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Performance and meat quality of broiler chickens fed diets containing hydroethanolic extract of guavira seed or peel

P.O. Nesello[1], C. Eyng[1#], R.V. Nunes[1], E.J.S. Argandona[2], C.A.C. Correia[2], J. Broch[2], N. Rohloff Junior[1], V.D.L. Savaris[1], E.C. dos Santos[1]

[1]State University of Western Parana, Marechal Candido Rondon, Brazil
[2]Federal University of Grande Dourados, Dourados, Brazil

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Abstract

The dietary inclusion of hydroethanolic extract of guavira seed or peel was evaluated on broiler performance, intestinal morphometry, carcass yield, and meat quality. A total of 1,680 male chicks, one day old, were distributed in a 2×5+2 factorial design (hydroethanolic extract of guavira seed (HEGS) or hydroethanolic extract of guavira peel (HEGP); 100; 200; 300; 400 and 500 mg/kg of inclusion and a positive control (PC) with antibiotics and negative control (NC) without growth promoter), with seven replicates and 20 birds each. A better feed conversion ratio (FCR) was observed from days 1–7 and days 1–21 on the PC diet than on the NC diet. FCR from 1–7d of broilers on PC was better than those on HEGS or HEGP, and this better FCR was maintained until 21 d for the HEGS treatment. The HEGP diet showed lower average feed intake and better FCR for the total period compared to NC diets. Higher carcass yield and a higher L_{15} value for breast meat was observed in the HEGP group compared to HEGS. Broilers fed the PC diet showed a higher a* value 15 min and 24 h *postmortem,* higher water retention capacity in breast meat compared to birds fed HEGS, and a higher value of a* 24 h *postmortem* than those receiving NC. The inclusion of 100 to 500 mg/kg of HEGP or HEGS did not improve performance, intestinal morphometry, and carcass characteristics when compared to the performance-enhancing antibiotic. However, the HEGP diet provided better performance of broilers than the non-use of growth promoter additives in the diet.

Keywords: antioxidant, cerrado fruits, natural additive
[#]Corresponding author: cinthiaeyng@hotmail.com

Introduction

The use of subtherapeutic doses of antibiotics in broiler diets improves the assimilation and absorption of nutrients, helps in maintaining the intestinal health of animals and, consequently, reduces production costs (Broom, 2018). For decades, it has been a common practice to add antibiotics to animal feed in the Brazilian poultry industry in order to ensure competitive prices on the world market. However, the occurrence of pathogenic bacteria resistant to these compounds, along with consumers' exposure to residues in the final product, has led several countries to ban the use of antibiotics as growth promoters in broiler diets (Raphae *et al*., 2017).

As a result, nutritionists have intensified the search for natural additives to maintain broiler productivity without posing a risk to public health. A wide range of plant extracts have demonstrated potential benefits due to the presence of biologically active compounds such as phenolic compounds, mainly flavonoids, and carotenoids (Loetscher *et al*., 2013; Morovat *et al*., 2016). Biologically active compounds may enhance animal performance and the quality of the final product due to their antimicrobial, antioxidant, and immunostimulant activity in the animals' metabolism (Windisch *et al*., 2008; Pourhossein *et al*., 2015). Prihambodo *et al*. (2021) evaluated 42 articles using a meta-analysis regarding levels of inclusion of flavonoids and observed a positive influence of the compounds on average daily gain of broilers in the finisher phase, and for feed conversion rate both in the starter and finisher phases.

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Guavira (*Campomanesia* spp.), a fruit of the Brazilian Cerrado, also known as guabiroba, has aroused the interest of researchers due to the presence of flavonoids. Extracts produced from guavira fruit have high *in vitro* antioxidant activity and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical-scavenging activity ranging from 52–92.2% (Coutinho *et al*., 2008). Moreover, studies suggest that essential oils extracted from guavira fruit have antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Candida albicans*, and moderate activity against *Escherichia coli* (Coutinho *et al*., 2009; dos Santos *et al*., 2019). Lohmann et al. (2021) observed that the dietary inclusion of 219 mg/kg of HEGP resulted in better feed conversion rate for broilers in the finishing phase. In this context, the use of natural additives in animal feed has become a feasible alternative to antibiotics because they protect the intestinal mucosa and favour digestion and absorption processes, which contributes to a balanced gut microbiota. Moreover, these additives improve the oxidative profile and stability of poultry meat (Christaki *et al*., 2012).

Despite evidence of beneficial effects of compounds present in guavira peel and seed, studies on their effects in animals, especially broilers, are scarce. Based on this information, we hypothesized that the inclusion of extracts of guavira seed or peel in broiler diets would improve broiler performance and meat quality considering the antimicrobial and antioxidant activity of the extracts. Therefore, this study aimed to evaluate the dietary inclusion of the extract of guavira peel or seed (*C. adamantium*) on performance, intestinal morphometry, carcass yield, and meat quality of broilers.

Material and Methods

This study was carried out at the Poultry Research Center of Western Paraná State University, Unioeste, Marechal Cândido Rondon, Brazil. The experimental protocol was approved by the University Animal Use Ethics Committee (number 04/2019).

A total of 1,680 Cobb 500®, male, one-day-old broiler chicks were distributed in a completely randomized design, in a 2 x 5 + 2 factorial design [(two types of extract - hydroethanolic extract of guavira seed (HEGS) and hydroethanolic extract of guavira peel (HEGP); five inclusion levels - 100, 200, 300, 400, and 500 mg/kg; and two control diets - positive with antibiotic addition (avilamycin) and negative (without growth promoter)], with seven replicates and 20 birds per experimental unit (EU). The lighting program used was following the lineage manual recommendation. Exhaust fans and evaporative plates performed the environment cooling and the renewal of the air. The minimum and maximum average temperature and relative humidity were monitored daily and kept within the recommended thermal comfort range for each phase.

