



# Performance, nutrient digestibility, and intestinal histomorphometry of broilers fed diet supplemented with guava extract standardized in phenolic compounds

Raiana Almeida Noleto-Mendonça<sup>1</sup> , Julyana Machado da Silva Martins<sup>2\*</sup> , Deborah Pereira Carvalho<sup>1</sup> , Itallo Conrado Sousa de Araújo<sup>3</sup> , José Henrique Stringhini<sup>1</sup> , Edemilson Cardoso da Conceição<sup>4</sup> , Marcos Barcellos Café<sup>1</sup> , Nadja Susana Mogyca Leandro<sup>1</sup> 

<sup>1</sup> Universidade Federal de Goiás, Escola de Veterinária e Zootecnia, Departamento de Zootecnia, Goiânia, GO, Brasil.

<sup>2</sup> Universidade do Estado de Minas Gerais, Unidade Ituiutaba, Ituiutaba, MG, Brasil.

<sup>3</sup> Universidade Federal de Minas Gerais, Escola de Veterinária, Departamento de Zootecnia, Belo Horizonte, MG, Brasil.

<sup>4</sup> Universidade Federal de Goiás, Faculdade de Farmácia, Goiânia, GO, Brasil.

**ABSTRACT** - The objective of this study was to evaluate the effects of guava extract standardized in phenolic compounds (SGE) on broiler performance, nutrient digestibility, and intestinal histomorphometry. A total of 300 one-day-old male Cobb-500<sup>®</sup> broiler chicks were distributed in a completely randomized design with five treatments (basal diet supplemented with 120 mg vitamin E/kg and basal diet supplemented with 0, 600, 800, or 1,000 mg SGE/kg) and six replicates of ten birds each. Performance was evaluated at seven and 21 days of age; digestibility of nutrients was determined by total excreta collection from 18 to 21 days of age; and histomorphometry of the small intestine was assessed at 21 days of age. Broilers fed diets supplemented with SGE or vitamin E had higher body weight and weight gain and better feed conversion than those fed unsupplemented diet at seven days of age. At the same age, there was a quadratic effect of SGE levels on body weight and weight gain, with better weights for 715 and 716 mg SGE/kg, respectively; and a decreasing linear effect for feed conversion. At 21 days of age, body weight and weight gain increased linearly with the inclusion of SGE in diet. Digestibility of feed nutrients was not influenced by treatments. Broilers fed diet supplemented with 800 or 1,000 mg SGE/kg had greater villus height and villus: crypt ratio of the duodenum than those fed unsupplemented diet. Villus height in the jejunum of broilers fed diet supplemented with 600 mg SGE/kg was lower than that of broilers that received vitamin E. Guava extract standardized in phenolic compounds can be used in diets for broilers in the starter phase, considering that the extract increases weight gain, reduces feed conversion, and helps in the development of the intestinal mucosa.

**Keywords:** animal nutrition, functional additive, *Gallus gallus domesticus*, intestinal health, *Psidium guajava* L.

\*Corresponding author:  
julyanamachado\_zoo@hotmail.com

Received: February 5, 2021

Accepted: June 24, 2021

**How to cite:** Noleto-Mendonça, R. A.; Martins, J. M. S.; Carvalho, D. P.; Araújo, I. C. S.; Stringhini, J. H.; Conceição, E. C.; Café, M. B. and Leandro, N. S. M. 2021. Performance, nutrient digestibility, and intestinal histomorphometry of broilers fed diet supplemented with guava extract standardized in phenolic compounds. Revista Brasileira de Zootecnia 50:e20210026. <https://doi.org/10.37496/rbz5020210026>

**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.



## 1. Introduction

The guava industrialization process generates waste comprising mainly seeds and residual pulp, which corresponds to 10 to 15% of the total mass of the fruit (Packer et al., 2015). Because there is no suitable destination, such waste is often discarded in landfills, where it accumulates and contributes to environmental pollution.

Agroindustrial guava waste is rich in phenolic compounds, which are substances with antimicrobial and antioxidant properties (Melo et al., 2011). Thus, such waste has the potential for use in animal nutrition as a functional additive, which would add value to this byproduct and minimize negative economic and environmental impacts. The liquid extract obtained from guava agroindustrial waste shows high antioxidant activity *in vitro* (Sousa et al., 2011; Araújo et al., 2014). However, little is known about the effects that phenolic compounds from guava waste have on the animal organism.

Phenolic compounds can improve the gut morphology of broilers, with positive effects on villus height and crypt depth (Kamboh and Zhu, 2014; Oliveira et al., 2018). Increased villus height is associated with improved digestive and absorptive functions in the intestine, which consequently can improve performance (Viveros et al., 2011). Phenolic compounds have been reported to inhibit inflammation in the intestine, reflecting positively on its barrier function, thereby assisting with intestinal health (Noda et al., 2012).

Vitamin E is an excellent antioxidant. When used as a supplement in poultry feed, it improves the immune response (Bhatti et al., 2016) and protects tissues and cells from oxidative damage (Khan et al., 2011; Voljč et al., 2011). It also prevents inflammation in the intestine, with improvement in its barrier function (Wang et al., 2021), thus contributing to improved performance.

