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Citrus sinensis essential oil as a modulator of intestinal morphology in broiler chickens

[Óleo essencial de *Citrus sinensis* como modulador da morfologia intestinal em frangos de corte]

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Abstract: This study evaluated the effects of supplementation with *Citrus sinensis* essential oil, obtained from juice industry waste, on the intestinal morphology of broiler chickens. The experiment used 150 Cobb chickens, divided into three treatments: control, antibiotic (200 ppm amoxicillin), and orange essential oil (200 ppm). At 21 and 42 days, histomorphometric analyses of the villi and crypts of the small intestine portions (duodenum, jejunum, and ileum) were performed. At 21 days, villi and crypts were larger in the negative control treatment compared to the positive control treatment (amoxicillin) and the treatment with orange essential oil, while in the ileum, greater villus height was observed in the treatment with essential oil. At 42 days, supplementation with *Citrus sinensis* promoted a significant increase in villus height and greater crypt depth in the duodenum and ileum of the chickens, compared to the negative control group. In the jejunum, no statistical differences were observed between treatments. The villus/crypt ratio was higher in the supplemented groups, indicating better absorptive function. The use of *Citrus sinensis* essential oil improved intestinal integrity and may be a viable and sustainable alternative in poultry farming, promoting better nutrient utilization and valorization of agro-industrial waste.

Keywords: alternative; antibiotics; supplementation; valorization; villi.

Resumo: O presente estudo avaliou os efeitos da suplementação com óleo essencial de *Citrus sinensis*, obtido a partir de resíduos da indústria de suco, sobre a morfologia intestinal de frangos de corte. Foram utilizadas 150 aves da linhagem Cobb, divididas em três tratamentos: controle, antibiótico (200 ppm de amoxicilina) e óleo essencial de laranja (200 ppm). Aos 21 e 42 dias, foram realizadas análises histomorfométricas das vilosidades e criptas das porções do intestino delgado (duodeno, jejuno e íleo). Aos 21 dias as vilosidades e criptas foram maiores no tratamento controle negativo em comparação ao tratamento controle positivo (amoxicilina) e ao com óleo essencial de laranja, enquanto no íleo ocorreu maior altura de vilos no tratamento com óleo essencial, aos 42 dias a suplementação com *Citrus sinensis* promoveu aumento significativo da altura das vilosidades e profundidade de cripta do duodeno e íleo dos frangos e maior profundidade das criptas nessas regiões, em comparação ao grupo controle negativo. No jejuno, não foram observadas diferenças estatísticas entre os tratamentos. A relação vilo/cripta foi superior nos grupos suplementados, indicando melhor funcionalidade absortiva. O uso do óleo essencial de *Citrus sinensis* melhorou a integridade intestinal e pode ser uma alternativa viável e sustentável na avicultura, promovendo melhor aproveitamento dos nutrientes e valorização de resíduos agroindustriais.

Palavras-chave: alternativa; antibióticos; suplementação; valorização; vilosidades.

1. Introducion

With the continuous development of the broiler production chain over recent decades, the adoption of sustainable alternatives in production systems has gained increasing attention [1, 2]. This is because antibiotics are still used as growth promoters and for clinical treatment to control bacterial infections [3]. However, the continuous use of antibiotics exerts selective pressure that leads to the emergence of resistant bacteria, which is a major concern for scientists, as it poses a risk to both human and animal health [4, 5].

Among the various alternatives, the use of essential oils (EOs) stands out. These compounds are plant-specific and are responsible for the characteristic flavor and fragrance of plants. They are traditionally extracted by boiling water and steam distillation; however, other methods such as solvent extraction, supercritical CO_2 extraction, and expression are also employed [6–8]. The use of phytogenic additives, such as essential oils, has shown positive effects on the zootechnical performance of broiler chickens [8]. These improvements are associated with modulation of the intestinal microbiota, stimulation of digestive enzyme secretion, and enhancement of the immune system, resulting in improved feed conversion and weight gain [9].

Among the most extensively studied essential oils, orange essential oil (*Citrus sinensis*) has gained prominence and is considered a viable alternative due to its natural origin, efficacy, residue-free nature, and economic accessibility [10,11]. Essential oils derived from citrus fruits have demonstrated positive effects on growth performance, intestinal microbiota, and jejunal morphological characteristics [12–14]. In addition to their benefits to poultry health, essential oils also exert a significant impact on zootechnical performance [11]. According to Ren et al. [15], supplementation with phytogenic feed additives effectively improves meat quality parameters and increases added value by remodeling the intestinal microbiota ecology and modulating postmortem glycolytic metabolism and lipid peroxidation pathways in these animals.

Therefore, the objective of this study was to evaluate the morphological and functional effects of dietary supplementation with *Citrus sinensis* essential oil on the duodenum, jejunum, and ileum of broiler chickens slaughtered at 21 and 42 days of age.

2. Material and methods

2.1 Biological assay

The experiment was conducted at the Poultry Production Sector and the Laboratory of Biochemistry and Animal Metabolism of the Instituto Federal Goiano (IF Goiano), Campus Rio Verde, Goiás, Brazil. All procedures were approved by the Institutional Committee for the Care and Use of Animals (CEUA; protocol no. 5622220718) and were performed in accordance with guidelines for the ethical use of animals in research.

