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Buriti Oil (*Mauritia flexuosa* L.) as Functional Feed for Broiler Chickens

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Abstract: This experiment evaluated the effects of including buriti oil (BO) in the diet of broilers on growth performance, nutrient digestibility, carcass yield, meat quality, blood parameters, intestinal morphometry, the proliferation of collagen fibers in the skin and collagen concentration in the breast muscle. A total of 180 one-day-old Ross male chicks were distributed in a completely randomized experimental design with three treatments (0%, 1% and 2% BO) and six replications of 10 birds each. Nutrient digestibility was evaluated from 8 to 21 days, while the performance, carcass yield, meat cut yields, abdominal fat, meat quality, blood parameters, intestinal morphometry and skin histology of the birds were evaluated after 21 days. The inclusion of BO significantly affected ($p \leq 0.05$) performance, digestibility, meat color, lymphocyte count, duodenal morphometry and collagen concentration. In addition, it increased the metabolizable energy of the diet and reduced the birds' performance, indicating that the nutraceutical effect of BO improved nutrient use. However, it caused an excess of energy that had to be metabolized by the birds, consequently affecting their performance. BO increased the pigmentation of the breast meat and reduced the lymphocyte count, probably due to its antimicrobial action, consequently decreasing the recruitment of defense cells. An increase in the height of the crypt of the duodenum was observed as the inclusion of BO increased, with the opposite being observed in the jejunum. The inclusion of BO increased the histological proliferation of collagen from minimal (0%) to medium (1%) and moderate (2%), as well as the collagen concentration (CC = 35.933 + 4.677BO; $R^2 = 0.80$). Thus, due to functional/nutraceutical effects, adding BO in the diet of broilers can be a promising alternative to antibiotic growth promoters to improve poultry production.

Keywords: additives; antimicrobial; functional oils; natural antioxidants; oleic acid

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1. Introduction

With the fast growth of the world's population, projected to reach almost 10 billion people by 2050, food safety has become one of the main public health concerns of the 21st century [1]. It is estimated that global demand for food will increase by 56% by 2050, with

a significant growth in demand for animal products such as poultry, pork and beef [2]. To meet this growing need, animal production systems have become increasingly intensive, requiring efficient and sustainable solutions. Historically, antibiotic growth promoters (AGPs) have been widely used to improve animal performance and mitigate the risks of infectious diseases. However, the prolonged and extended use of AGPs has resulted in the development of antimicrobial resistance, which has led to strict regulations and the search for safe and effective alternatives [3]. Several studies have explored alternatives to AGPs, such as probiotics, prebiotics, essential oils and bioactive compounds, as well as antimicrobial peptides of plant origin, due to their potential to improve intestinal health, animal performance and product quality [1–9].

Among these alternatives, vegetable oils rich in bioactive compounds are gaining prominence as functional ingredients, combining nutritional and therapeutic properties. Buriti oil (BO), extracted from the fruit of the *Mauritia flexuosa* L. palm, is rich in natural antioxidants such as tocopherols, carotenoids, provitamin A, phenolic compounds and vitamin C [10–13]. It also has a high concentration of oleic acid, a predominant component of BO, known for its antimicrobial and antioxidant properties [10,14–16]. These characteristics make BO a promising candidate as a functional substitute for AGPs, simultaneously contributing to animal nutrition and health.

Although BO is widely used in the cosmetics industry due to its antioxidant, antimicrobial, anti-inflammatory and regenerative properties [14–19]; however, its potential as an additive in broiler nutrition has been explored mainly in relation to production parameters and intestinal morphometry. Previous studies indicate that diets supplemented with 0.45% BO can offer similar productive performance to diets containing antibiotics, in addition to being economically viable [20]. On the other hand, higher levels (0.75% and 1.5%) had no significant differences in performance parameters but did have an impact on carcass yield, with greater deposition of abdominal, thigh and heart fat, an effect often associated with the use of oils in the diet [21]. We hypothesized that because BO is rich in oleic acid, a nutrient with antimicrobial and antioxidant action, as well as β-carotene, tocopherols and phenolic compounds, known for their potent antioxidant properties, it has the potential to reduce or replace the use of antibiotics in broiler diets. This hypothesis is based on the combination of the oil's bioactive properties, which can contribute to improving the intestinal health and productive performance of the animals. The practical relevance of this approach lies in the possibility of significantly reducing the use of antibiotics in animal production, meeting the demands for more sustainable and safer systems, while minimizing the risks associated with antimicrobial resistance. Thus, the aim of this study was to explore the effects of buriti oil in the diet of broilers from 1 to 21 days of age, assessing its impact on growth performance, nutrient digestibility, carcass and cut yield, the physical quality of meat, blood parameters, intestinal morphometry, the proliferation of collagen fibers in the skin, collagen concentration in the breast muscle and economic viability.