Considering that, when added to broiler feed, phenolic compounds can contribute to improving performance (Starčević et al., 2015), nutrient digestibility (Chamorro et al., 2013), and gut morphology (Viveros et al., 2011) and have similar beneficial effects to those of vitamin E, which is a substance with well-documented effects in the literature (Yoo et al., 2016; Selvam et al., 2017), the present study aimed to evaluate performance, feed nutrient digestibility, and small intestine histomorphometry of broilers fed diets supplemented with guava extract standardized in phenolic compounds (SGE).

## 2. Material and Methods

The research was conducted in accordance with the institutional committee on the use of animals (030/2015). The experiment was carried out in Goiânia, state of Goiás, Brazil (16°35'33" S, 49°16'51" W; 710 m altitude).

A total of 300 one-day-old male broiler chicks of the Cobb-500® commercial lineage, with initial weight of 45.2±0.2 g, were acquired from a commercial hatchery. The birds were housed in batteries composed of galvanized wire cages (0.50 × 0.40 × 0.40 m), each one equipped with a drinking fountain, a trough-type feeder, and an excreta collection tray. The batteries were located in a masonry building with a clay tile roof, a concrete floor, and sides with short walls, screen, and curtains.

The experimental design was completely randomized, consisting of five treatments with six replicates of 10 birds each. The birds were weighed, distributed in plots with uniform weight, and reared from one to 21 days of age. The treatments consisted of basal diet (negative control), basal diet supplemented with 120 mg vitamin E/kg (positive control), and basal diet supplemented with 600, 800, or 1,000 mg SGE/kg.

The SGE was obtained from agroindustrial guava waste composed mainly of seeds and pulp remains provided by a commercial company. To obtain the extract, the waste was dried in an oven at 40 °C (Model MA 035/5, Marconi Equipamentos for Laboratório Ltda., Piracicaba, São Paulo, Brazil) for a period of 72 h and ground in a knife mill (Model TE-650, Moinho Tipo Willye, Tecnal®, Piracicaba, São Paulo, Brazil), resulting in powdered plant material. The powder was then subjected to percolation using 50% alcohol (vol vol<sup>-1</sup>) resulting in the liquid extract. Percolation was carried out according to the method described in Brazilian Pharmacopoeia Phytotherapeutic Form (ANVISA, 2011) with the following adaptations. A 2-kg aliquot of powdered plant material and 10 L of solvent were added to a percolator and subjected to 24 h of maceration; intense dripping then started and continued until the solvent was exhausted; so as not to leave the powder without solvent and dry out, the liquid collected from the plant material that was percolated was again added to the percolator, with this process being repeated ten times. The extract was subsequently concentrated by means of air flow at room temperature until

complete evaporation of the solvent. The liquid guava extract was standardized as to its content in phenolic compounds dosed by spectrophotometry, presenting 0.45% total phenols, 0.85% total tannins, and 5.97% flavonoids.

The experimental diets were isonutritive and formulated based on corn and soybean meal to meet the nutritional requirements of chickens during each rearing phase, according to the recommendations of Rostagno et al. (2011) (Table 1). The feeding program comprised two phases: pre-starter ration (1-7 days old) and starter ration (8-21 days old). Vitamin E and SGE were added to the feed in substitution of inert material (starch). To incorporate the extract into the feed, it was first added to the corn, which was, after homogenizing, added to the other ingredients in the mixer.

Performance variables (feed intake, body weight, weight gain, and feed conversion) were evaluated at seven and 21 days of age. To this end, the birds and the supply and surplus of rations were weighed weekly and the number and weight of birds killed daily was recorded.

Determination of digestibility coefficients of nutrients and energy in the ration was carried out using the total excreta collection method, as described by Sibbald and Slinger (1963) and adapted by Sakomura and Rostagno (2016), performed in the period of 18 to 21 days of age of the birds, corresponding to four days of collection. During the experimental period, feed intake, total excreta produced, and weight gain of birds were measured. Excreta was collected twice a day (morning and afternoon) and stored in identified plastic bags, weighed, and frozen for further analysis.

At the end of the collection period, samples were thawed and homogenized, and aliquots were removed and weighed. Aliquots were then pre-dried in a ventilated oven (Modelo MA 035/5, Marconi Equipamentos for Laboratório Ltda., Piracicaba, São Paulo, Brazil) at 55 °C for 72 h. Dry matter content analyses were then performed in a rectilinear oven (Modelo 315/3, Fanen®, Guarulhos, São Paulo, Brazil)

**Table 1 - Ingredients and calculated nutritional composition of pre-starter (1-7 days of age) and starter (8-21 days of age) diets**

Item	Pre-starter	Starter
Ingredient (%)		
Corn	54.73	58.89
Soybean meal (45%)	38.34	34.84
Soy oil	2.33	2.28
Bicalcium phosphate	1.81	1.43
Limestone	0.98	0.97
Salt	0.51	0.48
DL-Methionine (99%)	0.33	0.26
L-Lysine HCL (78%)	0.28	0.21
L-Threonine (98.5%)	0.11	0.06
Vitamin and mineral supplement <sup>1</sup>	0.40	0.40
Starch	0.18	0.18
Total	100.00	100.00
Calculated nutritional composition (%)		
Metabolizable energy (kcal/kg)	2,950	3,000
Crude protein	22.20	20.80
Calcium	0.92	0.81
Available phosphorus	0.47	0.39
Sodium	0.22	0.21
Digestible methionine + cystine	0.94	0.84
Digestible methionine	0.64	0.55
Digestible lysine	1.31	1.17
Digestible threonine	0.85	0.76