Prior to the arrival of the flock, both the poultry house and the cage batteries underwent cleaning and disinfection of all facilities (screens, curtains, floor, external areas, and equipment) over a seven-day period, consisting of two days for cleaning and five days for sanitary downtime, as the facility does not operate under an intermittent production system. Disinfection was performed by spraying a disinfectant based on quaternary ammonium and glutaraldehyde.

2.2 Broiler chicken rearing

A total of 150 one-day-old male Cobb broiler chicks were used and housed in galvanized wire cages measuring 0.90 m × 0.60 m × 0.45 m. The experimental design was completely randomized, with three treatments and five replicates of ten birds each. The treatments (T) were as follows: T1: basal diet (BD; corn and soybean meal) without additives (negative control, NC); T2: BD supplemented with 200 ppm of amoxicillin (positive control, PC); and T3: BD supplemented with 200 ppm of orange essential oil.

Each cage was equipped with a trough-type feeder and drinker. Feed and water were provided *ad libitum* throughout the experimental period. The drinkers were cleaned twice daily or as needed using a sponge and sodium hypochlorite solution. A 24-hour lighting program was adopted, considering both natural and artificial light. Diets were formulated and prepared at the institution according to the feeding phases (1–7 days, 8–21 days, 22–35 days, and 36–42 days of age), following the nutritional recommendations of Rostagno et al. [16], as shown in Table 1.

The mean maximum and minimum temperatures and relative humidity values recorded throughout the experimental period are presented in Table 2.

Table 1. Ingredient composition and calculated nutritional levels of the control diet supplemented with 200 ppm of antibiotic or 200 ppm of *Citrus sinensis* essential oil

Ingredients (g/kg)	1-7 days	8-21 days	22-35 days	36-42 days
Corn 7.88 %	55.300	56.020	59.100	66.000
Soybean meal 46 %	39.371	37.930	32.000	26.490
Soybean oil	0.800	1.700	3.700	2.600
Limestone	2.200	1.120	0.190	0.020
Dicalcium phosphate	0.065	1.250	2.900	2.780
Vitamin–mineral premix	1.000	1.000	0.800	1.000
DL-methionine	0.265	0.220	0.500	0.200
L-lysine	0.300	0.200	0.300	0.400
L-threonine	0.194	0.070	0.100	0.070
Salt	0.506	0.490	0.480	0.440
Total	100.000	100.000	100.070	100.000
Calculated levels				
Metabolizable energy (Kcal/kg)	3000	3047.119	3155.094	3199.171
Crude protein (%)	25.310	23.422	20.584	19.014
Digestible lysine (%)	1.364	1.262	1.181	1.138
Digestible methionine (%)	0.548	0.541	0.779	0.465
Calcium (%)	1.011	0.882	0.914	0.804
Available phosphorus (%)	0.482	0.351	0.634	0.620
Sodium (%)	0.227	0.219	0.209	0.199

Vitamin–mineral premix (nutritional levels per kilogram of product): methionine (min) 300 g/kg; iron (min) 6,000 mg/kg; copper (min) 1,850 mg/kg; manganese (min) 16.8 g/kg; zinc (min) 14.5 g/kg; iodine (min) 330 mg/kg; selenium (min) 84 mg/kg; vitamin A (min) 1,500,000 IU/kg; vitamin D_3 (min) 500,000 IU/kg; vitamin E (min) 3,600 IU/kg; vitamin K_3 (min) 240 mg/kg; vitamin B_1 (min) 300 mg/kg; vitamin B_2 (min) 1,100 mg/kg; vitamin B_6 (min) 500 mg/kg; vitamin B_{12} (min) 3,600 µg/kg; niacin (min) 7,000 mg/kg; calcium pantothenate (min) 2,000 mg/kg; folic acid (min) 320 mg/kg; biotin (min) 6 mg/kg; choline chloride (min) 65 mg/kg. The experimental treatments were as follows: T1: basal diet (BD) without antibiotic or essential oil; T2: negative control (NC), consisting of BD supplemented with 200 ppm of amoxicillin; and T3: positive control (PC), consisting of BD supplemented with 200 ppm of orange essential oil.

Table 2. Temperature (°C) and relative humidity (%) inside the poultry house during each production phase.

CYCLE	TEMPERATURE °C		RELATIVE HUMIDITY%	
	Maximum	Minimum	Maximum	Minimum
21 days	29.77	18.22	76.00	58.37
42 days	27.83	17.21	67.50	58.87

2.3 Intestinal histomorphometry

At the end of the experimental period, at 21 and 42 days of age, five birds per treatment (one bird per replicate), with body weight within ±5 % of the flock mean, were selected and fasted. After approximately 8 h of fasting, euthanasia was performed by cervical dislocation, following the humane slaughter method in accordance with Ordinance No. 365/2021 of the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA).

Intestinal segments approximately 4.0 cm in length were carefully collected, immediately rinsed with distilled water, identified, and fixed in 10 % formalin until slide preparation. For histological processing, intestinal samples were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Subsequently, serial sections of 5 μm thickness were obtained, mounted on glass slides, stained with hematoxylin and eosin, and covered with glass coverslips fixed with mounting medium.

