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Effects of *Curcuma longa* on the intestinal health of chicks infected with *Salmonella* Typhimurium

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ABSTRACT - This study was conducted to evaluate the effects of *Curcuma longa* on the growth performance, intestinal integrity, and antimicrobial activity of chicks colonized by *Salmonella* Typhimurium. The study included 672 one-day-old male chicks of the Cobb™ lineage, which were divided into eight treatment groups with 14 birds per group and six repetitions, for a total of 48 experimental units per treatment. A randomized 4×2 factorial design scheme (*C. longa* levels × inoculation by *Salmonella* Typhimurium) was used. Chicks were orally inoculated with 1.2×10^4 cfu/mL of *Salmonella* Typhimurium in 0.5 mL of 0.85% buffered saline solution. *Curcuma* was added to the feed of chicks at 0, 1, 2, and 3% for 35 days. Data were analyzed by analysis of variance and Tukey's test. Optimal feed conversion was observed in chicks given feed supplemented with 1% *C. longa*, regardless of infection, and 1% *C. longa* prevented intestinal colonization by *Salmonella* Typhimurium. Supplementation and bacterial infection influenced the histomorphometry and pH of the intestine. Bacterial infection reduced the intestinal pH, whereas *C. longa* supplementation increased the pH, but only in infected chicks. Thus, supplementation with 1% *C. longa* favors feed conversion, inhibits intestinal colonization by *Salmonella* Typhimurium, and does not alter intestinal integrity. In contrast, supplementation with 3% *Curcuma longa* decreases feed intake, affecting the performance of 35-day-old chicks.

Keywords: avian microbiology, intestinal histomorphometry, performance, poultry

Introduction

Additives are added to animal feed to improve performance and control pathogens in the gastrointestinal tract. However, in some consumer markets in the European Union (Huyghebaert et al., 2011) and Brasil (Brasil, 1998), the use of antibiotics of growth modulators is prohibited. These restrictions have prompted a search for alternative additives, including phytogenic compounds that exhibit antimicrobial properties.

The intestine contains diverse bacteria that have various beneficial health effects, such as the promotion of maturation and intestinal integrity, antagonistic activity against pathogens through competitive exclusion, and immunomodulatory activity. Regulation of microbiota physiology is important for preventing the pathological effects of undesirable bacteria (Lan et al., 2005).

Among the various pathogenic bacteria that can adversely affect animal production and/or the humans that consume animal products, *Salmonella* spp. are epidemiologically complex, because they are widespread in nature and have diverse serotypes, making them capable of infecting different animal species (Chambers and Gong, 2011). *Salmonella* Typhimurium, a zoonotic serotype, is a human pathogen, and infection can occur through the consumption of contaminated meat (Hur et al., 2012; Pickler et al., 2012).

Curcuma longa (Zingiberaceae) is a rhizomatous plant containing a volatile oil composed of monoterpenes and sesquiterpenes as the main chemical constituents. These terpenes possess pharmacological activities, such as anti-inflammatory, antimicrobial, anti-parasitic, antioxidant, immunostimulant, and hepatoprotective activities (El-Hakim et al., 2009; Eevuri and Putturu, 2013) and exhibit potential antimicrobial activities in chicks. In poultry feed, turmeric has been extensively used in different concentrations, dosages, and durations (Khan et al., 2012). Studies have shown that some phytogenic additives promote the beneficial modulation of the intestinal microbiota, have trophic effects on intestinal mucous, and stimulate immunomodulators, resulting in improved digestion, nutrient absorption, and performance (Nunes et al., 2009).

This study was conducted to evaluate the utility of *C. longa* as a phytogenic additive in feed by determining its effects on the performance, integrity, and intestinal colonization by *Salmonella* Typhimurium in infected chicks.

Material and Methods

The experimental protocol used in this study was approved by the local Ethics Committee for the Use of Animals (CEUA; approval no. 127/14) and was carried out in Goiânia, GO, Brazil (−16.67926° N, −49.25629° W).

The experimental design was entirely randomized in a 4×2 factorial scheme (*C. longa* levels × inoculation with *Salmonella* Typhimurium; Table 1). The study included 672 one-day-old male chicks of the Cobb™ lineage (sexing performed in the hatchery), which were divided into eight treatment groups with six repetitions and 14 chicks per experimental unit, totaling 48 experimental units per treatment.

Feed containing 0, 1, 2, and 3% *C. longa* was offered to chicks from one to 35 days old (Table 2). Both infected and non-infected chicks were housed separately in similar sheds. The sheds were equipped with feeders, drinkers, and heaters until they were 14 days old. At the time of lodging, the one-day-old chicks were orally inoculated with 0.5 mL of 0.85% buffered saline containing 1.2×10^4 cfu/mL of *Salmonella* Typhimurium.

The inoculum was prepared using *Salmonella* Typhimurium isolated from chicks provided by the Laboratory of Bacteriology and typed by the Laboratory of Oswaldo Cruz Foundation-FIOCRUZ (RJ). The isolate was cultivated on xylose-lysine-tergitol-4 (XLT4) agar and incubated at 37 °C for 24 h. *Salmonella* Typhimurium cells were recovered from the agar and suspended in 0.85% buffered saline solution and adjusted to a concentration of 1.2×10^4 cfu/mL using the McFarland standard. The concentration was confirmed by quantifying the cfu of serial dilutions plated on XLT4 agar incubated at 37 °C.

