; Applegate, T. J.; Kogut, M. H. and Selvaraj, R. K. 2021. Effects of *Salmonella enterica* ser. Enteritidis and Heidelberg on host $CD4^{+}CD25^{+}$ regulatory T cell suppressive immune responses in chickens. PLoS One 16:e0260280. https://doi.org/10.1371/journal.pone.0260280

Silva, M. C.; Souza, V. B.; Thomazini, M.; Silva, D. R.; Smaniotto, T.; Carvalho, R. A.; Genovese, M. I.; Favaro-Trindade, C. S. 2014. Use of the jabuticaba (*Myrciaria cauliflora*) depulping residue to produce a natural pigment powder with functional properties. Food Science and Technology 55:203-209. https://doi.org/10.1016/j.lwt.2013.08.026

Silveira, R. F.; Roque-Borda, C. A. and Vicente, E. F. 2021. Antimicrobial peptides as a feed additive alternative to animal production, food safety and public health implications: An overview. Animal Nutrition 7:896-904. https://doi.org/10.1016/j.aninu.2021.01.004

Sivamani, R. K.; Chakkalakal, M.; Pan, A.; Nadora, D.; Min, M.; Dumont, A.; Burney, W. A. and Chambers, C. J. 2024. Prospective randomized, double-blind, placebo-controlled study of a standardized oral pomegranate extract on the gut microbiome and short-chain fatty acids. Foods 13:15. https://doi.org/10.3390/foods13010015

Soares, L. S. G.; Casella, T., Kawagoe, E. K.; Benetti Filho, V.; Omori, W. P.; Nogueira, M. C. L.; Wagner, G.; Oliveira, R. R.; Stahlhofer, S. R.; Ferreira, F. A.; Tondo, E. C. and Lindner, J. D. D. 2023. Phenotypic and genotypic characterization of antibiotic resistance of *Salmonella* Heidelberg in the south of Brazil. International Journal of Food Microbiology 391-393:110151. https://doi.org/10.1016/j.ijfoodmicro.2023.110151

Souza, T. M. 2007. Estudo farmacognóstico e avaliação da atividade antimicrobiana e citotóxica de preparações cosméticas contendo o extrato de folhas de *Myrciaria cauliflora* O. Berg. e de casca de *Stryphnodendron adstringens* (Mart.) Coville. Dissertação (M.Sc.). Universidade Estadual Paulista Júlio de Mesquita Filho, Araraquara.

Tan, Z.; Deng, J.; Ye, Q. and Zhang, Z. 2022. The antibacterial activity of natural-derived flavonoids. Current Topics in Medicinal Chemistry 22:1009-1019. https://doi.org/10.2174/1568026622666220221110506

Tarradas, J.; Tous, N.; Esteve-Garcia, E. and Brufau, J. 2020. The control of intestinal inflammation: A major objective in the research of probiotic strains as alternatives to antibiotic growth promoters in poultry. Microorganisms 8:148. https://doi.org/10.3390/microorganisms8020148

Thompson, N. T.; Kitzenberg, D. A. and Kao, D. J. 2023. Persister-mediated emergence of antimicrobial resistance in agriculture due to antibiotic growth promoters. AIMS Microbiology 9:738-756. https://doi.org/10.3934/microbiol.2023038

Vellano, I. H. B.; Milbradt, E. L.; Polizel, D. M.; Oro, C.; Paz, I. C. L. A.; Padovani, C. R.; Gross, L.; Silva, T. M.; Hataka, A.; Okamoto, A. S. and Filho, R. L. A. 2022. Comparison of microbiological, pathological, and zootechnical aspects between broiler chickens and turkeys challenged with *Salmonella* Heidelberg. Journal of Applied Poultry Research 31:100266. https://doi.org/10.1016/j.japr.2022.100266

Voss-Rech, D.; Kramer, B.; Silva, V. S.; Rebelatto, R.; Abreu, P. G.; Coldebella, A. and Vaz, C. S. L. 2019. Longitudinal study reveals persistent environmental *Salmonella* Heidelberg in Brazilian broiler farms. Veterinary Microbiology 233:118-123. https://doi.org/10.1016/j.vetmic.2019.04.004

Walker, G. K.; Suyemoto, M. M.; Hull, D. M.; Gall, S.; Jimenez, F.; Chen, L. R.; Thakur, S.; Crespo, R. and Borst, L. B. 2021. Genomic characterization of a nalidixic acid-resistant *Salmonella* Enteritidis strain causing persistent infections in broiler chickens. Frontiers in Veterinary Science 8:725737. https://doi.org/10.3389/fvets.2021.725737

Yang, X.; Liu, Y.; Yan, F.; Yang, C. and Yang, X. 2019. Effects of encapsulated organic acids and essential oils on intestinal barrier, microbial count, and bacterial metabolites in broiler chickens. Poultry Science 98:2858-2865. https://doi.org/10.3382/ps/pez031

Zhang, L.; Yan, Y.; Zhu, J.; Xia, X.; Yuan, G.; Li, S.; Deng, B. and Luo, X. 2023. Quinone pool, a key target of plant flavonoids inhibiting gram-positive bacteria. Molecules 28:4972. https://doi.org/10.3390/molecules28134972