Duodenal villi and crypts were also evaluated microscopically after slaughter following a methodology adapted from Almeida et al. [3]. Histomorphometric analyses of the duodenal mucosa were performed through images captured at 4× magnification with an optical microscope and analyzed with Image-Pro Plus® software. The evaluated variables included intestinal villus height, crypt depth, and the villus-to-crypt ratio. Villus height was measured from the basal region coinciding with the upper portion of the crypts to the villus apex, whereas crypt depth was measured from the basal region of the villi to the boundary with the muscularis mucosae. Statistical analyses were performed with the R software, and mean comparisons were conducted with the use of Tukey's test.

3. Results and discussion

The histomorphometric data of the small intestine (duodenum, jejunum, and ileum) of 21-day-old broiler chickens are presented in Table 3.

Table 3. Histomorphometry of the small intestine of 21-day-old broiler chickens fed orange essential oil: villus height, crypt depth, and villus-to-crypt ratio in the duodenum, jejunum, and ileum.

Trat	Duodenum			Jejunum			Ileum		
	VI (μm)	Cr (μm)	VI/Cr (μm)	VI (μm)	Cr (μm)	VI/Cr (μm)	VI (μm)	Cr (μm)	VI/Cr (μm)
NC	629.40^{a}	68.60^{b}	9.20^{a}	417.80	63.00^{b}	6.66^{a}	253.20	49.80^{b}	5.09^{a}
PC	541.00^{b}	84.20^{a}	6.48^{b}	386.80	80.80^{a}	4.79^{b}	247.20	62.20^{a}	3.99^{b}
EOs		67.40^{b}	9.30^{a}	398.80	68.20^{b}	5.85^{a}	267.80	61.00^{a}	4.42^{ab}
VC	8.810	9.870	9.820	8.800	7.960	9.340	9.100	8.280	10.000
P %	0.050	0.005	0.001	0.403	0.001	0.001	0.385	0.003	0.008
SEM	8.608	2.361	0.192	9.101	2.357	0.236	6.029	1.879	0.162

Treatment: treatment; NC: negative control; PC: positive control (amoxicillin); EOs: essential oil; CV: coefficient of variation; P: p-value; SEM: standard error of the mean; VI: villus height; Cr: crypt depth; VI/Cr: villus-to-crypt ratio. Means followed by different lowercase letters within the same row differ significantly at the 5 % significance level (P < 0.05). Means followed by the same or shared letters (e.g., ab) do not differ significantly from each other at the 5 % significance level.

According to the results presented in Table 3, significant differences were observed among treatments for the duodenum. Regarding villus height, the highest values were observed in the negative control and in the treatment supplemented with orange essential oil, with no significant difference between them. In contrast, the positive control showed lower villus height compared with the other treatments. For crypt depth, the positive control exhibited the highest values, whereas the essential oil treatment showed villus height, crypt depth, and villus-to-crypt ratio similar to those of the negative control and significantly different from the positive control (amoxicillin). The essential oil and negative control treatments were characterized by greater villus height and lower crypt depth. Concerning the villus-to-crypt ratio, the negative control and the orange essential oil treatments showed superior results, while the positive control presented lower values compared with the other treatments.

Intestinal crypt depth is a parameter widely used to evaluate gut health, as these structures are responsible for the renewal of enterocytes, which are essential cells for nutrient absorption located in the villi. Deeper crypts indicate greater proliferative activity and, consequently, a higher rate of cell renewal. In contrast, shallower crypts may suggest lower efficiency of this process. However, crypt size should be interpreted with caution, as it is also associated with the functional capacity of the intestinal tissue [(23)].

Crypt depth reflects the rate of epithelial renewal, with deeper crypts suggesting greater cellular turnover, whereas shallower crypts indicate lower proliferative activity or less efficient renewal. The balance between these processes defines intestinal turnover, which ensures the maintenance of villus size and, consequently, the functional integrity of the mucosa [(17)]. Gilani et al. [(18)] ,observed that deeper crypts were associated with higher epithelial renewal rates, resulting in improved dietary digestibility in broiler chickens. In contrast, Nguyen et al. [(19)] reported positive correlations between the villus-to-crypt ratio (V:C) and populations of beneficial bacteria, such as Lactobacillus spp., as well as improved productive performance indicators in modern poultry production.

In the present study, the negative control and the group treated with orange essential oil exhibited superior histomorphometric parameters, characterized by a higher V:C ratio and preserved mucosal integrity, suggesting greater absorptive efficiency and robust intestinal health, in agreement with recent evidence. According to Wang et al. [(20)], the size and number of intestinal villi are directly related to the number of constituent cells. Thus, greater cellular density tends to result in taller and more numerous villi, thereby enhancing nutrient absorption efficiency.

A meta-analysis conducted by Rocha et al. [(21)], who evaluated evaluating the supplementation of broiler chickens with various essential oils, found no significant differences in villus height ($p = 0.335$) or crypt depth ($p = 0.451$) in the duodenum, nor were changes observed in the villus-to-crypt ratio ($p = 0.094$), indicating that this intervention did not alter intestinal architecture

Figure 1 shows the histomorphometry of the small intestine (duodenum) of 21-day-old broiler chickens fed orange essential oil.