Guavira fruits were acquired in Ponta Porã city, MS, Brazil and processed at the Faculty of Biological Sciences of the Federal University of Grande Dourados, MS, Brazil. The fruit was washed and sanitized in 0.66% sodium dichloroisocyanide solution for 15 min and then pulped. The peel and seed were dehydrated at 40 ^{o}C for 36 h in a dehydrator with an airflow of 0.5 $m^3\ s^{-1}$ and stored in low-density polyethylene packaging at room temperature (25 ^{o}C) until the production of the extracts.

The extracts were obtained in a Soxhlet using 150 grams of the sample (peel or seed) in 750 mL of 70% absolute ethyl alcohol (1: 5 ratio). The extractions were carried out at a temperature of 80 ^{o}C for 3 h and then the solvent (ethanol) was removed under vacuum in a rotary evaporator (Fisatom, model 810) to produce the aqueous extracts. The aqueous extracts were placed in amber glass bottles and stored at 7 ^{o}C until use.

To determine the total polyphenol and flavonoid content, the bioactive compounds were extracted according to the method of Singleton & Rossi (1965), with modifications. An amount of 2 mL of each extract was homogenized with 2 mL of methanol in Falcon tubes. Subsequently, the tubes were placed in a homogenizer for 10 min and then centrifuged (Centrifuge Kasvi K14-4000, Kasvi, São Paulo, BR) for 20 min at 8,568 × *g*. The supernatant was used directly for the measurements.

The concentration of total polyphenols in the extracts was determined according to the method of Singleton & Rossi (1965), with modifications. An aliquot of the supernatant previously extracted (125 µl) was homogenized with 125 µl of Folin-Ciocalteu reagent (1: 1 deionized water) and sodium carbonate (28 g L^{-1}) in a total volume of 2.25 mL. The samples were incubated in the dark at room temperature (25 °C) for 30 min and, subsequently, the absorbance was measured in a spectrophotometer at 750 nm. The results were expressed in mg equivalent of gallic acid (EAG) mL^{-1} of the extracts, using a standard curve developed with concentrations between 0 to 300 mg L^{-1} of gallic acid. The analysis was performed in triplicate.

The flavonoid content was measured using the aluminium chloride method of Buriol *et al*. (2009), with modifications. An amount of 300 µl of the supernatant extracted previously was homogenized with 150 µl of aluminium chloride (5% mass $volume^{-1}$ in methanol) and the final volume was adjusted to 3000 µl with methanol. The samples were incubated in the dark at room temperature (25 °C) for 30 min and the absorbance was measured on a spectrophotometer at 425 nm. A standard

curve based on quercetin was prepared and the results were expressed as mg equivalent of quercetin (EQ) mL^{-1} of the extracts. The analysis was performed in triplicate.

The experimental diets, based on corn and soybean meal, were supplied in mash form and formulated to meet the bird`s nutritional requirements (regular–medium performance) recommended by Rostagno *et al*. (2017) for phases 1–7, 8–21, and 22–42 days of age (Table 1). The birds received feed and water *ad libitum* throughout the experimental period. The inclusion of extracts and the growth promoter in the positive control diet (avilamycin) was performed in substitution to the inert substance, kaolin.

Table 1 Composition and specifications of experimental feed for broiler chickens

Ingredients (g/kg)	1 to 7 days	8 to 21 days	22 to 42 days
Corn	535.93	553.95	639.79
Soybean meal (46%)	383.35	361.93	279.98
Soybean oil	32.26	39.65	41.59
Monocalcium phosphate	18.27	16.17	13.14
Limestone	12.18	11.02	9.08
Salt	5.34	5.17	4.84
L-lysine sulphate (51.7%)	4.57	4.57	5.04
DL-methionine (98%)	3.86	3.67	3.09
L- threonine (98%)	1.34	1.27	1.15
Vitamin supplement[1]	1.30	1.00	0.70
Mineral supplement[2]	0.50	0.50	0.50
Choline chloride (60%)	0.60	0.60	0.60
Inert[3]	0.50	0.50	0.50
Nutrient specifications (g/kg)			
Crude Protein	219.40	210.90	180.00
Metabolizable Energy (MJ/kg	12.45	12.77	13.25
Calcium	9.71	8.78	7.17
Available Phosphorus	4.63	4.19	3.49
Digestible Met + Cist	9.67	9.29	8.05
Digestible Lysine	13.07	12.56	10.88
Digestible Threonine	8.63	8.29	7.18
Digestible Tryptophan	2.51	2.39	1.98

[1]Vitamin supplement (content per kg of product): Vit. A (min) 11.000.000 U.I.; Vit. D3 (min) 4.000.000 U.I.; Vit. E (min) 55.000 U.I.; Vit. K3 (min) 3.000 mg; Vit. B1 (min) 2.300 mg; Vit. B2 (min) 7.000 mg; Vit. B6 (min) 4.000 mg; Vit. B12 (min) 25.000 mg; Pantothenic Acid (min) 12g; Nicotinic Acid (min) 60 g; Folic Acid (min) 2.000 mg; Biotin (min) 250 mg; [2]Mineral Supplement (content per kg of product): Iron (min) 100 g; Copper (min) 20 g; Manganese (min) 130 g; Selenium (min) 300 mg; Zinc (min) 130 g; Iodine (min) 2.000 mg; [3] The hydroethanolic extract of guavira seed, the hydroethanolic extract of guavira peel, and the antibiotic (avilamycin) in the positive control diet was added in replacement for inert substance (kaolin)

At seven, 21, and 42 d of age, the birds as well as the leftover feed, were weighed for performance evaluation (weight gain, average feed intake, and feed conversion ratio). Mortalities that occurred during the period were recorded for later correction of data on average feed intake and feed conversion ratio, according to Sakomura & Rostagno (2016).