<sup>1</sup>Vitamin and mineral supplement (composition per kg product): vitamin A, 5,500,000 IU; vitamin D3, 1,000,000 IU; vitamin E, 3,750 mg; vitamin K3, 1,250 mg; vitamin B1, 500 mg; vitamin B2, 2,500 mg; vitamin B6, 750 mg; vitamin B12, 7,500 mg; niacin, 17,500 mg; calcium pantothenate, 6,500 mg; folic acid, 250 mg; choline chloride 50%, 150,000 mg; selenium, 100 mg; antioxidante, 2,000 mg; vehicle (sufficient quantity to), 1,000 g; manganese, 32,500 mg; zinc, 22,500 mg; iron, 25,000 mg; copper, 3,000 mg; iodine, 500 mg.

at 105 °C; nitrogen content was analyzed in a nitrogen distiller (Modelo TE-036/1, Tecnal®, Piracicaba, São Paulo, Brazil), using the Kjeldahl method (INCT-CA N-001/1) according to Detmann et al. (2012); ether extract was determined using a fat extractor (Model TE 044 Eight Proof, Tecnal®, Piracicaba, São Paulo, Brazil) according to the methodology described by Silva and Queiroz (2006); and gross energy was determined using a calorimetric pump (Model C-200, Ika, Wilmington, North Carolina, USA).

The digestibility coefficient of nutrients was calculated using the equations proposed by Sakomura and Rostagno (2016), while that of energy, according to Matterson et al. (1965).

At 21 days of age, one bird from each repetition, totaling six birds per treatment, representing the average weight of the plot ( $\pm 5\%$ ), were subjected to 6 h of fasting and then euthanasia by cervical dislocation for histological evaluation of the small intestine.

To make histological slides, 2.0-cm segments of the duodenum (in the distal portion of the duodenal loop), jejunum (2.0 cm before the ileal diverticulum), and ileum (2.0 cm after the ileal diverticulum) were collected and fixed in 10% buffered formaldehyde solution for 24 h. After fixation, the segments were stored in 70% alcohol, processed according to the methodology of Luna (1968), and stained using Hematoxylin-Eosin (H&E). Semi-serial sections of 5  $\mu$  in thickness were made using an electronic rotary microtome (Model RM2255, Leica Biosystems, Buffalo Grove, Illinois, USA).

Images were obtained under 5X magnification with the aid of an optical microscope (DM4000B, Leica Microsystems, Wetzlar, Hessen, Germany) coupled to a microcomputer. The images were analyzed using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA; freeware, <https://imagej.net/Welcome>), in which the height of ten villi and the depth of ten crypts were measured per repetition for a total of 180 measurements per treatment. Villus height was measured from the basal region of the villus to its apex, while crypts were measured from their base to the villus/crypt transition region (Fukayama et al., 2005). The villus:crypt ratio was calculated by dividing villus height by crypt depth.

Data were subjected to analysis of variance and comparison of means by Tukey test (5%). Homogeneity of variances (Barlett test) and normality of the residuals (Shapiro-Wilk test) were considered. Polynomial regression analysis was performed among levels of inclusion of SGE. Statistical analyses were performed using R software, version 3.2.3 (R Core Team, 2015).

The following statistical model was used:

$$Y_{ij} = \mu + T_i + e_{ij}$$

in which  $Y_{ij}$  = value observed for the response variable  $Y$  in treatment  $i$  and its repetition  $j$ ,  $\mu$  = general average of all observations,  $T_i$  = treatment effect (0, 600, 800, and 1,000 mg SGE/kg or 120 mg vitamin E/kg), and  $e_{ij}$  = experimental error associated with the observed value  $Y_{ij}$ .

### 3. Results

Body weight, weight gain, and feed conversion of broilers in the period from one to seven days of age were influenced by treatments ( $P < 0.001$ ) (Table 2). Broilers fed diet supplemented with any level of SGE or vitamin E showed greater weight gain and body weight and better feed conversion compared with those fed unsupplemented diet. The regression analysis revealed a quadratic effect for weight gain and body weight, with better weights with 715 and 716 mg SGE/kg, respectively, and a decreasing linear effect for feed conversion ( $P < 0.05$ ).

In the period from one to 21 days of age, there were no differences among treatments for feed intake, weight gain, body weight, and feed conversion ( $P > 0.05$ ) (Table 3). However, according to the equations [weight gain at 21 days =  $840.8703 + 0.0562EGP$  ( $R^2 = 0.86$ ); body weight at 21 days =  $885.9459 + 0.0563EGP$  ( $R^2 = 0.85$ )], as extract levels were increased, weight gain and body weight increased.

The digestibility coefficients of feed nutrients and metabolizable energy were not influenced by SGE or vitamin E levels ( $P > 0.05$ ) (Table 4).

Villus height, crypt depth, and villus:crypt ratio for the duodenum and villus height and villus:crypt ratio for the jejunum were influenced by levels of SGE and by vitamin E ( $P < 0.05$ ); however, the regression analysis revealed no effect of SGE levels ( $P > 0.05$ ) (Table 5).