2. Materials and Methods

This research was carried out in the Poultry Sector of the Professor Cinobelina Elvas Campus of Federal University of Piauí (UFPI) in Bom Jesus, Piauí, Brazil, located at a latitude of 09°04′28″ S and longitude of 44°21′31″ W and an altitude of 277 m [22]. This study followed the ethical research protocols according to Resolution No. 879/08 of the National Council for the Control of Animal Experimentation (CONCEA) and No. 007/13, approved by the Ethics and Animal Experimentation Committee of UFPI (CEEA-UFPI) on 19 July 2013 (Supplementary Figure S1).

2.1. Experiment I

Determining the Nutritional Composition and Metabolizable Energy of BO

A previous study was carried out to evaluate the chemical composition of BO, which was obtained from farmers in Formosa village, in Bom Jesus–Piauí. BO was analyzed for dry matter, crude protein, gross energy, ether extract and fatty acid contents. To determine the metabolizable energy, a metabolism trial was conducted in metal batteries (1 × 1 × 0.5 m). Forty-eight male broiler chickens of the Cobb line, of 11 to 22 days of age, were distributed according to their average weight. The birds were housed in metabolism cages in groups (experimental units) equipped with trough feeders and nipple drinkers.

The experimental diets consisted of a reference diet (RD) based on corn and soybean meal to meet the nutritional requirements of the birds and a test diet containing 90% RD and 10% BO to obtain the metabolizable energy of BO. Six replicates with four birds each were defined for each diet.

The reference diet was formulated to meet the nutritional requirements of the birds for the phase, considering the requirements and chemical composition of the ingredients, as described by Rostagno et al. [23]. To determine the metabolizable energy of BO, the method of total excreta collection was used according to Sakomura and Rostagno [24]. The adaptation period lasted for five days, and the excreta were collected twice a day. After collection, the excreta were packed in plastic bags and identified by replication, and at the end of the experimental period, the feed intake was determined.

At the end of the experimental period, the excreta were thawed, homogenized and dried in a forced-air circulation oven at 55 °C for 72 h and then were ground and analyzed with the provided diets. The nitrogen, dry matter and gross energy contents were determined by AOAC [25]. With the obtained results, the values of the apparent metabolizable energy (AME) of BO were determined using the equations proposed by Sakomura and Rostagno [24].

2.2. Experiment II

2.2.1. Birds and Experimental Design

For the experiment, 180 male Ross broilers were obtained from the commercial hatchery CIALNE (CNPJ:07.220.874/0001-01), which is duly registered with the Ministry of Agriculture. The chicks were randomly allocated to one of 18 pens, measuring 1 × 2 m, in a stocking rate of 10 birds/pen, allowing for 0.200 m^2/bird. All pens were bedded with 8 cm of used rice hulls. The chicks were randomly allocated to 3 dietary treatments, each with 6 replications of 10 birds. The treatments consisted of three levels of BO (0, 1 and 2%) added in all phases. All birds had ad libitum access to both feed and water throughout the study period. All birds were fed a pre-starter (days 1–7, crumble) and starter (days 8–21, crumble) diet.

2.2.2. Diet

Feeds were formulated following the broiler genetic and feed nutritional composition of the Brazilian table of poultry and swine (Table 1), except for the nutritional BO composition (AME), which was obtained in the first experiment described previously. The nutritional adjustment for the metabolizable energy of the diets was made with the addition of BO (1 and 2%, replacing soybean oil), as well as adjustments with inert material (purified sand) in the diets according to Table 1.

Table 1. The ingredient and nutrient composition of the control diet, as fed.