Table 1 - Design randomized in a 4×2 factorial scheme (*C. longa* levels × inoculation by *Salmonella* Typhimurium)

Treatment	*Curcuma longa L.* (Factor 1)	*Salmonella* Typhimurium (Factor 2)
Control	No	No - 0.85% buffered saline
CL1	1%	No - 0.85% buffered saline
CL2	2%	No - 0.85% buffered saline
CL3	3%	No - 0.85% buffered saline
Control + ST	No	Yes - 1-day-old chicks (orally inoculated)
CL1 + ST	1%	Yes - 1-day-old chicks (orally inoculated)
CL2 + ST	2%	Yes - 1-day-old chicks (orally inoculated)
CL3 + ST	3%	Yes - 1-day-old chicks (orally inoculated)

CL - Curcuma longa; ST - *Salmonella* Typhimurium.
CL1 - 1% *Curcuma longa*; CL2 - 2% *Curcuma longa*; CL3 - 3% *Curcuma longa*.

Three different experimental feeds were used: pre-starter, starter, and grower. The mashed animal feeds, containing ground maize and soybean meal, were formulated according to the composition and nutritional requirements recommended by Rostagno et al. (2011). Feed was provided *ad libitum* throughout the experimental trial. The feed did not contain growth moderators or anti-coccidian agents. The added *C. longa* powder replaced the starch, which is an inert material.

Dried and sprayed rhizomes of *C. longa* (as powder) were acquired from Mara Rosa, GO, Brazil (July 2013). Pharmacogenetic evaluation of the botanical material was carried out for quality control. Macroscopic analysis and thin-layer chromatography of the pulverized rhizomes were performed along with a curcumin standard (Merck, Billerica, MA, USA) as described by the Brazilian Pharmacopoeia (Brasil, 2010).

Performance was evaluated by determining the average weight, weight gain, feed intake, feed conversion (measured as the total weight of the dead chicks), viability, pH, and intestinal histomorphometry of chicks at 21 and 35 days of age. The average weight was obtained by dividing the total weight of birds by their average number. Weight gain was calculated by the difference between final and initial weight of birds added to the weight of the dead bird and by dividing by the average number of birds. Feed intake was calculated by the ratio between the total feed intake and the average number of birds. Feed conversion ratio was calculated by the feed intake:weight gain ratio, corrected for the total weight of dead birds. Viability was calculated by subtracting mortality from 100 (100 − mortality).

Table 2 - Ingredients, nutritional values (dry matter calculated), and inclusion levels of *Curcuma longa* in feed of 1 to 35-day-old chicks

Item	Diet (g kg^{-1} as fed)		
	Pre-starter (1-7 days)	Starter (8-21 days)	Grower (22-35 days)
Ingredient			
Corn	513.8	532.9	562.5
Soybean meal (45%)	361.2	337.9	301.5
Plant oil	36.3	45.5	54.9
Dicalcium phosphate	19.2	18.2	16.8
Calcium	8.4	8.1	7.70
Salt	4.5	4.4	4.2
L-lysine HCl	3.8	2.2	2.0
DL-methionine	3.7	2.6	2.4
L-threonine	1.6	0.7	0.5
Vitamin supplement[1]	2.0	2.0	2.0
Mineral supplement[2]	0.5	0.5	0.5
Inert/saffron (1, 2, and 3%)[3]	45.0	45.0	45.0
Total	1,000.00	1,000.00	1,000.00
Nutrient (of dry matter)			
ME (kcal kg^{-1})	2,960	3,050	3,150
Calcium (%)	0.942	0.899	0.837
Avaliable phosphorus (%)	0.471	0.449	0.418
Sodium (%)	0.224	0.218	0.208
Lysine (%)	1.503	1.316	1.212
Methionine + cysteine (%)	0.968	1.005	0.947
Methionine (%)	0.790	0.673	0.632
Crude protein (%)	22.11	21.14	19.73

[1] Vitamin supplement (provided per kg of diet): vitamin A, 1,680,000 IU; vitamin D3, 400,000 IU; vitamin E, 3500 mg; vitamin K, 360 mg; vitamin B1, 436.50 mg; vitamin B2, 1,200 mg; vitamin B6, 624 mg; vitamin B12, 2,400 mcg; folic acid, 200 mg; pantotenic acid, 3,120 mg; niacin, 8,400 mg; D-biotin, 10,000 mcg.
[2] Mineral supplement (provided per kg of diet): zinc, 17,500 ppm; iron, 12,500 ppm; copper, 2,000 ppm; iodine, 187.50 ppm; selenium, 75 ppm (without growth promoter and coccidian).
[3] Saffron powder was added to the feed instead of the inert material (starch) in 1, 2, and 3%, according to Abbas et al. (2010).

At each time point, chicks were weighed, desensitized, and sacrificed by inner section of the neck vessels, and subsequently necropsied. Fragments of the duodenum were collected from one chick per plot, and the fragments were placed in vials containing 10% buffered formaldehyde to prepare histological sections according to the method of Junqueira and Carneiro (1999). After flushing with hematoxylin and eosin (H&E), samples were subjected to histomorphometric analysis to measure villus height and crypt depth using ImageJ 1.45 (Rasband, 1997). Thirty measurements of villus height and 30 sequential measurements of crypt depth were obtained per section and per fragment of each tissue, always from the right to the left of the cut, totaling 180 readings per treatment. The imaging was performed using an optical microscope (DM 4000 B; Leica, Wetzlar, Germany) coupled to a microcomputer.

To test for the presence of *Salmonella*, swabs were collected from six chicks per treatment. The swabs were transferred to test tubes containing 9 mL of selenite-cystine (SC) broth and incubated at 37 °C for 18-24 h. Aliquots of the incubated broth were transferred to bright green (BG) and XLT4 agar and incubated at 37 °C for 18-24 h. Plates with 3-5 cfu showing the morphological characteristics of *Salmonella* were transferred to tubes containing triple sugar iron broth and incubated at 37 °C for 18-24 h. Tubes showing *Salmonella* growth were tested for urease, indole, methyl red, motility, decarboxylase, and lysine. Those showing *Salmonella*-compatible biochemical reactions were analyzed by using a serological test with *Salmonella* anti-O-antigen serum (Probac do Brasil, São Paulo, Brazil).