At seven and 21 days of age, one bird per EU, with a representative weight (mean ± 5%), was slaughtered to collect a 2-cm fragment of the jejunum segment for morphometric analysis. The samples were opened longitudinally, washed with saline solution, fixed in buffered formalin solution (10%), and dehydrated in a series of increasing alcohol concentrations, diaphanized in xylol, and embedded in paraffin (Luna, 1968). After the semi-serial microtomy (7-µm sections), the sections were stained using the haematoxylin and eosin technique. Morphometric analyses (30 readings $sample^{-1}$) were performed using the Pro-Plus Image System (Version 5.2, Cybernetic Average). The villi heights were taken from the basal region, which coincides with the upper portion of the crypts to the apex; and the crypts, from the base of the crypt to the villus transition region. The villus height and width measurements and crypt depth were used to calculate the absorption surface area of the intestinal mucosa (Kisielinski *et al*., 2002).

To determine carcass yield, cut yield (breast, legs, and wings), and meat quality at 42 d of age, two birds were selected (mean weight ± 5%) and slaughtered by cervical dislocation with subsequent bleeding, plucking, and evisceration. For carcass yield, the eviscerated carcass weight (without head, feet, neck, and abdominal fat) was used in relation to the weight of the live bird. For the yield of cuts, the weight of the eviscerated carcass was considered. Abdominal fat was considered to be deposited around the gizzard, abdomen, and cloacal pouch.

For meat quality analyses, the breast muscle (*Pectoralis major*) and the right thigh were used to assess pH and colour at 15 min and 24 h *postmortem*. The pH meter used was HI 99163, which was calibrated using the 2-point method against standard buffer solutions with pH values of 4.0 and 7.0. The pH meter probe was inserted into the muscle at an angle of 45° and washed with deionized water between each measured sample. The colour was expressed in the CIELAB dimensions of luminosity (L - brightness - dark to light level), redness (a* - red / green intensity) and yellow (b* - yellow / blue intensity) using the portable colorimeter (Konica Minolta Sensing CR -400). The colorimeter was calibrated against black and white reference titles before use. The colour measurements were determined at room temperature (20–25 ºC) on the surface of each muscle sample, in three randomly selected locations, using an angle of 0º.

Subsequently, water holding capacity (WHC), cooking loss (CL) and shear force (SF) analyses were performed on breast meat. The left thigh was used to determine lipid oxidation (TBARS). The WHC was performed according to the centrifugation method, proposed by Nakamura & Katok (1985). Samples of 1 g of breast muscle *in natura* were wrapped in filter paper, centrifuged (Centrifuge Baby I 206 BL) at 2,142 × *g* for 4 min, weighed, dried in an oven at 70 ºC for 12 h and weighed again to calculate the WHC, in percentage:

WHC (%) = (sample weight of meat after centrifugation - weight of the sample after drying / initial weight of the fresh sample multiplied by 100).

To determine the CL, the breast fillets were weighed, packed in laminated paper, and cooked on a commercial electric plate with heating up to 180 °C, until they reached an internal temperature of 80 °C. Then, the samples were kept at rest until they stabilized at room temperature and weighed again, thus obtaining the weight after cooking (Honikel, 1998). The SF analysis was performed with the fillets used for CL. The samples were cut into three rectangles (1.0 × 1.0 × 1.3 cm) and placed with the fibres oriented perpendicular to the blade to determine the SF in kilogram force (kgf cm^{-2}) using the TA-XT2i Texturometer equipment (Stable Micro System, Jarinu, Brazil), coupled with the Warner–Bratzler Shear Force probe (mechanical, calibrated with a standard weight of 5 kg and speed of the disconnector of 20 cm min^{-1}) (Fronning & Uijttenboogaart, 1988).

The determination of the value of substances reactive to thiobarbituric acid (TBARS) was carried out on the meat of the broiler thigh immediately after slaughter (day 0) and after storage at -20 °C after seven, 30, and 60 d of storage. The analyses were carried out according to the adapted methodology described by Vyncke (1970) and Sørensen & Jørgensen (1996). The aldehydes were extracted by homogenizing 10 mL of a solution of trichloroacetic acid (7.5%) and BHT (0.2%) with 2.5 g of sample. Then, the obtained supernatant was filtered on a qualitative filter paper and 3 mL of thiobarbituric acid solution (0.02 M) were added to 3 mL of the previously extracted solution (1:1). The obtained solution was maintained for 40 min at 80 °C, for the formation of the coloured complex, and the absorbance was measured using a spectrophotometer at 538 nm. A standard curve using 1,1,3,3 tetraethoxypropane (TEP) was prepared, and the results were expressed as malonaldehyde (MDA) mg^{-1} meat.

The data were subjected to analysis of variance, using the statistical software SAS® (University Edition), excluding control treatments, to determine the effects of the type of extract and levels of inclusion, as well as the interaction between these factors. The effect of the levels on the evaluated characteristics was determined using polynomial regression. The F-test was used to compare the means of the experimental groups that received each type of extract. The comparison between the diets containing peel and seed extract, regardless of the level of inclusion and the positive and negative control diets, was performed using an F-test for contrasts.

Results and Discussion

The content of total polyphenols and flavonoids in the hydroethanolic extract of guavira seed (HEGS) and guavira peel (HEGP) was 5.86 mg GAE mL^{-1} and 1.04 mg QE mL^{-1}; and 8.39 mg GAE mL^{-1} and 1.12 mg QE mL^{-1}, respectively.