	Reference Diet *	Pre-Starter	Starter
Corn (7.8% CP)	65.434	52.491	56.321
Soybean meal (48% CP)	30.023	38.232	35.511
Soybean oil	0.659	3.429	3.528
Dicalcium phosphate	1.474	1.857	1.641
Limestone	0.994	0.986	0.903
Salt	0.481	0.534	0.517
Vitamin and mineral premix [1]	0.400	0.500	0.400
L-Lysine HCL	0.263	0.229	0.248
DL-Methionine	0.272	0.356	0.3396
L-Threonine	-	0.086	0.085
L-Arginine	-	-	0.007
Buriti oil	-	0.000	0.000
Inert	-	1.300	0.500
Metabolizable Energy (Mcal/kg)	3002	2975	3050
Linoleic acid	1.835	3.101	3.205
Ca	0.860	0.971	0.878
Available P	0.384	0.463	0.419
K	0.823	0.975	0.929
Cl	0.341	0.385	0.377
Na	0.210	0.225	0.225
Crude Protein (%)	20.00	23.00	22.00
Digestible Lysine	1.141	1.307	1.256
Digestible Methionine + Cysteine	0.822	0.967	0.929
Digestible Methionine	0.539	0.657	0.630
Digestible Arginine	1.117	1.413	1.344

[1] Composition per kilogram of feed: vit A, 3,000,000 IU; vit E, 9500 IU; vit B1, 588 mg; vit B2, 1160 mg; vit B6, 792 mg; vit B12, 4150 µg ; vit K3, 520 mg; vit D3, 800 IU; vit B5, 3230 mg; vit B3, 9800 mg; vit B9, 200 mg; biotin, 20 mg; zinc, 13 g; iron, 13 g; Mn, 15 g; Cu, 3120 mg; I, 254 mg; Co, 48 mg; Se, 88 mg; ethoxyquin, 52 mg; B.H.A, 40 mg; Q.S.P, 1000 mg. The inclusion of 1 and 2% buriti oil in the treatments was intended to replace soybean oil (SO) and inert material (IN) by 2.661 and 1.887% (SO) and 1.068 and 0.842% (IN) for the pre-starter phase and 2.748 and 1.968% (SO) and 0.280 and 0.060% (IN) for the starter phase, respectively, in relation to the basal diet showed in the table (Supplementary Tables S1 and S2). * The metabolizable energy of the buriti trial (first study).

2.2.3. Performance and Carcass Yield

The daily feed intake (FI) for each pen was recorded. The average body weight gain (BWG) and FI were adjusted for mortality until d21 and were used to calculate the feed conversion rate (FCR). At 21 days of age, three birds weighing close to the average weight of the box were slaughtered after being deprived of feed for eight hours for the evaluation of the carcass and meat cut yield. After the feed-deprivation period, the birds were weighed individually to determine their live weight and then were slaughtered, bled and plucked. The carcass yield was defined as the ratio between the eviscerated carcass weight and the weight of the feed-deprived bird. To define the eviscerated carcass weight, the weight of the feed-deprived slaughtered bird without feathers, organs, a head, a neck and feet was considered. Soon after, the breast, drumstick, thigh and wings were cut and weighed. The yield of the meat cuts was determined in relation to the weight of the eviscerated carcass.

2.2.4. Nutrient Digestibility

A total of 90 male broilers with similar body weights were transferred to metabolic cages (1 × 1 × 0.5 m) and randomly divided into 18 groups (six replications per treatment). The treatments were the same used in the performance trial. The birds were acclimated to experimental diets (free access) for 4 days (days 9 to 13). Excreta were collected for four days by the total collection method. The feed was weighed before and after collection periods to determine feed intake and marked with 1% ferric oxide to determine the beginning

and end of the excreta collections. Samples were collected twice daily in trays covered with plastic material. After collection, the excreta were weighed, packed, identified and frozen at 18 °C until the end of the collection period, when they were thawed, weighed and homogenized; then, a sample was removed, weighed and dried in a forced-ventilation oven at 65 °C for 72 h. The oven-dried samples were weighed, ground and stored for laboratory analysis. The contents of dry matter (DM), mineral matter (MM), crude protein (CP) and gross energy (GE) were determined in all samples following the methods described by AOAC [25]. With the laboratory results, the apparent metabolizable energy (AME), dry matter digestibility coefficient (DMDC), crude protein digestibility coefficient (CPDC) and fat digestibility coefficient (FDC) were determined according to Sakomura and Rostagno [24] by the difference between the gross energy (GE) or nutrient intake (NI) and GE or NI output from the excreta, as described by Matterson et al. [26].