Performance, pH, and intestinal histomorphometry data were subjected to analysis of variance, and the averages were compared by Tukey's test (P = 0.05). When significance was detected, the polynomial regression model was adjusted. To evaluate intestinal colonization by *Salmonella* Typhimurium, a descriptive test that considered the frequency was used. For the contamination factor, Snedecor's F distribution was used to describe the difference in the effects of the bacterium.

Results

Macroscopic analysis of the pulverized rhizomes of *C. longa* showed robust yellowish coloration with whitish granulations and its characteristic smell. Illumination of a thin-layer chromatograph of the curcumin standard under ultraviolet light (365 nm), showed green fluorescence (Rf = 0.68) in the upper third of the plate; green fluorescence with the same characteristics as those of the standard was also observed in the chromatograph of *C. longa* samples (Rf = 0.68; Figure 1).

The performance analysis (Table 3) revealed no interaction (P>0.05) between the factors studied (i.e., *C. longa* levels and *Salmonella* Typhimurium infection) and final weight, feed intake, and feed conversion on days 1-21. However, supplementation with 3% *C. longa* resulted in decreased final weight, weight gain, and feed intake compared with the values in the control group (0% *C. longa*).

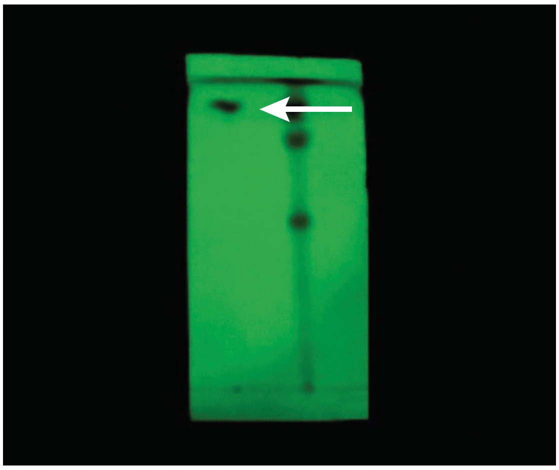

Figure 1 - Thin-layer chromatography of the sprayed rhizomes of *Curcuma longa* highlighting fluorescent green spot corresponding to curcumin (arrow).

Regression analysis of *C. longa* levels in 21-day-old chicks revealed negative linear effects on weight gain (Y = 22.485x − 817.65; R^2 = 0.1909) and feed intake (Y = 5.8139x − 155.65107; R^2 = 0.1912), demonstrating that a higher concentration of *C. longa* in the feed resulted in decreased weight gain and feed intake. Infection by *Salmonella* Typhimurium (P<0.05) also affected performance, as inoculation negatively influenced the final weight, weight gain, and feed intake (P<0.05).

In 35-day-old chicks (Table 4), no interaction was observed between the studied factors (*C. longa* levels and *Salmonella* Typhimurium contamination; P>0.05); however, a difference (P<0.05) was observed between *C. longa* levels in the feed and final weight, weight gain, and feed conversion. Chicks fed 1% *C. longa* presented higher final weights and weight gains than chicks fed 2 and 3% *C. longa* but did not significantly differ from the control. Feed conversion was higher in the group of chicks fed diet supplemented with 1% *C. longa* than in the groups fed diet supplemented with 0, 2, and 3% *C. longa*.

Table 3 - Performance variables of chicks inoculated by *Salmonella* Typhimurium and fed diet with *Curcuma longa,* up to 21 days old

Curcuma longa level	Performance variable					
	IW (g)	FW (g)	WG (g)	FI (g)	FC (g/g)	Viability (%)
0%	43.90	849.18a	805.27a	1,186.97a	1.47bc	83.93
1%	43.93	859.38a	815.46a	1,184.22ab	1.42c	79.16
2%	43.90	813.10ab	769.24ab	1,188.59a	1.55a	84.41
3%	43.99	789.72b	745.73b	1,143.22b	1.54ab	83.93
Regression	NS	NS	Linear[1]	Linear[2]	NS	NS
Contamination						
With *Salmonella*	43.93	804.54b	760.63b	1,142.14b	1.51	83.54
Without *Salmonella*	43.93	851.15a	807.22a	1,208.43a	1.48	82.14
	P-value					
Levels	0.802	0.0017	0.0017	0.0169	<0.0001	0.0801
Contamination	0.907	0.001	0.001	<0.0001	0.1057	0.3762
Levels × contamination	0.487	0.7078	0.7079	0.9174	0.1976	0.1104
CV (%)	0.55	4.50	5.80	3.21	3.97	6.59

IW - initial weight; FW - final weight; WG - weight gain; FI - feed intake; FC - feed conversion; NS - no significance; CV - coefficient of variation.
a,b - Averages with different letters differ statistically from each other by the Tukey test (P<0.05).
[1] Y = 22.485x − 817.65; R^2 Adjusted = 0.1909.
[2] Y = 5.8139x − 155.6510741; R^2 Adjusted = 0.1912.

Table 4 - Performance variables of chicks contaminated by *Salmonella* Typhimurium and fed diet with *Curcuma longa,* up to 35 days old

Curcuma longa level	Performance variable					
	IW (g)	FW (g)	WG (g)	FI (g)	FC (g/g)	Viability (%)
0%	43.90	1,786.01ab	1,742.11ab	5,500.09	2.04a	76.79
1%	43.93	1,861.20a	1,817.27a	5,613.46	1.93b	72.02
2%	43.90	1,753.44b	1,709.54b	5,514.31	2.02a	77.27
3%	43.99	1,724.34b	1,680.35b	5,412.49	2.02a	76.19
Regression	NS	NS	Quadratic[1]	Quadratic[2]	NS	NS
Contamination						
With *Salmonella*	43.93	1,736.88b	1,692.95b	5,347.43b	1.98	76.40
Without *Salmonella*	43.93	1,824.93a	1,780.99a	5,672.99a	2.02	74.70
	P-value					
Levels	0.802	0.005	0.0050	0.1255	0.0125	0.0993
Contamination	0.907	0.002	0.0020	<0.0001	0.1154	0.2912
Levels × contamination	0.487	0.5198	0.5180	0.6223	0.1315	0.1416
CV (%)	0.55	5.13	5.26	3.62	4.16	7.31

IW - initial weight; FW - final weight; WG - weight gain; FI - feed intake; FC - feed conversion; NS - no significance; CV - coefficient of variation.
a,b - Averages with different letters differ statistically from each other by the Tukey test (P<0.05).
[1] $Y = -33.883x^2 + 75.495x - 1{,}794.4$; R^2 Adjusted = 0.0967.
[2] $Y = 33.907x^2 + 75.547x - 1{,}750.5$; R^2 Adjusted = 0.0962.