The presence of total polyphenols, and specifically flavonoids, in fresh fruits and plants, as well as in their respective extracts, has aroused the interest of researchers due to the biological activity of these substances. According to Pascoal *et al*. (2011), flavonoids, including isoquercitrin and quercitrin, are the major compounds in extracts of fruit species belonging to the genus *Campomanesia*, popularly known as guavira.

Flavonoids may act in a wide range of biological systems due to variations in their chemical structure. These compounds affect animal performance by modulating the microbiota, favour intestinal health (Yang *et al*., 2019), and stimulate the immune system (Ouyang *et al*., 2016; Kishawy

et al., 2019). Lescano *et al*. (2016) observed an *in vitro* anti-inflammatory activity of extract from guavira peel, with total polyphenol and flavonoid levels of 1.35 and 0.96 mg GAE g^{-1}, respectively.

There was a interaction (P <0.05) between type and inclusion level of the extract for weight gain (WG) from 1–21 d of age (Table 2). The sliced data showed that broilers fed diets containing 300 mg/kg HEGS showed higher WG (P <0.05) than birds receiving the same level of HEGP (Table 3). On the other hand, there was no significant interaction or isolated effect (P >0.05) on other performance parameters in all periods.

The results of the contrasts (Table 4) showed that broilers fed the positive control diet (with the inclusion of antibiotics) from 1–7 d (P <0.05) and 1–21 d (P <0.05) had a better feed conversion ratio (FCR) than those fed a negative control diet (without the inclusion of a growth-promoting additive), although they did not differ at 42 d (P >0.05). FCR was better for broilers fed positive control (PC) treatment than counterparts fed diets with HEGS from 1–7 d (P <0.05) and from 1–21 d (P <0.05). The inclusion of HEGP negatively influenced the FCR from 1–7 d (P <0.05) compared to the PC treatment. The WG was lower in broilers fed diets containing HEGS from 1–21 d (P <0.05) than in animals fed a negative control (NC) diet. The dietary inclusion of HEGS in the total period (1–42 d) resulted in higher average feed intake (AFI) (P <0.05) and better FCR (P <0.05) compared to treatments containing HEGP. Moreover, broilers fed diets with HEGP showed lower AFI (P <0.05) and better FCR (P <0.05) in the total period compared to those receiving NC diets.

Table 2 Performance of broiler chickens fed diets containing levels of hydroethanolic extract of guavira seeds (HEGS) and peel (HEGP)

	Level	1 to 7 days			1 to 21 days			1 to 42 days		
	(mg/kg)	AFI[1]	WG[2]	FCR[3]	AFI	WG	FCR	AFI	WG	FCR
HEGS	100	169.2	145.6	1.163	1.354	0.999	1.355	4.737	3.085	1.536
	200	169.3	144.7	1.170	1.306	0.956	1.367	4.776	3.062	1.561
	300	167.9	145.1	1.158	1.328	0.987	1.345	4.746	3.109	1.527
	400	171.5	147.0	1.167	1.320	0.960	1.375	4.705	3.054	1.541
	500	169.3	145.8	1.161	1.329	0.987	1.345	4.725	3.120	1.515
Average		169.4	145.6	1.163	1.327	0.978[A]	1.357	4.737	3.086	1536
SEM		1.252	0.829	0.007	0.006	0.006	0.005	0.024	0.017	0.007
HEGP	100	169.7	146.1	1.161	1.299	0.958	1.357	4.619	3.012	1.534
	200	170.5	147.7	1.155	1.335	0.972	1.373	4.831	3.117	1.550
	300	169.1	144.9	1.167	1.274	0.928	1.372	4.576	2.974	1.539
	400	168.4	146.7	1.149	1.315	0.970	1.356	4.794	3.111	1.526
	500	170.0	146.8	1.159	1.318	0.967	1.363	4.750	3.039	1.563
Average		169.5	146.5	1.158	1.308	0.959[B]	1.364	4.705	3.051	1.542
SEM		1.131	0.757	0.008	0.008	0.007	0.004	0.033	0.019	0.005
Type		0.94	0.49	0.67	0.07	0.04	0.30	0.41	0.16	0.45
Level		0.98	0.89	0.99	0.56	0.56	0.50	0.19	0.66	0.43
Type *vs* Level		0.93	0.89	0.95	0.06	0.05	0.21	0.26	0.06	0.14

[1]AFI = Average feed intake (g); [2]WG = Weight gain (g); [3]FCR = Feed conversion ratio; [A,B]Values with different upper letters are significantly different using the F-test (P <0.05)

Table 3 Interaction between inclusion level and type of hydroethanolic extract of guavira on weight gain of broilers from 1 to 21 days

Level (mg/kg)	HEGS[1]	HEGP[2]	*P*-value
100	0.999	0.958	0.086
200	0.956	0.972	0.503
300	0.988[a]	0.929[b]	0.014
400	0.960	0.971	0.646
500	0.988	0.967	0.120
P-value	0.106	0.292	

[1]HEGS = hydroethanolic extract of guavira seed; [2]HEGP = hydroethanolic extract of guavira peel; [a,b]In the same line, means followed by different lowercase letters are significantly different using the F-test (P <0.05)

Table 4 Contrast analysis of the performance parameters of broiler chickens fed diets containing levels of hydroethanolic extract of guavira seed (HEGS) and hydroethanolic extract of guavira peel (HEGP)