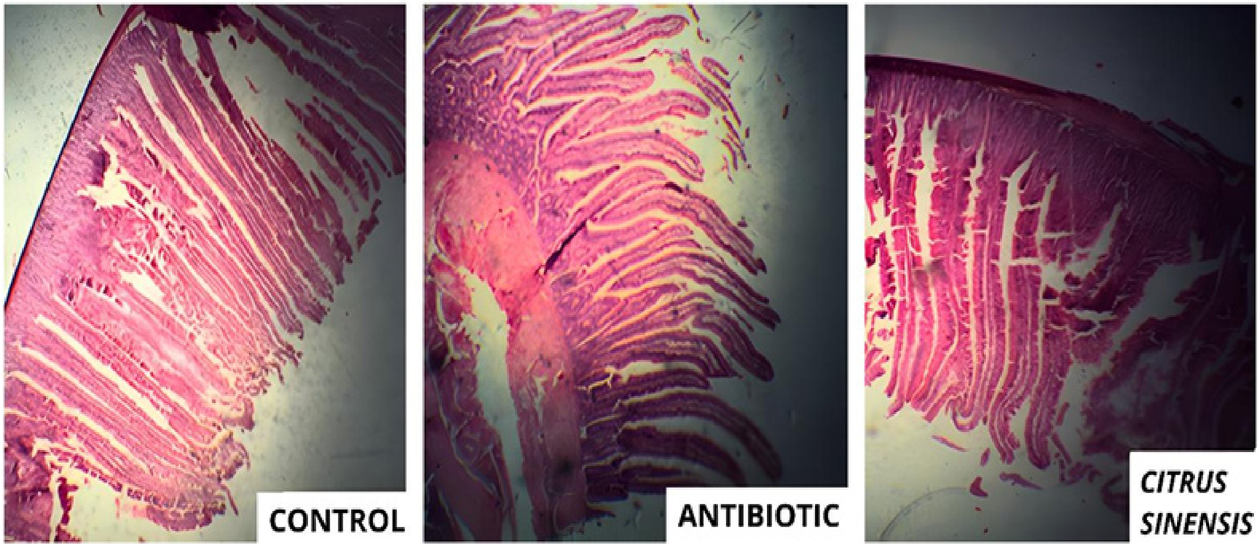

Figure 1. Histological micrographs of the duodenum of 21-day-old broiler chickens from the control group, 200 ppm antibiotic group, and 200 ppm orange essential oil group.

In the jejunum, no significant differences were observed in villus height among treatments, with the negative control showing a villus height of 417.80 µm, the positive control 386.80 µm, and the essential oil treatment 398.80 µm. Regarding crypt depth, the antibiotic-treated group presented the highest numerical value, whereas no significant differences were observed among the other treatments. The villus-to-crypt ratio did not differ between the negative control and the orange essential oil treatments, which exhibited superior results compared with the positive control.

In the present study, no significant differences were observed among treatments in the jejunum. However, Basir and Toghyani [22] , reported a significant increase in villus height and crypt depth in the jejunum, which may be attributed to the presence of tannins in dried lemon pulp. The mean villus length and mean crypt depth were greater and indicated improved jejunal morphology in the group supplemented with 5 % citrus by-products. Supporting these findings, Xu et al. [23] , observed that supplementation with tannic acid may protect the mucosal layer of the proventriculus, reduce villus atrophy, and enhance growth performance by positively influencing intestinal microbiota, villus morphology, and intestinal barrier function.

Hong et al. [24], while evaluating diets supplemented with a mixture of essential oils (125 ppm, including oregano essential oil, anise, and citrus peel), reported increased villus height in the duodenum, whereas villus height and crypt depth in the jejunum and ileum were not affected. Based on the available literature, there are considerable controversies regarding the benefits of essential oils.

According to Zeng et al. [25], essential oils are complex mixtures, and factors such as botanical species, ecological conditions, plant part used, harvest time, and extraction or storage methods markedly affect their composition and, consequently, their effects on animals, resulting in highly inconsistent outcomes on performance and intestinal health.

Figure 2 shows the histomorphometry of the small intestine (jejunum) of 21-day-old broiler chickens fed orange essential oil.

Figure 2. Histological micrographs of the jejunum of 21-day-old broiler chickens from the control group, 200 ppm antibiotic group, and 200 ppm orange essential oil group.

In the ileum, no significant differences were observed in villus height among treatments. However, crypt depth was greater in the orange essential oil and amoxicillin treatments compared with the negative control. This increase also affected the villus-to-crypt ratio, in which the negative control showed higher values compared with the positive control, while the orange essential oil treatment exhibited intermediate values relative to both control treatments (Table 3).

Fascina et al. [26], evaluated the use of phytogenic additives, organic acids, and antibiotics in broiler diets and reported no differences in villus development across the different intestinal segments, except for ileal villus height. In contrast, Zeechan et al. [27] found that supplementation of organic acids (citric acid and malic acid) combined with phytogenic compounds (*Origanum vulgare and Cinnamomum verum*) in drinking water improved growth performance by enhancing intestinal histomorphometry.

Oyeagu et al. [28], observed that broiler chickens supplemented with a *Bacillus*-derived protease showed significant increases in villus height and reductions in crypt depth, particularly in the ileum. These effects were attributed to an increase in enterocyte numbers, implying a greater intestinal absorptive surface and improved nutrient absorption, supporting the concept that a higher number of epithelial cells enhances the functional potential of the intestinal mucosa.

Figure 3 shows the histomorphometry of the small intestine (ileum) of 21-day-old broiler chickens fed orange essential oil. Histomorphometric data of the small intestine (duodenum, jejunum, and ileum) of 42-day-old broiler chickens are presented in Table 4.