Similarly, as was observed in the 21-day-old chicks, *Salmonella* Typhimurium infection in 35-day-old chicks compromised their performance and resulted in lower final weight, weight gain, and feed intake.

In the infected poultry groups (Table 5), *Salmonella* Typhimurium was not recovered from chicks fed diet supplemented with 1% *C. longa* at any time point (7, 14, 21, or 35 days), indicating that the inoculated bacterium could not colonize their intestines. However, *Salmonella* Typhimurium was not recovered from any of the inoculated groups after 21 days of age.

Histomorphometry of the jejunum in 35-day-old chicks (Table 6) revealed no interaction with the concentration of *C. longa* (P>0.05). Similarly, *C. longa* supplementation did not influence villus height or crypt depth (P>0.05) at 21 days. However, regression analysis revealed a quadratic effect on villus height ($Y = -45.828x^2 + 254.28x + 624.29$; $R^2 = 0.7137$), demonstrating that supplementation of feed with 2.7% *C. longa* promoted villus height. Furthermore, regression analysis revealed a significant (P<0.05) linear effect on crypt depth ($Y = 27.226x + 129.85$; $R^2 = 0.3799$), demonstrating increased crypt depth with increasing *C. longa* supplementation. *Salmonella* Typhimurium showed significant effects (P<0.05) on villus height and crypt depth in both the control and infected groups of 21- and 35-day-old chicks (Figure 2).

Thirty-five-day-old chicks fed 1% *C. longa* presented significantly greater crypt depth (P<0.05) in the jejunum than the control group, irrespective of *Salmonella* Typhimurium inoculation. However, *C. longa* supplementation had no effect (P>0.05) on villus height or villus:crypt ratio.

Table 5 - Frequency of *Salmonella* Typhimurium, in cloacal Swab, of inoculated chicks and chicks fed diet supplemented with different levels of *Curcuma longa*, at 7, 14, 21, and 35 days old

Curcuma longa level	Contamination by *Salmonella* Typhimurium (n/N (%))			
	7 days	14 days	21 days	35 days
0%	4/6 (66.6%)*	3/6 (50%)	0/6 (0%)	0/6 (0%)
1%	0/6 (0%)	0/6 (0%)	0/6 (0%)	0/6 (0%)
2%	2/6 (33.3%)	2/6 (33.3%)	0/6 (0%)	0/6 (0%)
3%	1/6 (16.6%)	2/6 (33.3%)	0/6 (0%)	0/6 (0%)

* n = positive; N = total analyzed; (%) = percentage of the total.

Table 6 - Average of villus height (VH), crypt depth (CD), and villus:crypt ratio of the jejunum of 21- and 35-day-old chicks inoculated by *Salmonella* Typhimurium and fed diet supplemented with *Curcuma longa*

Curcuma longa level	21 days			35 days		
	VH (µm)	CD (µm)	Villus:crypt	VH (µm)	CD (µm)	Villus:crypt
0	728.24c	169.20	4.46	1,197.25	224.70	5.83
1%	808.31b	155.67	5.34	1,195.34	235.84	5.20
2%	935.70a	170.38	5.68	1,116.79	253.58	4.55
3%	909.02a	189.88	5.26	1,165.28	228.09	5.39
Regression	Quadratic[1]	Linear[2]	NS	NS	NS	NS
Contamination						
With *Salmonella*	952.80a	201.19a	4.95	1,213.71a	245.72	5.11
Without *Salmonella*	737.84b	141.38b	542	1,128.93b	224.25	5.43
			P-value			
Levels	<0.0001	0.159	0.203	0.205	0.593	0.153
Contamination	<0.0001	<0.0001	0.122	0.005	0.167	0.331
Levels × contamination	<0.0001	0.707	0.039	0.097	0.531	0.544
CV (%)	8.35	21.10	24.09	7.56	24.41	25.03

NS - no significance; CV - coefficient of variation.
a,b - Averages with different letters differ statistically from each other by the Tukey test (P<0.05).
[1] $Y = -45.828x^2 + 254.28x + 624.29$; R^2 Adjusted = 0.7137.
[2] $Y = 27.226x + 129.85$; R^2 Adjusted = 0.3799.