For the duodenum, broilers that received diets containing 800 or 1,000 mg SGE/kg had greater villus height compared with those that received 0 or 600 mg SGE/kg ( $P < 0.001$ ). Crypt depth was shallower in broilers fed diet supplemented with vitamin E compared with those that did not receive

**Table 2** - Performance of broilers from 1 to 7 days of age fed diets containing guava extract standardized in phenolic compounds (SGE)

Treatment	FI (g)	BW (g)	WG (g)	FC (g/g)
0 mg/kg	152.7	163.9b	118.8b	1.287c
600 mg/kg	159.5	184.6a	139.5a	1.142b
800 mg/kg	161.3	187.4a	142.2a	1.135b
1,000 mg/kg	150.6	182.1a	136.9a	1.100a
120 mg/kg vit. E	160.8	187.3a	142.2a	1.130ba
P-value	0.071	<0.001	<0.001	<0.001
SEM	1.55	2.00	1.97	0.01
P-value regression	ns	Q (0.008) <sup>1</sup>	Q (0.006) <sup>2</sup>	L (<0.001) <sup>3</sup>

FI - feed intake; BW - body weight; WG - weight gain; FC - feed conversion; Q - quadratic; L - linear; SEM - standard error of the mean; ns - not significant.

a-c - Means followed by different letters in the column differ between each other by Tukey's test at 5% probability.

<sup>1</sup>  $y = 163.7999 + 0.0626x - 0.00004369x^2$  ( $R^2 = 0.98$ ).

<sup>2</sup>  $y = 118.7182 + 0.0626x - 0.00004379x^2$  ( $R^2 = 0.98$ ).

<sup>3</sup>  $y = 1.2783 - 0.0002x$  ( $R^2 = 0.96$ ).

**Table 3** - Performance of broilers from 1 to 21 days of age fed diets containing guava extract standardized in phenolic compounds (SGE)

Treatment	FI (g)	BW (g)	WG (g)	FC (g/g)
0 mg/kg	811.2	888.9	843.8	1.000
600 mg/kg	929.6	908.2	863.1	1.119
800 mg/kg	866.1	939.2	894.0	1.002
1,000 mg/kg	917.9	942.6	897.4	1.028
120 mg/kg vit. E	893.6	932.5	887.4	1.007
P-value	0.106	0.207	0.203	0.367
SEM	15.52	8.47	8.43	0.02
P-value regression	ns	L (0.039) <sup>1</sup>	L (0.038) <sup>2</sup>	ns

FI - feed intake; BW - body weight; WG - weight gain; FC - feed conversion; L - linear. SEM - standard error of the mean; ns - not significant.

<sup>1</sup>  $y = 885.9459 + 0.0563x$  ( $R^2 = 0.85$ ).

<sup>2</sup>  $y = 840.8703 + 0.0562x$  ( $R^2 = 0.86$ ).

**Table 4** - Nutrient digestibility and apparent metabolizable energy corrected for nitrogen balance (AMEn) of broiler feed containing guava extract standardized in phenolic compounds, in the period of 18 to 21 days of age

Treatment	DMDC (%)	NDC (%)	EEDC (%)	AMEn (kcal/kg DM)
0 mg/kg	66.7	53.1	82.0	3,077
600 mg/kg	68.5	47.9	79.7	3,078
800 mg/kg	66.9	53.0	79.4	3,147
1,000 mg/kg	68.3	53.8	83.0	3,147
120 mg/kg vit. E	66.3	54.8	82.5	3,066
P-value	0.853	0.459	0.132	0.750
SEM	0.72	1.21	0.57	25.06
P-value regression	ns	ns	ns	ns

DMDC - dry matter digestibility coefficient; NDC - nitrogen digestibility coefficient; EEDC - ether extract digestibility coefficient; SEM - standard error of the mean; ns - not significant.

supplementation. The vilus:crypt ratio was higher in the duodenum of broilers fed diet supplemented with 800 or 1,000 mg SGE/kg or vitamin E compared with that of birds fed unsupplemented diets. For the jejunum, villus height for chickens fed diet supplemented with 600 mg SGE/kg was lower than that for chickens that received vitamin E and other levels of SGE, and was similar to the villus height of chickens fed unsupplemented diets. In addition, broilers fed diets containing 800 or 1,000 mg SGE/kg had a higher villus:crypt ratio than those that received 600 mg SGE/kg; however, they were statistically similar to those that did not receive supplementation or those that received vitamin E in their diet ( $P < 0.001$ ).

Villus height, crypt depth, and villus:crypt ratio for the ileum were not influenced by treatments ( $P > 0.05$ ) (Table 5), and the regression analysis revealed no effect of SGE levels ( $P > 0.05$ ).

**Table 5** - Villus height, crypt depth, and villus:crypt ratio of the duodenum, jejunum, and ileum of broilers fed diets containing guava extract standardized in phenolic compounds (SGE), at 21 days of age

Treatment	Villus height ( $\mu\text{m}$ )	Crypt depth ( $\mu\text{m}$ )	Villus:crypt ratio
		Duodenum	
0 mg/kg	980.9c	302.0a	3.29c
600 mg/kg	1,038.0c	272.1ab	3.89bc
800 mg/kg	1,346.6a	259.4ab	4.84a
1,000 mg/kg	1,220.9ab	259.9ab	4.71ab
120 mg/kg vit. E	1,078.3bc	238.8b	4.51ab
P-value	<0.001	<0.001	<0.001
SEM	28.72	6.39	0.14
P-value regression	ns	ns	ns
		Jejunum	
0 mg/kg	888.2ab	233.2	3.89ab
600 mg/kg	777.6b	250.9	3.02b
800 mg/kg	952.6a	214.2	4.55a
1,000 mg/kg	918.5a	211.6	4.35a
120 mg/kg vit. E	909.9a	246.2	3.72ab
P-value	0.005	0.060	<0.001
SEM	15.62	5.51	0.14
P-value regression	ns	ns	ns
		Ileum	
0 mg/kg	612.7	198.9	3.09
600 mg/kg	621.2	196.9	3.18
800 mg/kg	679.8	216.1	3.17
1,000 mg/kg	656.3	187.8	3.49
120 mg/kg vit. E	660.7	191.8	3.45
P-value	0.391	0.073	0.283
SEM	12.26	3.42	0.07
P-value regression	ns	ns	ns

SEM - standard error of the mean; ns - not significant.

a-c - Means followed by different letters in the column differ between each other by Tukey's test at 5% probability.