	1 to 7 days			1 to 21 days			1 to 42 days		
Treatments[4]	AFI[1]	WG[2]	FCR[3]	AFI	WG	FCR	AFI	WG	FCR
NC	168.6	144.4	1.169	1.328	0.981	1.355	4.931	3.083	1.600
PC	166.8	149.0	1.119	1.320	0.989	1.335	4.795	3.067	1.567
HEGS	169.4	145.6	1.164	1.327	0.978	1.354	4.738	3.086	1.536
HEGP	169.5	146.5	1.158	1.308	0.959	1.364	4.705	3.051	1.542
SEM	0.760	0.530	0.005	0.005	0.004	0.003	0.020	0.010	0.005
Contrasts									
HEGS: HGEP	0.75	0.29	0.60	0.28	0.19	0.39	0.002	0.49	0.003
HEGS: PC	0.34	0.20	0.05	0.53	0.07	0.01	0.20	0.72	0.20
HEGS: NC	0.94	0.47	0.64	0.07	0.05	0.30	0.42	0.19	0.55
HEGP: PC	0.62	0.07	0.05	0.72	0.71	0.18	0.14	0.79	0.16
HEGP:NC	0.78	0.52	0.08	0.96	0.87	0.79	0.007	0.95	0.001
PC: NC	0.36	0.09	0.03	0.68	0.51	0.04	0.42	0.69	0.11

[1]AFI = Average feed intake (g); [2]WG = Weight gain (g); [3]FCR = Feed conversion ratio; [4]NC = negative control (without growth promoter); PC = positive control (with antibiotic, avilamycin); HEGS = hydroethanolic extract of guavira seed; HEGP = hydroethanolic extract of guavira peel

Improvements in animal performance due to natural substances are associated with the capacity of bioactive compounds to favour digestive and absorptive processes due to their antimicrobial activity (Peric *et al*., 2009). According to Tufarelli *et al*. (2017), polyphenols can be converted by the intestinal microbiota into bioactive molecules that affect intestinal ecology and host health. According to the same authors, there is evidence that specific doses of selected polyphenols can alter the composition of the microbial population in the intestine. Moreover, these compounds may increase the efficiency of digestive processes and, consequently, nutrient absorption, by stimulating the activity of enzymes such as amylase, sucrase, and phosphatase (Seven *et al*., 2012). However, the actions of these compounds on the animals' metabolism seem to be dose-dependent. It has been shown that higher levels of flavonoids can reduce enzyme activity involved in the metabolism of carbohydrates and lipids (Oteiza *et al*., 2018), which may result in lower animal performance.

Despite the reports in the literature about the biological properties of guavira components, the inclusion of extracts from guavira peel and seed in broiler diets did not improve animal performance. Although benefits from the dietary inclusion of extracts have not been observed in comparison to antibiotic-based treatments, it is essential to highlight the positive results on AFI and FCR from 1 to 42 days of age in broilers receiving HEGP vs. the additive-free treatment. In an antibiotic-free scenario, the inclusion of HEGP could constitute an alternative in broiler diets.

The influence of natural compounds on animal metabolism can also be affected by the conditions under which the study is conducted. Chegini *et al*. (2019) observed that the effects of the dietary inclusion of propolis on nutrient digestibility were higher for broilers kept under thermal stress.

The unsatisfactory results reported in the literature regarding the inclusion of plant extracts in animal feed may be related to the environment to which animals are exposed (Dalólio *et al*., 2015). Improvements in performance are achieved by killing microorganisms and pathogens that impair the digestion and absorption of nutrients. According to Simitzis *et al*. (2010) and Park *et al*. (2015), well-nourished and healthy animals do not respond to growth-promoting additives when housed in clean and disinfected environments at moderate stocking densities. In the present study, the broilers were raised in clean environments; therefore, growth-promoting agents such as HEGS and HEGP extracts may have had more significant impacts if animals were housed in environmental conditions with greater health challenges. Moreover, the antimicrobial, antioxidant, or immunomodulatory activity of the compounds may vary according to the plant or fruit used, as well as the extraction process, which influences the concentration of active principles (Christaki *et al*., 2012).

For intestinal morphometry, there was no interaction (P >0.05) between extract type and inclusion level on villus height, crypt depth, villus:crypt ratio, and absorptive area at 7 and 21 days of age (data not shown). When comparing different extract types with control diets regardless of inclusion levels, we observed that broilers fed the PC diet showed greater crypt depth (P <0.05) at seven days of age than counterparts fed diets containing HEGP, with values of 80.06 µm vs. 75.73 µm, respectively. Moreover, broilers fed the PC diet showed greater villus height (P <0.05) and decreased crypt depth (P <0.05) at seven days of age compared to animals receiving the NC diet,

with values of 416.80 µm vs. 404.90 µm for villus height and 80.06 µm vs. 82.61 µm for crypt depth, respectively (Table 5).

Table 5 Contrast analysis of villus height (µm), crypt depth (µm), villus:crypt ratio, and absorptive area (µm^2) of jejunum at seven and 21 days of age of broiler chickens fed diets containing levels of hydroethanolic extract of guavira seeds (HEGS) and peel (HEGP)

Treatments[1]	Villus height		Crypt depth		Villus:crypt ratio		Absorptive area	
	7 days	21 days	7 days	21 days	7 days	21 days	7 days	21 days
NC	404.90	821.10	82.61	70.71	4.87	11.66	7.41	12.39
PC	416.80	774.15	80.06	72.87	5.17	10.62	7.26	12.28
HEGS	398.84	885.00	79.70	68.72	4.99	12.82	6.86	14.73
HEGP	362.32	714.97	75.73	61.18	4.80	11.93	7.78	11.74
SEM	8.61	39.51	0.86	2.16	0.08	0.43	0.19	0.76
Contrasts								
HEGS: HEGP	0.77	0.49	0.52	0.25	0.42	0.87	0.87	0.82
HEGS: PC	0.18	0.63	0.16	0.09	0.80	0.34	0.42	0.82
HEGS: NC	0.84	0.06	0.35	0.12	0.67	0.37	0.58	0.09
HEGP: PC	0.09	0.79	0.03	0.82	0.20	0.60	0.55	0.97
HEGP:NC	0.57	0.68	0.90	0.81	0.53	0.50	0.45	0.44
PC: NC	0.05	0.39	0.03	0.55	0.25	0.12	0.02	0.32