Histomorphometric analysis of the duodenum did not show a significant effect (P>0.05) of the interaction between *C. longa* supplementation and *Salmonella* Typhimurium infection (Table 7) on crypt depth in 21-day-old chicks (Figure 3). However, there was a significant difference

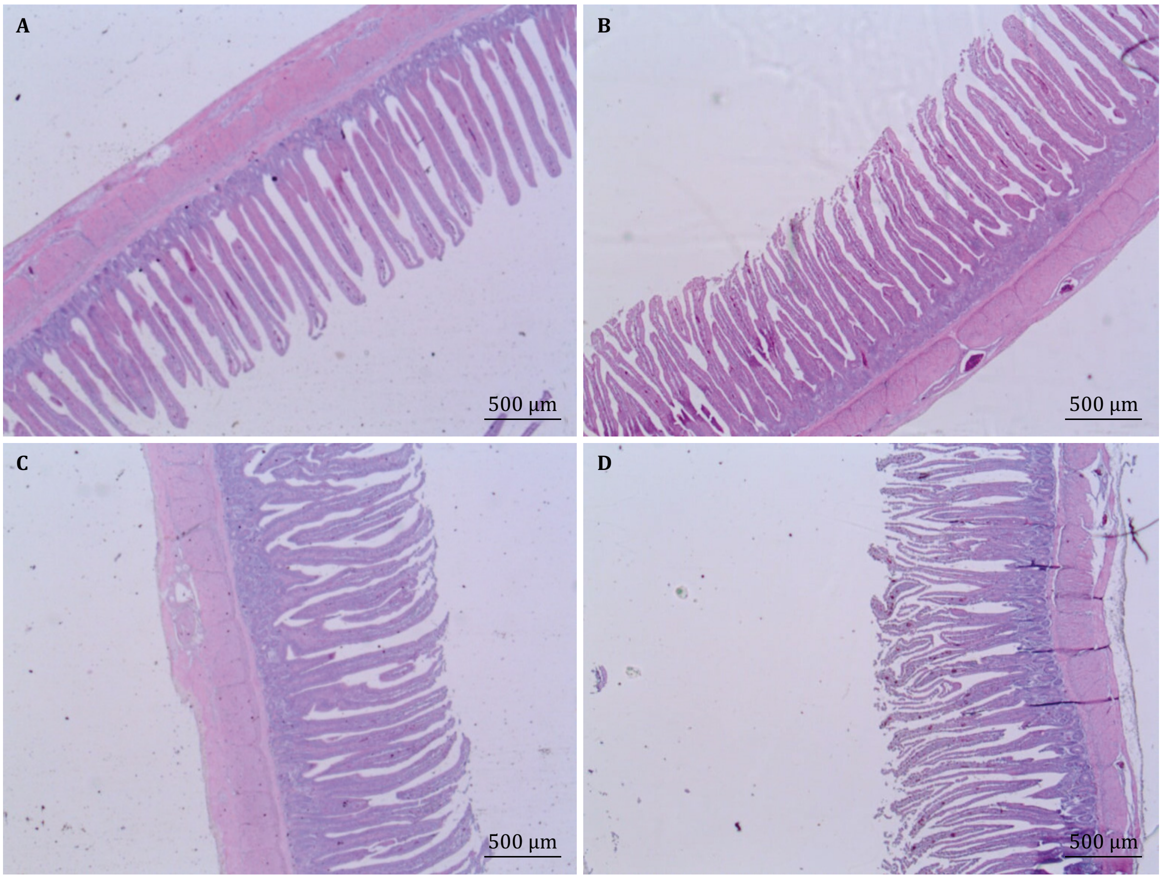

Figure 2 - Histological photos (villi height and crypt depth) of the jejunum of 21-day-old chicks uninoculated (A) and inoculated by *Salmonella* Typhimurium (B), and of 35-day-old chicks uninoculated (C) and inoculated by *Salmonella* Typhimurium (D), fed diet supplemented with 2% *Curcuma longa*.

Table 7 - Average of villus height (VH), crypt depth (CD), and villus:crypt ratio of the duodenum of 21- and 35-day-old chicks inoculated by *Salmonella* Typhimurium and fed diet supplemented with *Curcuma longa*

Curcuma longa level	21 days			35 days		
	VH (µm)	CD (µm)	villus:crypt	VH (µm)	CD (µm)	Villus:crypt
0%	1,466.0	251.81	6.18	1,421.8	257.0b	5.68
1%	1,395.0	255.67	5.55	1,489.4	324.0a	4.68
2%	1,476.5	233.1	6.43	1,565.8	317.4ab	5.14
3%	1,500.6	246.95	6.2	1,651.4	317.2ab	5.45
Regression	Quadratic[1]	NS	Quadratic[2]	NS	NS	NS
Contamination						
With *Samonella*	1,542.5a	264.8a	5.97	1,558.5	308.45	5.28
Without *Salmonella*	1,372.2b	2287b	6.20	1,505.7	299.43	5.19
			P-value			
Levels	0.610	0.660	0.368	0.093	0.017	0.221
Contamination	0003	0.011	0.558	0.424	0.578	0.803
Levels × contamination	0.054	0.478	0.055	0.200	0.258	0.404
CV (%)	13.34	19.07	19.36	14.8	18.37	23.15

NS - no significance; CV - coefficient of variation.
a,b - Averages with different letters differ statistically from each other by the Tukey test (P<0.05).
[1] $Y = 67.061x^2 - 122.13x + 1,405.4$; R^2 Adjusted = 0.1994.
[2] $Y = 0.3918x^2 - 1.0963x + 6.2807$; R^2 Adjusted = 0.7137.

(P<0.05) in villus height in the presence of *Salmonella* Typhimurium, and the values were lower in non-infected chicks.

Supplementation with *C. longa* significantly affected villus height (P<0.05). Groups of infected chicks fed feed supplemented with 2% *C. longa* showed increased villus height (Table 8). In non-infected