## 4. Discussion

In this study, supplementation with guava extract resulted in improved weight gain, body weight, and feed conversion at seven days of age, in addition to increased weight gain and final weight with increased extract levels at 21 days of age. Guava extract may have determined the increase in weight gain and body weight and the consequent reduction in feed conversion, probably due to the improvement in intestinal morphology also verified in the present study.

Guava extract has a high concentration of phenolic compounds, which, according to Oliveira et al. (2018), can act as trophic agents in the intestine, increasing the villus:crypt ratio, promoting greater integrity and intestinal health and consequent improvement in performance. According to Furlan et al. (2004), trophic agents stimulate the mitotic process in the crypt-villus region, stimulating the development of the mucosa; as a result, the number of cells and villus size increases.

Starčević et al. (2015) supplemented broiler diet with 5 g/kg of the phenolic compound tannic acid and observed an improvement in weight gain in the initial rearing period. The authors attributed this result to the better utilization of feed by the birds that received the phenolic compound. Research by Oliveira et al. (2018) showed a linear increase in body weight and weight gain of chickens fed diets supplemented with 0, 0.5, 1.0, and 1.5% of guava waste byproduct containing 0.17% of phenolic compounds, at seven days of age. On the other hand, Pascariu et al. (2017) found a reduction in weight gain of chickens that received, via drinking water, 15 mL/L of grape pomace polyphenolic extract containing 1.82 g polyphenols in gallic acid equivalent (GAE)/100 mL.

The increase in villus height and villus:crypt ratio observed in the present study for chickens fed diet supplemented with 800 or 1,000 mg/kg guava extract containing 0.45% phenolic compounds may be related to the stimulation of epithelial cell mitosis by phenolic compounds. According to Kamboh and Zhu (2014), longer villi are associated with activated cell mitosis. Similar results were found by Viveros et al. (2011), who added 60 g/kg of grape pomace concentrate containing 48.7 g (0.48%) phenolic compounds in chicken feed and found greater villus:crypt ratio in the jejunum at 21 days of age. Oliveira et al. (2018) found no significant differences for villus height, crypt depth, and villus:crypt ratio in the duodenum and jejunum of chickens fed diet supplemented with 0, 0.5, 1.0, and 1.5% of guava byproduct; however, they observed a reduction in crypt depth and a linear increase for the villus:crypt ratio of the ileum.

Works by Silva et al. (2010), Bona et al. (2012), and Amad et al. (2013) indicated that the use of compounds from plant secondary metabolism as additives can beneficially assist the intestinal epithelium. Phenolic compounds can protect epithelial cells and prevent inflammation in the intestine, improving intestinal barrier function (Noda et al., 2012). Liu et al. (2018) evaluated the effects of oral administration of 0, 200, 300, or 400  $\mu$ L of essential oils of a phenolic compound (carvacrol) to broilers on intestinal barrier function. These authors observed increase in goblet cell content and increased expression of proteins that make up the intestinal barrier (occludin, claudin-1, claudin-5, and ZO-2) in the intestinal mucosa of broilers that received carvacrol in relation to the control. It was concluded that carvacrol essential oils have positive effects on the intestinal barrier function of broilers.

In addition, phenolic compounds can assist in the development and health of the intestinal mucosa through their antioxidant action. Procházková et al. (2011) reported that phenolic compounds, such as flavonoids, sequester free radicals by donating a hydrogen atom. Thus, phenolic compounds can protect intestinal cells from the inflammation caused by reactive species resulting from oxidative stress. According to Mishra and Jha (2019), oxidative stress in birds can result in intestinal inflammation, while the addition of antioxidant substances in the diet reduces free radicals in the intestine and helps maintain the integrity of the intestinal mucosa.

The improvement in intestinal morphology, that is, the increase in the area of digestion and absorption of nutrients in chickens fed diet supplemented with guava extract was not reflected in the digestibility of nutrients. However, it may have resulted in less energy expenditure for the maintenance of the intestinal epithelium and, consequently, better performance for chickens fed diet supplemented with SGE. According to Porto et al. (2015), the digestive system demands a large amount of energy to maintain intestinal integrity as it is metabolically one of the most active systems of the animal organism. Thus, the use of additives in feed that can help the development and integrity of intestinal cells can result in less energy expenditure with the recovery of the intestinal mucosa and provide improvement in the performance of chickens. Thus, by promoting greater intestinal health, the loss of energy is reduced, as, according to Dibner and Richards (2004), damage to the mucosa can increase the need for maintenance of the intestine, diverting nutrients that would be used for the growth of the animal.