Figure 3. Histological micrographs of the ileum of 21-day-old broiler chickens from the control group, 200 ppm antibiotic group, and 200 ppm orange essential oil group.

Table 4. Histomorphometry of the small intestine segments (duodenum, jejunum, and ileum) of 42-day-old broiler chickens fed *Citrus sinensis*.

Trat	Duodenum			Jejunum			Ileum		
	Vl (µm)	Cr(µm)	Vl/	Vl (µm)	Cr (µm)	Vl/Cr (µm)	Vl (µm)	Cr (µm)	Vl/Cr (µm)
NC	509.00^{b}	92.00^{b}	5.55	493.00	85.40	5.78	318.2^{b}	68.2^{b}	4.68^{a}
PC	552.40^{ab}	114.40^{a}	4.92	491.80	78.60	6.34	271.8^{b}	77.6^{ab}	3.52^{b}
EOs	595.00^{a}	112.40^{a}	5.29	479.20	85.80	5.66	377.2^{a}	85.2^{a}	4.44^{a}
VC	6.960	5.950	6.910	7.240	11.000	12.360	9.30	9.400	10.730
P	0.014	0.001	0.052	0.795	0.405	0.329	0.001	0.008	0.004
SEM	13.134	2.977	0.111	8.608	2.361	0.192	13.572	2.494	0.172

Treatment: treatment; NC: negative control; PC: positive control (amoxicillin); EOs: essential oil; CV: coefficient of variation; P: p-value; SEM: standard error of the mean; Vl: villi; Cr: crypt; Vl/Cr: villus-to-crypt ratio. Means followed by different lowercase letters within the same row differ significantly at the 5 % significance level ($P < 0.05$). Means followed by the same or shared letters (e.g., ab) do not differ significantly from each other at the 5 % significance level.

In this segment of the small intestine, significant differences in villus height were observed in the duodenum and ileum. The greatest villus height was observed in the treatment supplemented with essential oil, followed by the amoxicillin treatment, whereas the negative control exhibited the lowest villus height values. Hosseinzadeh et al. [29] evaluated the effects of *Plectranthus amboinicus* essential oil (PAEO) and rosemary (*Rosmarinus officinalis* L.) essential oil (ROEO) in broiler chickens from 1 to 42 days of age and reported increased duodenal histomorphology at slaughter.

In the evaluation of duodenal crypts, the negative control group differed from the other treatments, presenting significantly lower values. In the ileum, crypt depth was greatest in the essential oil treatment, whereas the amoxicillin treatment showed higher values than the negative control but lower than the essential oil treatment. Thus, the lowest crypt depth was observed in the negative control group. However, deeper crypts indicate faster tissue turnover to ensure villus renewal, as required in response to natural epithelial desquamation or inflammation caused by pathogenic agents and their toxins [30].

Bondar et al. [31] evaluated dietary supplementation with *Curcuma longa* powder (0.5 % and 1 %) in 42-day-old broiler chickens. The authors reported increased duodenal villus height and reduced crypt depth in the duodenum, resulting in a more favorable villus height–to–crypt depth (VH:CD) ratio compared with the control group.

Intestinal morphology is closely associated with digestive capacity and gut health, particularly in response to supplementation with essential oils. Su et al. [32] observed that supplementation with a blend of essential oils (3.05 % thymol, 2.3 % carvacrol, and 0.26 % cinnamaldehyde) improved broiler performance by increasing nutrient digestibility, enhancing transporter protein expression, modulating intestinal morphology, and improving immune and antioxidant capacity. Carvacrol- and thymol-based essential oils act on the bacterial cell membrane, impairing mitotic division, causing cellular dehydration, and inhibiting the survival of pathogenic bacteria [33].

Figure 4 shows the histomorphometry of the small intestine (duodenum) of 42-day-old broiler chickens fed orange essential oil.