[1]NC = negative control (without growth promoter); PC = positive control (with antibiotic, avilamycin); HEGS = hydroethanolic extract of guavira seed; HEGP = hydroethanolic extract of guavira peel

The antioxidant activity of polyphenols modulates intestinal morphology and influences the absorptive capacity of the intestine in animals (Viveros *et al*., 2011; Kamboh & Zhu, 2014). However, no improvements in gut morphometrics were observed in broilers fed diets with HEGS or HEGP. Increased villus height is an indicator of increased surface area available for absorption, and this effect was observed in animals fed diets containing antibiotic, being correlated with the higher WG at 7 d of age. Likewise, decreased crypt depth is an indicator of the lowered production of immature enterocytes, with subsequent lower tissue turnover rate and fewer maintenance requirements (Attia *et al*., 2017). In this context, the villus height/crypt depth ratio is associated with efficient nutrient absorption and better performance. Adil *et al*. (2011) found no differences in villus height and crypt depth of broilers fed different levels of chamomile powder.

There was no interaction (P >0.05) between extract type and inclusion level on carcass traits. However, broilers fed diets containing HEGP showed higher carcass yield (%) (P <0.05) compared to birds that received HEGS (70.80% vs. 69.79%, respectively; data not shown), regardless of the inclusion level. The results of the contrasts show that broilers fed diets containing HEGS showed lower carcass yield (P <0.05) than birds receiving the NC diet (69.79% vs. 70.25%, respectively; data not shown).

The effectiveness of compounds from natural extracts on carcass characteristics has been extensively evaluated. However, the results are still contradictory. Several studies did not report an influence on carcass and cut yields with the use of plant extracts (Aji *et al*., 2011; Ma *et al*., 2015; Salajegheh *et al*., 2018). Surai (2014) mentions the likely binding of polyphenolic compounds to endogenous proteins through the interaction of their reactive hydroxyl groups with the carbonyl groups of proteins, resulting in reduced protein digestibility in diets containing polyphenols. However, broilers fed diets containing HEGP, which contains higher levels of active compounds, showed higher carcass yield compared to birds receiving HEGS. Therefore, this result cannot be related to lower protein digestibility.

In contrast to the limiting effects of natural extracts on carcass parameters, studies have shown improvements in the physical traits of meat. Phenolic compounds and flavonoids are responsible for the antioxidant activity of plant-based additives (Mehrparvar *et al*., 2016) and have been shown to have several physiological and biochemical functions in the body (Park *et al*., 2015). Therefore, they can be used to increase the antioxidant, antimicrobial, anti-browning, and protein cross-linking potentials, and to maintain or improve meat quality (Tufarelli *et al*., 2017).

There was no interaction (P >0.05) or isolated effect (P >0.05) of treatments on pH, colour, WHC, CL, and SF of breast meat (Table 6). The results of the contrasts (Table 7) showed that the lightness (L) of breast meat 15 min *postmortem* was higher (P <0.05) in broilers fed diets with HEGP than in birds receiving HEGS. The breast meat from broilers fed the PC had higher redness (a*) 15 min (P <0.05) and 24 h *postmortem* (P <0.05) and lower WHC (P <0.05) compared to birds fed the

HEGS treatment. Moreover, the breast meat of broilers receiving the PC diet had higher redness (a*) 24 h *postmortem* (P <0.05) than that of animals receiving the NC diet.

Table 6 The pH values, colour, water-holding capacity (WHC), cooking loss (CL), and shear force values (SF) of breast meat of broiler chickens at 42 days of age fed diets containing hydroethanolic extract of guavira seed (HEGS) and hydroethanolic extract of guavira peel (HEGP)

	Level	pH		*L		*a		*b				
	(mg/kg)	15 min	24 h	15 min	24 h	15 min	24 h	15 min	24 h	WHC	CL	SF
HEGS	100	6.39	5.83	47.03	53.39	2.85	2.59	3.73	5.03	67.96	30.56	3.16
	200	6.21	5.80	48.71	52.06	2.94	2.69	4.35	3.45	66.71	28.46	4.35
	300	6.05	5.74	47.28	50.58	2.83	2.81	4.36	4.55	68.32	25.85	3.43
	400	6.25	5.65	48.60	53.13	3.42	3.21	5.26	4.28	67.91	31.10	4.20
	500	6.14	5.88	47.90	52.69	3.69	3.14	4.82	4.28	69.91	26.93	2.85
Average		6.20	5.78	47.90	52.36	3.13	2.88	4.50	4.32	68.11	28.69	3.62
SEM		0.23	0.20	3.49	2.71	1.30	1.38	1.58	1.75	3.33	4.96	1.57
HEGP	100	5.73	5.78	49.87	55.37	3.07	2.10	5.60	5.01	68.27	31.34	4.33
	200	6.18	5.67	47.01	50.78	2.04	1.83	3.99	5.29	68.58	28.36	4.74
	300	6.13	5.73	49.60	53.08	2.28	2.36	3.62	2.75	68.83	27.24	2.35
	400	6.19	5.64	49.83	52.78	2.57	1.72	4.17	4.14	70.17	28.47	3.59
	500	6.12	5.77	46.73	53.04	3.97	3.70	3.74	4.82	70.06	28.00	3.65
Average		6.07	5.73	48.61	53.01	2.79	2.34	4.23	4.40	69.13	28.68	3.73
SEM		0.50	0.14	3.43	3.16	1.62	1.23	2.33	2.13	2.41	3.13	1.78
Extract		0.17	0.20	0.40	0.35	0.30	0.07	0.57	0.85	0.15	0.91	0.73
Level		0.71	0.15	0.68	0.06	0.13	0.14	0.86	0.44	0.29	0.03	0.08
Extract *vs* Level		0.15	0.69	0.32	0.36	0.71	0.33	0.28	0.17	0.82	0.67	0.32