It is noteworthy that the amount of tannins present in the extract of guava residue used in this work (0.85%) did not negatively affect the digestibility of nutrients, which demonstrates that the extract doses tested did not have a toxic or antinutritional effect for chickens, therefore, being safe to use. The presence of antinutritional factors in feeds, such as high polyphenol (tannins) content, can negatively interfere with the digestibility of nutrients, especially proteins, as they are able to complex with proteins and inhibit the action of digestive enzymes (Brenes et al., 2010). Chamorro et al. (2013) supplemented chicken diets with 5 g/kg of grape seed extract containing 14.8 g of tannins in equivalent of cyanidin/100 g of dry matter and found lower ileal digestibility of protein in relation to the chickens of the control group, at 21 days of age. On the other hand, Brenes et al. (2008) supplemented broiler ration with 0, 15, 30, and 60 g/kg of grape residue containing 15.09% tannins and found no change in the digestibility of protein; however, the digestibility of ethereal extract worsened at 42 days of age. The authors related this effect to the ability of the phenolic compound (tannins) to bind to bile salts and cholesterol, resulting in reduced absorption and increased fecal excretion of lipids.

The results for performance, digestibility of feed nutrients, and histomorphometry of the small intestine of broilers fed diet supplemented with vitamin E in the present study were similar to those of broilers that received SGE in their feed. There are several functions attributed to vitamin E in the animal organism, among them: as an immunostimulant, it protects the cells associated with the immune response against the effects of oxidation, increasing the function and proliferation of these cells (Khan et al., 2011); antioxidant protection of body tissues (Voljč et al., 2011) and cells (Khan et al., 2011); and improved intestinal health through anti-inflammatory (Pitargue et al., 2019) and antioxidant (Cheng et al., 2018) effects in the intestinal mucosa. The bioactivity of vitamin E is mainly related to its antioxidant potential; it works by interrupting the oxidation chain reaction, thus protecting cell membranes from oxidative damage (Carocho et al., 2013), and can contribute to improving animal performance. Selvam et al. (2017) found the inclusion of 70 g of vitamin E/ton in feed to positively affect broiler performance, immune response, and antioxidant status.

## 5. Conclusions

Guava extract standardized in phenolic compounds can be used in diets for broilers in the starter rearing phase as a functional additive, since levels of 800 to 1,000 mg/kg increase weight gain, reduce feed conversion, and aid in the development and maintenance of the intestinal mucosa.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Conceptualization: R.A. Noleto-Mendonça, N.S.M. Leandro and E.C. Conceição. Formal analysis: R.A. Noleto-Mendonça and N.S.M. Leandro. Funding acquisition: N.S.M. Leandro. Investigation: R.A. Noleto-Mendonça, J.M.S. Martins, D.P. Carvalho and I.C.S. Araújo. Methodology: R.A. Noleto-Mendonça, J.H. Stringhini and N.S.M. Leandro. Project administration: R.A. Noleto-Mendonça and N.S.M. Leandro. Resources: N.S.M. Leandro. Supervision: M.B. Café and N.S.M. Leandro. Validation: R.A. Noleto-Mendonça and N.S.M. Leandro. Writing-original draft: R.A. Noleto-Mendonça. Writing-review & editing: R.A. Noleto-Mendonça, J.M.S. Martins and N.S.M. Leandro.

## Acknowledgments

Authors thank the Predilecta Alimentos for having granted the guava residues; the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for the financial support; and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for scholar and research fellowships.