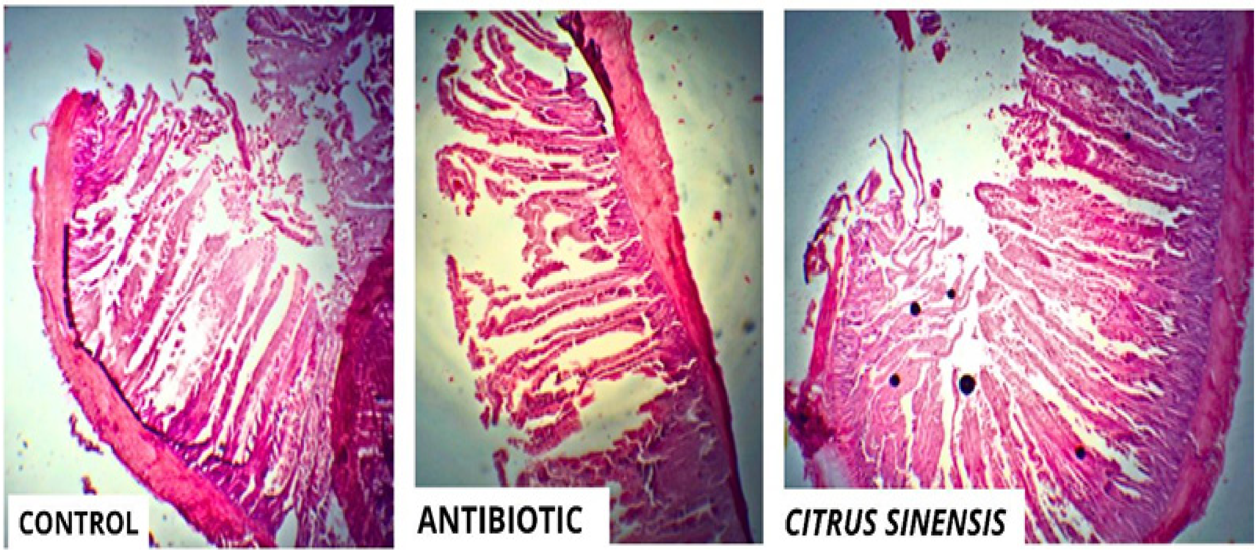

Figure 4. Duodenal microvilli of 42-day-old broiler chickens from the control group, 200 ppm antibiotic group, and 200 ppm orange essential oil group.

Figure 5. Histomorphometry of duodenal crypts of 42-day-old broiler chickens from the control group, 200 ppm antibiotic group, and 200 ppm *Citrus sinensis* group.

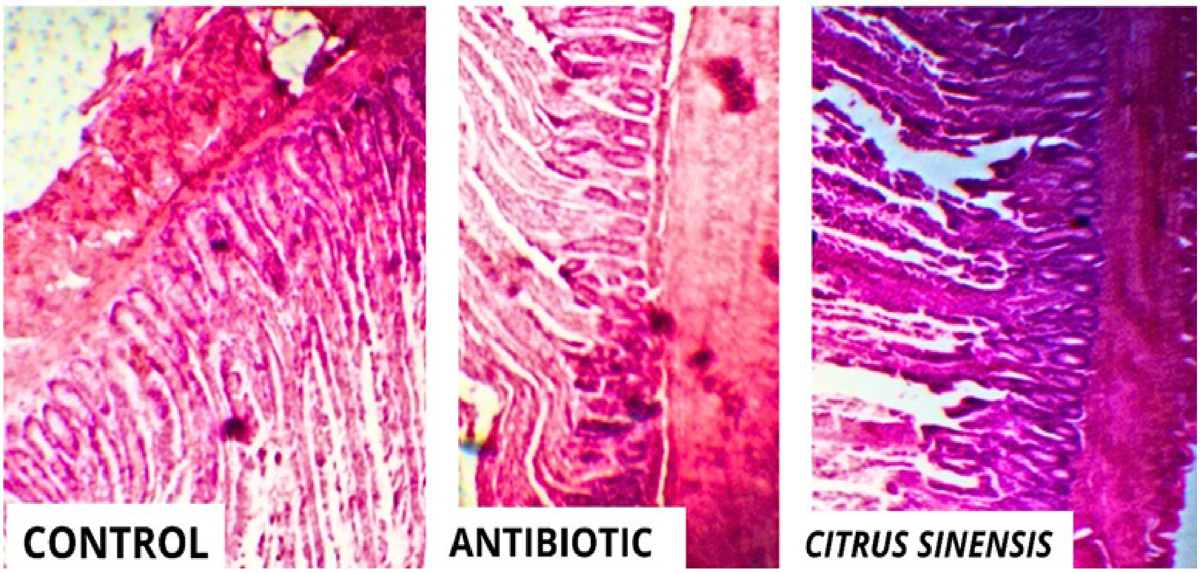

Figure 5. Histological section of the duodenal crypts at 42 days from the control group, 200 ppm antibiotic group, and 200 ppm orange essential oil group.

The jejunum is the site of maximum intestinal absorption and nutrient digestion, playing a key role in promoting growth performance in poultry (34). In the jejunal portion, no differences were observed in any of the traits evaluated in this study, similarly to Vukić-Vranješ et al. (35), who also did not observe changes in villus height or crypt depth in the jejunum of 42-day-old broilers fed a mixture of oregano, anise, and Citrus essential oils at a higher concentration (1000 g/t) than that used in the present study (200 ppm) compared to the Citrus treatment. When using a mixture of plant extracts, Attia et al. (36), did not achieve improvements in gastrointestinal morphology in 42-day-old broilers.

In line with those findings, Paschoal et al. (37), when investigating the effect of a mixture of essential oils from oregano, garlic, lemon, rosemary, thyme, eucalyptus, and sweet orange at concentrations of 200 or 300 ppm, observed no differences in villus height or crypt depth in the jejunum of broilers, similar to the results of the present study, where the addition of essential oils did not affect the villi and crypts in the jejunal portion.

Changes in jejunal mucosal morphology can impact broiler productivity, as the jejunum is responsible for the majority of intestinal nutrient absorption. The use of encapsulated essential oil blends is therefore of interest to enhance the efficiency of digestive enzymes (38).

Ding et al. (39), observed improvements in intestinal health of broilers fed lemongrass and mountain tea essential oils at doses of 400 to 600 mg/kg, where intestinal morphology exhibited taller villi and shallower crypts. According to the authors, this contributes to maintaining intestinal barrier integrity and reducing inflammation.

Figure 6 highlights the histomorphometry of the jejunum in the control group, the 200 ppm antibiotic group, and the 200 ppm *Citrus sinensis* group at 42 days. Figure 7 shows the histomorphometry of the jejunal crypts of 42-day-old broilers fed orange essential oil.