Table 7 Contrast analysis of the pH values, colour, water-holding capacity (WHC), cooking loss (CL), and shear force values (SF) of breast meat of broiler chickens at 21 days of age fed diets containing hydroethanolic extract of guavira seed (HEGS) and hydroethanolic extract of guavira peel (HEGP)

	pH		*L		*a		*b				
Treatments[1]	15 min	24 h	15 min	24 h	15 min	24 h	15 min	24 h	WHC	CL	SF
NC	5.97	5.77	45.45	51.96	3.53	3.12	4.63	5.31	67.44	27.21	3.64
PC	6.19	5.73	46.00	51.69	4.00	4.40	3.48	4.65	66.31	29.38	4.22
HEGS	6.20	5.77	47.90	52.36	3.13	2.88	4.50	4.32	68.11	28.62	3.62
HEGP	6.07	5.72	48.61	53.01	2.79	2.34	4.23	4.40	69.18	28.67	3.73
SEM	0.04	0.02	0.38	0.33	0.16	0.16	0.22	0.21	0.34	0.43	0.18
Contrasts											
HEGS:HEGP	0.53	0.57	0.03	0.43	0.24	0.20	0.65	0.28	0.20	0.40	0.90
HEGS:PC	0.45	0.90	0.06	0.29	0.04	0.001	0.36	0.74	0.02	0.66	0.46
HEGS:NC	0.15	0.22	0.38	0.37	0.32	0.11	0.58	0.86	0.14	0.95	0.77
HEGP:PC	0.29	0.71	0.76	0.86	0.56	0.10	0.30	0.54	0.50	0.32	0.52
HEGP:NC	0.16	0.92	0.10	0.76	0.53	0.69	0.88	0.24	0.62	0.42	0.97
PC:NC	0.93	0.55	0.17	0.59	0.15	0.01	0.22	0.67	0.15	0.64	0.37

[1]NC = negative control (without growth promoter); PC = positive control (with antibiotic, avilamycin); HEGS = hydroethanolic extract of guavira seed; HEGP = hydroethanolic extract of guavira peel

There was no interaction (P >0.05) between extract types and inclusion levels, or isolated effect (P >0.05), on the pH and colour of thigh meat (Table 8). The results of the contrasts were not different (P >0.05, data not shown). Meat colour is usually an indicator of meat quality, and changes in the visual aspect may influence qualitative aspects. Muscle pH is also a meat quality parameter and is considered one of the most critical post-slaughter factors that influence countless meat quality attributes, such as colour, tenderness, WHC, and CL, among other muscle characteristics (Aberle *et al*., 2001).

The WHC is influenced by pre- and postmortem factors. This attribute represents the muscle's ability to retain water and directly affects meat tenderness. Meat tenderness is influenced by many factors, with the content and chemical structure of connective tissue, the myofibrils, and sarcoplasmic reticulum being the material basis of tenderness (Wang *et al*., 2017). According to the same authors, increases in WHC can be related to an improvement in antioxidant effects in the muscle.

Table 8 Colour and pH values of leg meat of broiler chickens at 42 days of age fed diets containing hydroethanolic extract of guavira seed (HEGS) and peel (HEGP)

	Level	pH		*L		*a		*b	
	(mg/kg)	15 min	24 h	15 min	24 h	15 min	24 h	15 min	24 h
HEGS	100	6.03	5.73	47.29	53.00	4.14	4.47	3.05	5.03
	200	6.02	5.75	50.82	51.90	2.92	3.08	1.78	3.45
	300	6.31	5.84	48.78	51.53	3.73	3.96	3.13	4.55
	400	6.12	5.84	50.01	52.81	3.53	4.11	2.55	4.28
	500	6.01	5.74	50.11	51.86	3.58	4.29	2.37	4.28
Average		6.10	5.78	49.38	52.23	3.58	3.97	2.60	4.32
SEM		0.34	0.20	4.11	2.72	1.49	1.94	1.85	1.75
HEGP	100	5.94	5.84	50.40	54.27	3.41	3.49	2.19	5.01
	200	6.05	5.75	49.99	52.97	3.11	1.35	2.71	5.29
	300	6.03	6.05	49.85	51.11	3.53	3.02	2.21	2.75
	400	5.98	5.73	52.63	51.65	2.24	5.43	1.84	4.14
	500	6.09	6.01	49.39	52.00	3.51	3.45	2.83	4.82
Average		6.02	5.87	50.45	52.40	3.16	3.42	2.36	4.40
SEM		0.18	0.34	2.44	3.38	1.44	1.68	1.66	2.13
Extract		0.22	0.16	0.19	0.81	0.24	0.20	0.58	0.85
Level		0.45	0.35	0.33	0.39	0.42	0.42	0.91	0.44
Extract *vs* Level		0.44	0.39	0.39	0.83	0.70	0.70	0.52	0.17

Many studies on natural extracts corroborate our findings. Ri *et al*. (2017) found no effect of natural extracts on CL, drip loss, SF, pH, or meat colour. Lipiński *et al*. (2017) did not observe any effect of dietary inclusion of polyphenols on the colour, pH, or CL of the breast muscle. Simitzis *et al*. (2010) also observed no effects of natural extracts on SF, pH after 45 min and 24 h *postmortem*, colour parameters, and sensory attributes. Wang *et al*. (2017) and Olusola *et al*. (2018) found no difference in pH values.