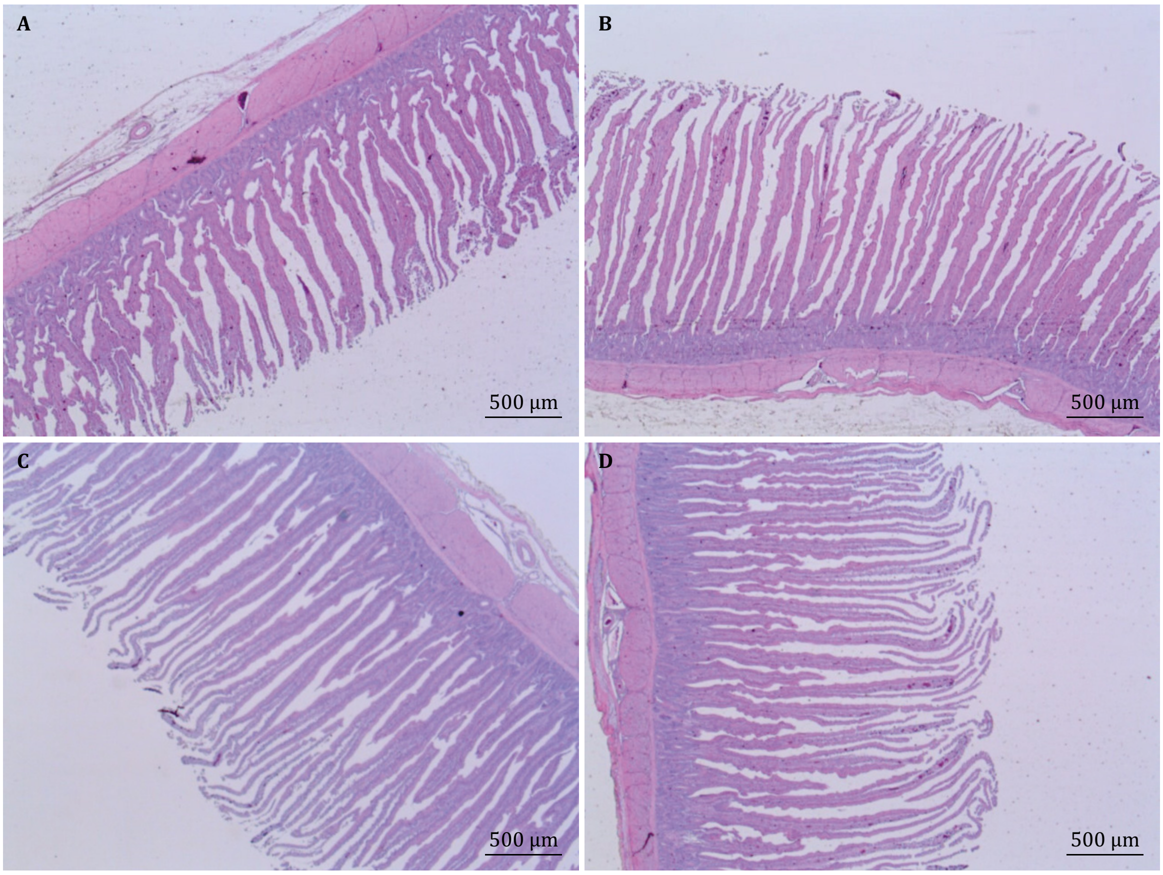

Figure 3 - Histological photos (villi height and crypt depth) at the duodenum of 21-day-old chicks uninoculated (A) and inoculated by *Salmonella* Typhimurium (B), and of 35-day-old chicks uninoculated (C) and inoculated by *Salmonella* Typhimurium (D), fed diet supplemented with 2% *Curcuma longa*.

Table 8 - Values of villus height and villus:crypt ratio in the jejunum of 21-day-old chicks of the control and inoculated with *Salmonella* Typhimurium and fed diet supplemented with different levels of *Curcuma longa*

Curcuma longa level	Villus height	
	With *Salmonella*	Without *Salmonella*
0%	866.87bA	589.62bB
1%	976.30abA	640.32bB
2%	1,049.92aA	821.47aB
3%	918.09bA	899.94aA
Regression	Quadratic[1]	NS
	Villus:crypt ratio	
0%	4.3aA	4.6aA
1%	5.5aA	5.2aA
2%	5.9aA	5.5aA
3%	4.1aA	6.4aA
Regression	Quadratic[2]	NS

NS - no significance; CV - coefficient of variation.
Averages followed by different uppercase letters in the same row and lowercase letters in the column indicate significant differences using Tukey test (P<0.05).
[1] $Y = -79.457x^2 + 329.34x + 642.76$; R^2 Adjusted = 0.7675.
[2] $Y = -0.6667x^2 + 1.6033x + 5.2283$; R^2 Adjusted = 0.1741.

chicks, supplementation with 2 and 3% *C. longa* also resulted in higher average villus heights. In 21-day-old infected chicks, regression analysis revealed a quadratic effect on villus height ($Y = -79.457x^2 + 329.34x + 642.76$; $R^2 = 0.7675$), demonstrating that supplementation with 2% *C. longa* resulted in increased villus height. No significant effect was observed in non-infected chicks.

In infected chicks, regression analysis showed a quadratic effect on the relationship between villi and crypts ($Y = -0.6667x^2 + 1.6033x + 5.2283$; $R^2 = 0.1741$; Table 8), and there was a strong relationship between villi and crypts in chicks fed diet supplemented 1.2% *C. longa*.

For intestinal pH, a significant interaction (P<0.05) was observed between *C. longa* supplementation and *Salmonella* Typhimurium infection in all segments of the intestine in 21-day-old chicks (Table 9). Analysis of the effects of the interaction between *C. longa* supplementation and *Salmonella* Typhimurium infection on the pH in the duodenum (Table 10) revealed that the pH values of infected chicks without *C. longa* supplementation were lower than the pH values of the infected chicks fed diet supplemented with 2 and 3% *C. longa*. In the regression analysis of different levels of *C. longa*, the infected groups showed a quadratic effect ($Y = -0.0667x^2 + 0.2867x + 5.97$; $R^2 = 0.3411$), demonstrating that 2.1% *C. longa* increased the duodenal pH. In addition, non-infected groups fed diet supplemented with 3% *C. longa* showed a higher pH than groups that received other concentrations of *C. longa*.

Salmonella Typhimurium-infected chicks given feed supplemented with 0 and 1% *C. longa* showed a lower pH in the duodenum (Table 10). Regression analysis of duodenal pH showed a linear effect ($Y = 0.11x + 6.01$; $R^2 = 0.3248$) for *C. longa* supplementation, revealing that the pH increased with increasing concentrations of *C. longa* in the feed.