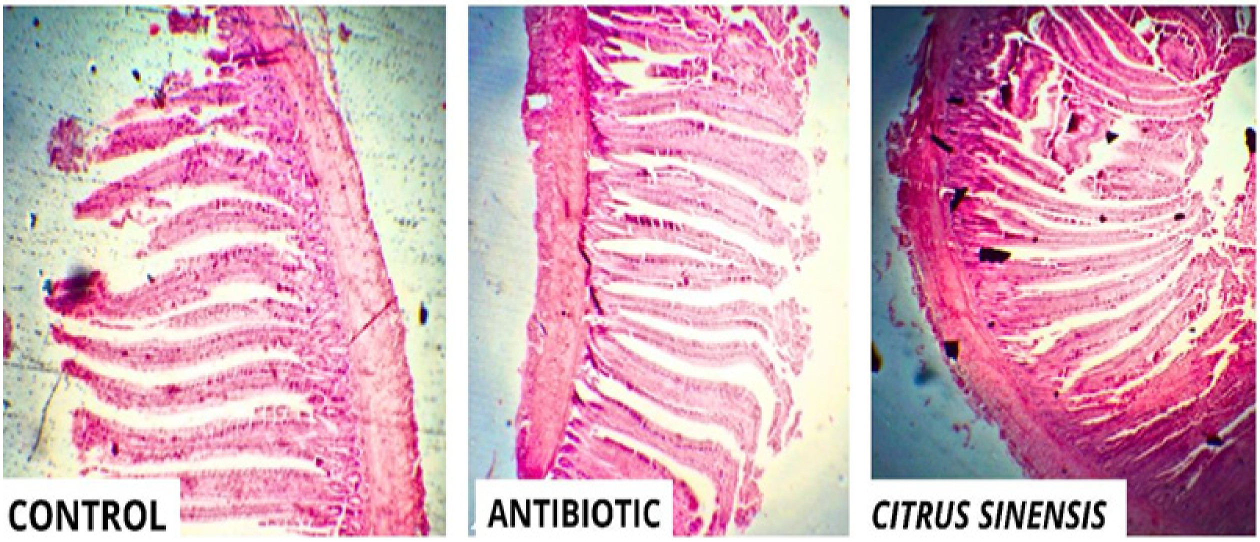

Figure 6. Jejunal microvilli at 42 days in the control group, 200 ppm antibiotic group, and 200 ppm orange essential oil group.

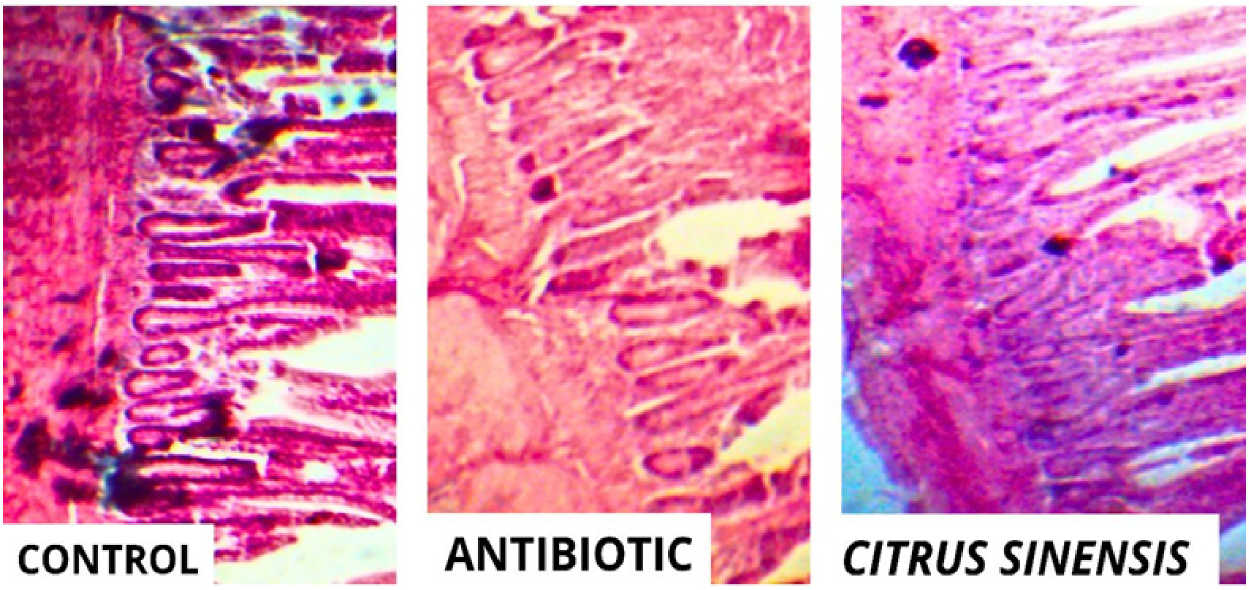

Figure 7. Histological section showing the crypt depth of the jejunum at 42 days in the control group, 200 ppm antibiotic group, and 200 ppm orange essential oil group.

The ileum is the final portion of the small intestine, responsible for nutrient absorption and bile salt reabsorption. The pH conditions are more neutral (6.3–7.2), and there is increased mucus production due to a higher number of goblet cells in this region compared to the duodenum and jejunum [40]. In the evaluation of ileal villi, the group supplemented with *Citrus sinensis* showed statistically better results than the negative control and the amoxicillin groups. Regarding crypt depth, the treatments with orange essential oil and amoxicillin exhibited better results compared to the negative control group. In the villus-to-crypt ratio assessment, the negative control and Citrus essential oil groups did not differ from each other but performed better than the amoxicillin group.