## References

- Amad, A. A.; Wendler, K. R. and Zentek, J. 2013. Effects of a phytogetic feed additive on growth performance, selected blood criteria and jejunal morphology in broiler chickens. *Emirates Journal of Food and Agriculture* 25:549-554. <https://doi.org/10.9755/ejfa.v25i7.12364>
- ANVISA - Agência Nacional de Vigilância Sanitária. 2011. Formulário de Fitoterápicos Farmacopéia Brasileira. 1.ed. ANVISA, Brasília, DF.
- Araújo, K. L. G. V.; Magnani, M.; Nascimento, J. A.; Souza, A. L.; Epaminondas, P. S.; Souza, A. L.; Queiroz, N. and Souza, A. G. 2014. Antioxidant activity of co-products from guava, mango and barbados cherry produced in the Brazilian Northeast. *Molecules* 19:3110-3119. <https://doi.org/10.3390/molecules19033110>
- Bhatti, N.; Hussain, Z.; Mukhtar, M.; Ali, A.; Imran, M.; Rafique, A.; Manzoor, S. and Rehman, S. 2016. Effects of vitamins E and C supplementation on the immune response of broiler chicks. *Journal of Antivirals & Antiretroviral* 8:151-154. <https://doi.org/10.4172/jaa.1000152>
- Bona, T. D. M. M.; Pickler, L.; Miglino, L. B.; Kuritza, L. N.; Vasconcelos, S. P. and Santin, E. 2012. Óleo essencial de orégano, alecrim, canela e extrato de pimenta no controle de *Salmonella*, *Eimeria* e *Clostridium* em frangos de corte. *Pesquisa Veterinária Brasileira* 32:411-418. <https://doi.org/10.1590/S0100-736X2012000500009>
- Brenes, A.; Viveros, A.; Goñi, I.; Centeno, C.; Sáyago-Ayerdy, S. G. and Saura-Calixto, F. 2008. Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. *Poultry Science* 87:307-316. <https://doi.org/10.3382/ps.2007-00297>
- Brenes, A.; Viveros, A.; Goñi, I.; Centeno, C.; Saura-Calixto, F. and Arija, I. 2010. Effect of grape seed extract on growth performance, protein and polyphenol digestibilities, and antioxidant activity in chickens. *Spanish Journal of Agricultural Research* 8:326-333. <https://doi.org/10.5424/sjar/2010082-1199>
- Carocho, M. and Ferreira, I. C. F. R. 2013. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology* 51:15-25. <https://doi.org/10.1016/j.fct.2012.09.021>
- Chamorro, S.; Viveros, A.; Centeno, C.; Romero, C.; Arija, I. and Brenes, A. 2013. Effects of dietary grape seed extract on growth performance, amino acid digestibility and plasma lipids and mineral content in broiler chicks. *Animal* 7:555-561. <https://doi.org/10.1017/S1751731112001851>
- Cheng, K.; Zhang, M.; Huang, X.; Zheng, X.; Song, Z.; Zhang, L. and Wang, T. 2018. An evaluation of natural and synthetic vitamin E supplementation on growth performance and antioxidant capacity of broilers in early age. *Canadian Journal of Animal Science* 98:187-193. <https://doi.org/10.1139/cjas-2017-0040>
- Detmann, E.; Souza, M. A.; Valadares Filho, S. C.; Queiroz, A. C.; Berchielli, T. T.; Saliba, E. O. S.; Cabral, L. S.; Pina, D. S.; Ladeira, M. M. and Azevedo, J. A. G. 2012. Métodos para análise de alimentos: Instituto Nacional de Ciência e Tecnologia de Ciência Animal. Suprema, Visconde do Rio Branco.
- Dibner, J. J. and Richards, J. D. 2004. The digestive system: challenges and opportunities. *Journal Applied Poultry Research* 13:86-93. <https://doi.org/10.1093/japr/13.1.86>
- Fukayama, E. H.; Bertechini, A. G.; Geraldo, A.; Kato, R. K. and Murgas, L. D. S. 2005. Extrato de orégano como aditivo em rações para frangos de corte. *Revista Brasileira de Zootecnia* 34:2316-2326. <https://doi.org/10.1590/S1516-35982005000700018>
- Furlan, R. L.; Macari, M. and Luquetti, B. C. 2004. Como avaliar os efeitos do uso de prebióticos, probióticos e flora de exclusão competitiva. p.6-28. In: Anais do 5º Simpósio Técnico de Incubação, Matrizes de Corte e Nutrição. Embrapa Suínos e Aves; ACAV, Balneário Camboriú.
- Kamboh, A. A. and Zhu, W-Y. 2014. Individual and combined effects of genistein and hesperidin on immunity and intestinal morphometry in lipopolysaccharide-challenged broiler chickens. *Poultry Science* 93:2175-2183. <https://doi.org/10.3382/ps.2014-03971>
- Khan, R. U.; Naz, S.; Nikousefat, Z.; Tufarelli, V.; Javdani, M.; Rana, N. and Laudadio, V. 2011. Effect of vitamin E in heat-stressed poultry. *World's Poultry Science Journal* 67:469-478. <https://doi.org/10.1017/S0043933911000511>
- Liu, S.; Song, M.; Yun, W.; Lee, J.; Lee, C.; Kwak, W.; Han, N.; Kim, H. and Cho, J. 2018. Effects of oral administration of different dosages of carvacrol essential oils on intestinal barrier function in broilers. *Journal of Animal Physiology and Animal Nutrition* 102:1257-1265. <https://doi.org/10.1111/jpn.12944>
- Luna, L. G. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd ed. McGraw-Hill, New York. 258p.
- Matterson, L. D.; Potter, L. M.; Stutz, M. W. and Singsen, E. P. 1965. The metabolizable energy of feeds ingredients for chickens. *Agricultural Experimental Station Research Report* 7. 11p.
- Melo, O. S.; Bergamaschi, K. B.; Tiveron, A. P.; Massarioli, A. P.; Oldoni, T. L. C.; Zanús, M. C.; Pereira, G. E. and Alencar, S. M. 2011. Composição fenólica e atividade antioxidante de resíduos agroindustriais. *Ciência Rural* 41:1088-1093. <https://doi.org/10.1590/S0103-84782011000600027>