Among non-infected chicks, the colon pH of chicks in groups fed diet supplemented with 3% *C. longa* was lower than that of chicks in groups fed diet supplemented with 2% *C. longa*. Regression analysis showed a significant relationship (P<0.05) between *C. longa* supplementation and colon pH, with a quadratic effect ($Y = -0.0917x^2 + 0.195x + 6.27$; $R^2 = 0.2318$), demonstrating that 1% *C. longa* supplementation resulted in a higher pH in the colon; no significant effect was observed in non-infected chicks.

In 35-day-old chicks, there was no effect of the interactions between the factors studied (*C. longa* levels and *Salmonella* Typhimurium infection). However, the extent of *Salmonella* Typhimurium infection affected the pH values in the duodenum and ileum, where significantly lower pH values were observed in the *Salmonella*-infected groups, regardless of *C. longa* supplementation (Table 11). In the jejunum and ileum, supplementation with 2 and 3% *C. longa* increased the pH to a higher level than in both the non-supplemented and 1% supplementation groups (P<0.05).

Table 9 - pH values in the intestinal segments of 21-day-old chicks inoculated by *Salmonella* Typhimurium and fed diet supplemented with *Curcuma longa*

Curcuma longa level	pH			
	Duodenum	Jejunum	Ileum	Colon
0%	6.18	6.14	6.23	6.23
1%	6.19	6.09	6.22	6.24
2%	6.25	6.33	6.33	6.33
3%	6.13	6.18	6.28	6.16
Regression	NS	NS	NS	NS
Contamination				
With *Salmonella*	6.17	6.18	6.23	6.24
Without *Salmonella*	6.21	6.19	6.30	6.24
	P-value			
Levels	0.2372	0.0366	0.5082	0.1629
Contamination	0.3024	0.7711	0.2176	0.9386
Levels × contamination	<0.0001	0.0115	0.0062	0.0136
CV (%)	2.23	2.18	3.12	2.98

NS - no significance; CV - coefficient of variation.
Averages followed by different letters in the same column indicate significant differences using Tukey test (P<0.05).

Table 10 - Values of intestinal pH: duodenum, jejunum, ileum, and colon of 21-day-old chicks in the control and inoculated with *Salmonella* Typhimurium and fed diet supplemented with different levels of *Curcuma longa*

Curcuma longa level	pH – duodenum	
	With *Salmonella*	Without *Salmonella*
0%	5.97bB	6.38aA
1%	6.20abA	6.18abA
2%	6.27aA	6.23abA
3%	6.23aA	6.03bA
Regression	Quadratic[1]	NS
	pH – jejunum	
0%	5.97bB	6.32aA
1%	6.13abA	6.05aA
2%	6.33aA	6.32aA
3%	6.27abA	6.08aA
Regression	Linear[2]	NS
	pH – ileum	
0%	6.05aA	6.40aA
1%	6.18aA	6.25aA
2%	6.27aA	6.38aA
3%	6.40aA	6.15aA
Regression	NS	NS
	pH – colon	
0%	6.15aA	6.30abA
1%	6.20aA	6.28abA
2%	6.28aA	6.38aA
3%	6.32aA	6.00bA
Regression	NS	Quadratic[3]

NS - no significance; CV - coefficient of variation.
Averages followed by different uppercase letters in the same row and lowercase letters in the same column indicate significant differences using Tukey test (P<0.05).
[1] $Y = -0.0667x^2 + 0.2867x + 5.97$; $R^2 = 0.3411$.
[2] $Y = 0.11x + 6.01$; $R^2 = 0.3248$.
[3] $Y = -0.0917x^2 + 0.195x + 6.27$; $R^2 = 0.2318$.

Table 11 - pH values of the intestinal segments of 35-day-old chicks inoculated by *Salmonella* Typhimurium and fed diet supplemented with *Curcuma longa*

Curcuma longa level	pH			
	Duodenum	Jejunum	Ileum	Colon
0%	5.99	6.03b	6.03b	6.18
1%	6.04	5.97b	6.04b	6.11
2%	6.14	6.18ab	6.25a	6.24
3%	6.11	6.26a	6.21a	6.26
Regression	NS	NS	NS	NS
Contamination				
With	6.00b	6.08	6.06b	6.14
Without	6.15a	6.14	6.20a	6.25
	P-value			
Levels	0.4846	0.0033	0.0348	0.3667
Contamination	0.0485	0.3238	0.0300	0.0840
Levels × contamination	0.2204	0.1680	0.2760	0.3733
CV (%)	4.20	3.31	3.55	3.68

NS - no significance; CV - coefficient of variation.
Averages followed by different letters in the same column indicate significant differences using Tukey test (P<0.05).

Discussion

The results of the pharmacogenetic evaluation agreed with the description in the Brazilian Pharmacopoeia (Brasil, 2010) for this plant species, indicating that the tested plant species was *C. longa*.

The performance of 21-day-old chicks decreased in the presence of supplementation with 3% of *C. longa* and in the absence of *Salmonella* Typhimurium infection. This result differed from the findings of Abou-Elkhair et al. (2014), who showed no significant difference in body weight and feed intake in a similar experiment, although they used lower concentrations of *C. longa* in combination with black pepper and oregano seed. Samarasinghe et al. (2003) and Al-Jaleel (2012) observed increased weight gain in birds fed diet supplemented with 0.2-0.3% *C. longa*. According to Al-Jaleel (2012), *C. longa* supplementation at <2% increased antioxidant activity. Abbas et al. (2010) used 1, 2, and 3% *C. longa* as an antimicrobial agent and observed increased feed intake, weight gain, and anti-coccidian activity in birds fed diet supplemented with 3% *C. longa*. Abdel-Rahman et al. (2014) observed a greater final weight of birds fed diet supplemented with a mixture of herbs, including *Curcuma* powder and fenugreek (*Trigonella foenum-graecum*), when compared with the control group.