In a similar study, Bona et al. [41], reported results comparable to villus height in the ileum: 335.04 μm for a plant-based compound containing oregano, rosemary, cinnamon essential oils, and red pepper extract; 249.10 μm for the antibiotic avilamycin; and 278.19 μm for the control group. These results were lower than the *Citrus sinensis* group in the present study, which measured 377.20 μm. Broilers treated with phytogenic additives also exhibited an increased number of goblet cells in the duodenum.

Hong et al. [24], when supplementing the diet with a combination of essential oils (125 ppm containing oregano, anise, and citrus peel), observed an increase in villus height in the duodenum, while in the jejunum and ileum segments, both villus height and crypt depth remained unchanged.

Toniazzo et al. [42], used essential oils and bioactive compounds in broiler diets, including cinnamaldehyde (cinnamon), carvacrol (oregano), thymol (thyme), eucalyptus extract, paprika oleoresin, cashew and castor oils, as well as additives such as organic acids, turmeric, tannins, vitamin E, and zinc. No effect (p>0.05) was observed on intestinal morphological parameters (villus height, crypt depth, villus:crypt ratio, absorptive area) at 14 and 28 days of age; however, significant improvements in intestinal health (lower lesion scores) were observed with the use of additives such as organic acids, turmeric, tannins, vitamin E, and zinc. This suggests that the benefits may be more related to mucosal function and integrity than to measurable histological structure.

Figure 8 shows the histomorphometry of the ileum in 42-day-old broilers fed orange essential oil. Figure 9 highlights the histomorphometry of the ileal crypts in the control group, 200 ppm antibiotic group, and 200 ppm *Citrus sinensis* group.

Figure 8. Ileal microvilli in the control group, 200 ppm antibiotic group, and 200 ppm orange essential oil group.

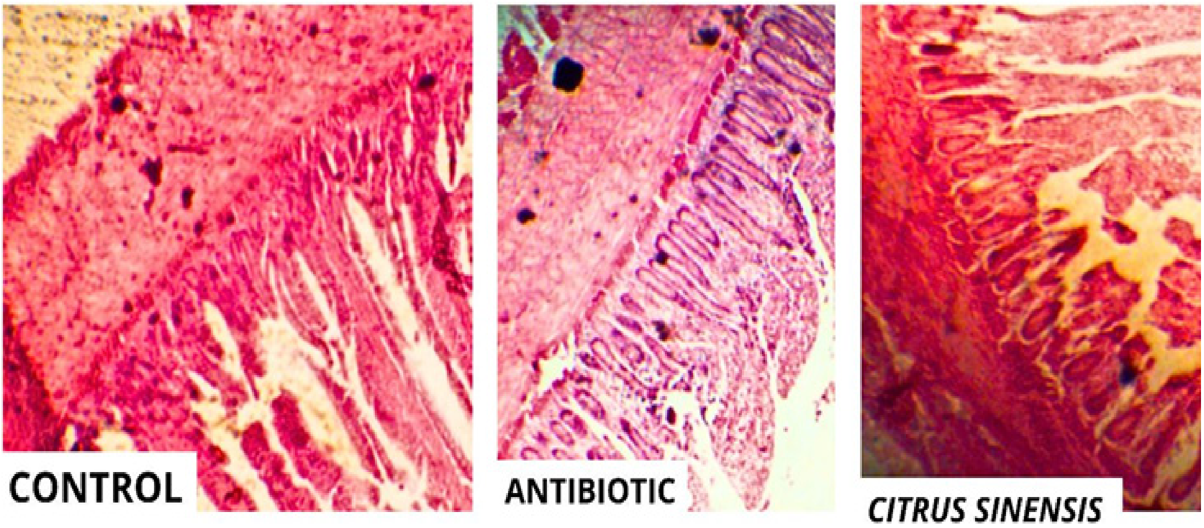

Figure 9. Histological micrographs of ileal crypts in the control group, 200 ppm antibiotic group, and 200 ppm orange essential oil group.

4. Conclusion

The inclusion of *Citrus sinensis* essential oil in broiler diets promoted significant improvements in small intestinal morphology, with increased villus height and crypt depth in the duodenum and ileum, reflecting greater absorptive capacity. The villus-to-crypt ratio was higher in the treatments with orange essential oil and in the negative control group. These results indicate the potential of orange essential oil as a promising natural alternative in broiler production, although further studies are needed to deepen the understanding of its properties and mechanisms of action in poultry.

Conflict of interest statement

The authors declare no conflict of interest.

Data availability statement

The complete dataset supporting the results of this study is published within the article.

Author contributions

Conceptualization: Junior, E. M. and Costa, K. O. Data curation: Alvarenga, J. C. Formal analysis: Sampaio, S. A. Methodology: Minafra, C. S. Supervision: Santos, F. R. and Minafra, C. S. Visualization: Silva, W. J. and Sampaio, S. A. Investigation: Morais, A. M. V. B. and Santos, F. R. Writing – original draft: Junior, E. M. and Costa, K. O. Writing – review & editing: Alvarenga, J. C. and Silva, W. J.

Generative AI use statement

The authors did not use any generative artificial intelligence tools or technologies in the creation or editing of any part of this manuscript.

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