- Mishra, B. and Jha, R. 2019. Oxidative stress in the poultry gut: potential challenges and interventions. *Frontiers in Veterinary Science* 6:60. <https://doi.org/10.3389/fvets.2019.00060>
- Noda, S.; Tanabe, S. and Suzuki, T. 2012. Differential effects of flavonoids on barrier integrity in human intestinal Caco-2 cells. *Journal of Agricultural and Food Chemistry* 60:4628-4633. <https://doi.org/10.1021/jf300382h>
- Oliveira, M. D.; Mello, H. H. C.; Stringhini, J. H.; Mascarenhas, A. G.; Arnhold, E.; Conceição, E. C.; Martins, J. M. S. and Silva Júnior, A. J. 2018. Antioxidant effect of the guava byproduct in the diet of broilers in the starter phase. *Revista Brasileira de Zootecnia* 47:e20160290. <https://doi.org/10.1590/rbz4720160290>
- Packer, V. G.; Melo, P. S.; Bergamaschi, K. B.; Selani, M. M.; Villanueva, N. D. M.; Alencar, S. M. and Contreras-Castillo, C. J. 2015. Chemical characterization, antioxidant activity and application of beetroot and guava residue extracts on the preservation of cooked chicken meat. *Journal of Food Science and Technology* 52:7409-7416. <https://doi.org/10.1007/s13197-015-1854-8>
- Pascariu, S. M.; Pop, I. M.; Simeanu, D.; Pavel, G. and Solcan, C. 2017. Effects of wine by-products on growth performance, complete blood count and total antioxidant status in broilers. *Brazilian Journal of Poultry Science* 19:191-202. <https://doi.org/10.1590/1806-9061-2016-0305>
- Pitargue, F. M.; Kim, J. H.; Goo, D.; Delos Reyes, J. D. and Kil, D. Y. 2019. Effect of vitamin E sources and inclusion levels in diets on growth performance, meat quality, alpha-tocopherol retention, and intestinal inflammatory cytokine expression in broiler chickens. *Poultry Science* 98:4584-4594. <https://doi.org/10.3382/ps/pez149>
- Procházková, D.; Bousová, I. and Wilhelmová, N. 2011. Antioxidant and prooxidant properties of flavonoids. *Fitoterapia* 82:513-523. <https://doi.org/10.1016/j.fitote.2011.01.018>
- Porto, M. L.; Givisiez, P. E. N.; Saraiva, E. P.; Costa, F. G. P.; Moreira Filho, A. L. B.; Andrade, M. F. S.; Brandão, P. A. and Guerra, R. R. 2015. Glutamic acid improves body weight gain and intestinal morphology of broiler chickens submitted to heat stress. *Brazilian Journal of Poultry Science* 17:355-362. <https://doi.org/10.1590/1516-635x1703355-362>
- R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. F.; Lopes, D. C.; Ferreira, A. S.; Barreto, S. L. T. and Euclides, R. F. 2011. Tabelas brasileiras para aves e suínos - Composição de alimentos e exigências nutricionais. 3.ed. Universidade Federal de Viçosa, Viçosa, MG. 252p.
- Sakomura, N. K. and Rostagno, H. S. 2016. Métodos de pesquisa em nutrição de monogástricos. 2.ed. Funep, Jaboticabal. 262p.
- Selvam, R.; Saravanakumar, M.; Suresh, S.; Sureshbabu, G.; Sasikumar, M. and Prashanth, D. 2017. Effect of vitamin E supplementation and high stocking density on the performance and stress parameters of broilers. *Brazilian Journal of Poultry Science* 19:587-594. <https://doi.org/10.1590/1806-9061-2016-0417>
- Sibbald, I. R. and Slinger, S. J. 1963. A biological assay for metabolizable energy in poultry feed ingredients together with findings which demonstrate some of the problems associated with the evaluation of fats. *Poultry Science* 42:313-325. <https://doi.org/10.3382/ps.0420313>
- Silva, D. J. and Queiroz, A. C. 2006. Análise de alimentos (métodos químicos e biológicos). 3.ed. Universidade Federal de Viçosa, Viçosa, MG. 235p.
- Silva, M. A.; Pessotti, B. M. S.; Zanini, S. F.; Colnago, G. L.; Nunes, L. C.; Rodrigues, M. R. A. and Ferreira, L. 2010. Óleo de aroeira-vermelha sobre o desempenho e a morfometria intestinal de frangos de corte. *Ciência Rural* 40:2151-2156. <https://doi.org/10.1590/S0103-84782010005000160>
- Sousa, M. S. B.; Vieira, L. M. and Lima, A. 2011. Fenólicos totais e capacidade antioxidante in vitro de resíduos de polpas de frutas tropicais. *Brazilian Journal of Food Technology* 14:202-210.
- Starčević, K.; Krstulović, L.; Brozić, D.; Maurić, M.; Stojević, Z.; Mikulec, Z.; Bajić, M. and Mašek, T. 2015. Production performance, meat composition and oxidative susceptibility in broiler chicken fed with different phenolic compounds. *Journal of the Science Food Agriculture* 95:1172-1178. <https://doi.org/10.1002/jsfa.6805>
- Viveros, A.; Chamorro, S.; Pizarro, M.; Arija, I.; Centeno, C. and Brenes, A. 2011. Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. *Poultry Science* 90:566-578. <https://doi.org/10.3382/ps.2010-00889>
- Voljč, M.; Frankič, T.; Levart, A.; Nemeč, M. and Salobir, J. 2011. Evaluation of different vitamin E recommendations and bioactivity of  $\alpha$ -tocopherol isomers in broiler nutrition by measuring oxidative stress in vivo and the oxidative stability of meat. *Poultry Science* 90:1478-1488. <https://doi.org/10.3382/ps.2010-01223>
- Wang, J.; Clark, D. L.; Jacobi, S. K. and Velleman, S. G. 2021. Effect of vitamin E and alpha lipoic acid on intestinal development associated with wooden breast myopathy in broilers. *Poultry Science* 100:100952. <https://doi.org/10.1016/j.psj.2020.12.049>
- Yoo, J.; Yi, Y. J.; Jung, S.; Yoon, J. U.; Kang, H. B.; Lee, D. H. and Heo, J. M. 2016. Growth performance, intestinal morphology, and meat quality in relation to alpha-lipoic acid associated with vitamin C and E in broiler chickens under tropical conditions. *Revista Brasileira de Zootecnia* 45:113-120. <https://doi.org/10.1590/S1806-92902016000300005>