In this study, feed intake decreased during the study period in all trials, resulting in lower weight gains and final weights of the chicks given feed supplemented with 3% *C. longa*. This reduction in feed intake can be attributed to the organoleptic characteristics of *C. longa*, such as its strong odor, present in the feed (Péret-Almeida et al., 2008; Sueth-Santiago et al., 2015). Moreover, the excretions from chicks in the groups given feed supplemented with higher concentrations of *C. longa* exhibited a more yellowish hue. These findings agree with those of Wahlstron and Blennow (1978), who showed that mice administered *C. longa* at a dose of 1 g kg^{-1} live weight eliminated 75% of the curcumin in feces. Additionally, Lao et al. (2006) observed more yellow stools in humans administered 12 g/day of *C. longa* in food.

Contamination with *Salmonella* Typhimurium on the first day of life led to poor performance. These results suggest that the inoculated bacteria affected the gastrointestinal tract by promoting an imbalance in the microbiota and injuring the mucous membranes, reducing digestion and feed absorption. This confirms the results of Borsoi et al. (2011), who reported reduced intestinal absorption of nutrients in the presence of enteropathogens.

Feed conversion by 35-day-old chicks was improved when 1% *C. longa* was included in the feed, regardless of *Salmonella* Typhimurium infection. In contrast, Emadi and Kermanshahi (2006) and Botelho (2014) did not observe any beneficial effects of *Curcuma* on performance at supplementation levels of 0.25, 0.50, 0.75% and 0.5, 1.0, 1.5, 2.0%, respectively. This variation in the effects of *C. longa* on the performance of chicks could be explained by variability in the quantity of phytochemicals in the plant, as well as other factors, such as the age and developmental stage of the plant, time of harvest, temperature, water availability, UV radiation, soil nutrients, altitude, and atmospheric composition. These factors can directly influence the relative proportions of these compounds in the plant.

In chicks given feed supplemented with 1% *Curcuma*, *Salmonella* was not recovered at any stage (7, 14, 21, and 35 days), despite inoculation with *Salmonella* Typhimurium on the first day of life. This suggests that feed containing 1% *Curcuma* prevented intestinal colonization by the inoculated bacterium. Thus, 1% supplementation showed antimicrobial activity, promoting alterations in the microbiota and acting against the inoculated bacterium (Lorenzi and Matos, 2002). Additionally, chicks may have eliminated *Salmonella* intermittently and in low numbers, thus limiting their isolation (Barancelli et al., 2012). Attia et al. (2017) also obtained significant results using 1% of *C. longa* demonstrating better performance feed as a phytogenic feed additive without negative effects on the productive and economic traits of broilers.

Salmonella Typhimurium was not recovered from any infected 21-day-old chick, which can be explained by the elimination of this bacterium accompanied by a gradual change in the intestinal microbiota and the development of intestinal lymphoid tissue, which occurs naturally with advancing age to increase resistance to microbial attack. Similarly, Beal et al. (2004) infected birds with *Salmonella* Typhimurium in the first, third, and sixth weeks of life and observed that the rates of persistent infection were lower in older infected birds. The resistance of the birds and the invasive capacity of the serovar also influence colonization by and excretion of *Salmonella* (Barrow, 2000; Andrade et al., 2007).

Intestinal histomorphometry showed that supplementation with 1% *Curcuma* increased crypt depth in the duodenum, indicating an attempt to recover the structure of the villi. These results agree with those of Abdel-Rahman et al. (2014), who also observed greater crypt depth in birds fed diet supplemented with a mixture of herbs composed of *C. longa* powder and *Trigonella foenum-graecum*.

Villus height and villus:crypt ratio are markers of mucosal integrity and intestinal function. As observed in this study, by 21 days, the pathogen had compromised the intestinal epithelium of the duodenum, resulting in increased villus height and a decreased villus:crypt ratio. Higher villi indicate greater digestive capacity; however, the weak relationship between these evaluations suggest that cellular proliferation had occurred to restore the villi that were destroyed. Similar results were observed by Viola et al. (2008) and Santos (2010), who reported that a lower villus:crypt ratio indicates the presence of destroyed villi and increased cellular proliferation in the crypts, resulting from an attempt to restore the damaged intestinal epithelium and overcome microbiological challenge in the intestine.

In non-infected chicks fed diet supplemented with 2 and 3% *C. longa*, trophic action was observed in the intestinal mucous of the duodenum, influencing cellular proliferation and increasing villus height to repair the damaged structure. Among the concentrations evaluated, the villi were highest in the *Salmonella* Typhimurium-infected group with 3% *C. longa* supplementation.

The level of *C. longa* supplementation as well as the presence of the pathogen altered the pH of the duodenum. The increased pH in the groups receiving *C. longa* supplementation facilitated bacterial proliferation to increase the diversity of the intestinal microbiota, particularly bacteria that are beneficial to the host. Huang et al. (2008) reported that the low pH values in *Salmonella*-infected chicks could be due to increased intestinal fermentation by *Salmonella*, which produces volatile fatty acids. A high dose of *Curcuma* (3%) likely increased the prevalence of acidophilus bacteria (i.e., *Lactobacillus*) in this segment of the intestine. This prediction agrees with the results of Lu et al. (2003), who reported that several factors might alter the pH and intestinal microbiota, such as age, antimicrobial supplementation, and infection by pathogenic microbes.

Conclusions

The inclusion of 1% *C. longa* in chicken feed improves performance, preserves intestinal integrity, and inhibits intestinal colonization by *Salmonella* Typhimurium when inoculated on the first day of life. Chicks provided feed containing 3% *C. longa* have worse performance, with reduced feed intake, irrespective of bacterial infection.

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