According to Faustman & Cassens (1990), the ultimate pH of normal meat is approximately 5.4–5.8, and our results are within this range. Lower values of redness (a*) but increased lightness (L*) and yellowness (b*) indicate pale meat and lower consumer acceptance. Overall, consumers tend to choose pink meat with a fresh appearance. The L-component in the breast meat of broilers was higher in animals fed diets containing HEGP than in those fed HEGS diets. This probably reflects the formation of methemoglobin in the breast muscle, which is associated with meat discoloration (Nieto *et al*., 2010). According to Nieto *et al*. (2010), meat redness (a*) is related to reduced iron (Fe^{3+}) and can be controlled by several strategies that prevent oxidation. Phenolic acids oxidize quinines, which in turn react with some amino acids in the myoglobin molecule, promoting myoglobin polymerization and exposing the heme moiety. Thus, natural additives with antioxidant capacity prevent red pigments from undergoing oxidation to brown metmyoglobin. However, in the present study, this effect was not observed because birds receiving PC treatment showed higher values of a* compared to those receiving HEGS.

There was no interaction (P >0.05) or isolated effect (P >0.05) of treatments on the peroxidation of thigh meat from broilers fed diets containing guavira extracts at 42 d. Thiobarbituric acid reactive substances were analysed at time 0 (time of slaughter) and stored at –20 °C for 7, 30, and 60 days (Table 9). The results of the contrasts showed no difference (P >0.05) in lipid oxidation between control diets and treatments with and without growth-promoting additive, regardless of inclusion levels. The means for mg of malondialdehyde per kg of meat for the NC, PC, HEGS, and HEGP treatments were 0.014, 0.016, 0.019, and 0.030, respectively (data not shown).

In the present study, the treatments did not affect TBARS values, corroborating the findings of Dabbou *et al*. (2017). According to North *et al*. (2019), although the effect on oxidative stability is clearly linked to the role of flavonoids as antioxidants, the exact mechanism is still uncertain; the most direct method would involve supplementing flavonoids, or their metabolites, in tissue, where they would protect molecules from oxidation.

Table 9 Values of thiobarbituric acid reactive substances (TBARS) (mg of malonaldehyde kg^{-1} meat) of thigh meat of broiler chickens at 42 days of age fed diets containing levels of hydroethanolic extract of guavira seed (HEGS) and peel (HEGP) and evaluated at different storage times (0, 7, 30, and 60 days)

	Level	Storage days			
	(mg/kg)	0	7	30	60
	100	0.028	0.011	0.011	0.016
	200	0.022	0.019	0.008	0.015
HEGS	300	0.022	0.015	0.008	0.013
	400	0.020	0.014	0.010	0.017
	500	0.019	0.017	0.013	0.075
	Average	0.022	0.015	0.010	0.027
	100	0.026	0.015	0.007	0.017
	200	0.022	0.005	0.011	0.013
HEGP	300	0.022	0.013	0.005	0.015
	400	0.027	0.014	0.006	0.012
	500	0.023	0.012	0.086	0.011
	Average	0.024	0.015	0.023	0.014
SEM		0.006	0.011	0.056	0.044
Type		0.460	0.925	0.620	0.449
Level		0.098	0.647	0.214	0.375
Type vs Level		0.339	0.809	0.297	0.266

Studies on the effects of extracts with total polyphenols, specifically flavonoids, on the quality of broiler meat are inconsistent. According to Kamboh *et al*. (2018), in addition to their antimicrobial activity, the compounds present in the extracts also have antioxidant capacity. Therefore, they can preserve meat quality and extend shelf life by inhibiting lipid oxidation. However, antioxidant activity is dependent on several factors and is related to the chemical structure of the compounds. The hydroxyl groups present in these compounds allow them to be electron donors; thus, they are capable of neutralizing free radicals and oxygen-reactive species (Tufarelli *et al*., 2017). Moreover, the different responses can be attributed to the type or dosage of the compounds, basal diet, diseases, and stressors such as room temperature and feeding conditions (Ma *et al*., 2015; Ri *et al*., 2017). According to Surai (2014), the antioxidant activity of polyphenols/flavonoids is not straightforward and depends on several factors, such as the efficiency of absorption, active concentrations in the target tissues, and metabolic transformation after absorption, which could decrease their antioxidant properties.

Thus, despite the several beneficial effects of polyphenols reported in the literature, the possible unpredictable behaviour of these compounds in the body must be considered, depending on the conditions (antioxidant *vs* pro-oxidant, mutagen *vs* antimutagenic). Thus, information on their absorption, metabolic fate in the body, and mechanism of action are necessary (Surai, 2014). Thus, our findings refute our hypothesis that the inclusion of extracts of guavira seed and peel in broiler diets could improve broiler performance and meat quality. However, it is important to emphasize that comparing the guavira extracts to the additive-free group, the birds that received the HEGP, independently of the inclusion level, showed better performance from 1–42 d without negative effects on meat quality. These results present a new possibility for testing different levels of the extracts analysed in this study.

Conclusion

The inclusion of 100–500 mg/kg of hydroethanolic extract of guavira peel and seed did not improve performance, intestinal morphometry, or carcass traits of broilers. Moreover, no effective antioxidant activity of guavira extracts was observed compared to the use of growth-promoting antibiotics. However, when considering the total breeding period and the non-use of growth-promoting additives in the feed, the inclusion of hydroethanolic extract from guavira peel resulted in better performance of broilers.

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Authors' Contributions

CE and RVN designed the study and were the supervisors. EJSA and CACC were in charge of the extract production. PON worked on the project, laboratory analysis, and received her MSc. JB, NRJ, VDLS, and ECS participated in management and discussion of the results, statistical analysis and writing, and corrected the manuscript.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work; there is no professional or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the content of this paper.

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