2.2.5. Meat's Physical Analyses

After slaughter and cooling, 12 carcasses per treatment were randomly collected and evaluated. At 24 h postmortem, the color of the breast (*pectoralis major*), cook loss and shear force values of the cooked meat were recorded. The color [lightness (L*), redness (a*) and yellowness (b*)] was evaluated before and after cooking. Measurements were taken in three spots of *Pectoralis major* muscle, considering the average of these values.

The filets from each whole breast were separated and used for the determination of cook loss, which was measured by cooking the samples in a convection oven on aluminum trays at 180 °C until 80 °C was reached in the core sample. The filets were then allowed to equilibrate with the room temperature and then were reweighed, and the cook loss was determined as the percentage of weight loss.

The shear force (SF) was determined with the cooked samples, after they were cut into pieces measuring 2 × 1 × 1 cm, with the longest length in the longitudinal direction of the muscular fibers. Then, 2 pieces of each sample (12 per treatment) were subjected to the SF test using the texturometer TexturePro CT (Brookfield, Middleboro, MA, USA) and tenderness meter, with a power cell of 10 kg, connected to a computer equipped with the Software Texture Pro CT (Brookfield, Middleboro, MA, USA). The samples were sheared in the transverse direction of the muscular fibers using a Warner Bratzler-type cutting blade at a cutting speed of 60 mm/min. The average SF value in each sample was expressed as kilogram-force (kgf).

The color measurement of the samples was assessed with a colorimeter (Minolta CR300, Tokyo, Japan) operating with the CIE system (L*, a* and b*), with L* being the luminosity, ranging from 0 (black) to 100 (white); a* being the red color intensity, ranging from green (−60) to red (+60); and b* being the yellow color intensity, ranging from blue (−60) to yellow (+60). The calibration of the machine was performed with a white ceramic plate, using the illuminant D65. Measurements were taken in the three spots of *Pectoralis major* muscle, considering the average of these values.

2.2.6. Blood Parameters

On the 21st day of age, 1.5 mL blood samples were collected from the ulnar vein of one bird per experimental unit, totaling six birds per treatment. The anticoagulant ethylenediaminetetraacetic acid (EDTA) was used in the proportion of 0.1 mL to 1.0 mL of blood for the collection, as described by Thrall et al. [27]. The leukocytes count was performed using the blood sample and 0.1% Toluidine Blue in a 1:200 dilution, with the count performed (N/mL) in a Neubauer chamber. The reading for the differential leukocyte count and evaluation of cell morphology was performed using an optical microscope with an immersion objective. The percentage of each type was observed.

2.2.7. Intestinal Morphometry

At 21 days of age, intestinal morphometric analysis was conducted according to Sousa et al. [28]. Two birds per experimental unit were euthanized for the collection of 2 cm-long segments of the duodenum and jejunum. These segments were carefully collected, washed in distilled water and fixed in a 10% neutral formalin buffer (37–40% formalin, distilled water, monobasic sodium phosphate and dibasic sodium phosphate) for 24 h. Following the fixation period, the segments were subjected to a series of procedures, including dehydration using ascending concentrations of alcohol (70%, 80%, 90% and 100% I, 100% II and 100% III), clearing with xylene (I and II), being embedded in histological paraffin and placement into paraffin blocks, as described by Prophet et al. [29].

Subsequently, the blocks were sectioned using a rotary microtome (LUPETEC™MRP09) to obtain histological sections with a thickness of 4 µm. Each animal yielded one slide, and on each slide, up to three semi-serial sections were placed, with the exclusion of ten sections between one section and the next. These sections were then stained with hematoxylin eosin and mounted between glass slides and coverslips using 500™ colorless glass varnish, as described by Paiva et al. [30].

Histomorphometric analysis, including measurements of the perimeter, height and width of the villi, as well as the height and width of the crypts, and internal and external muscle measurements was conducted using a trinocular optical microscope (Leica DM250) equipped with a Leica digital color camera (DFC7000T), with a 1920 × 1440 resolution (2.8 Pixel) and 4.54 µm × 4.54 µm pixel size, for a photographic record of the images. Measurements were taken using the Leica LAS Interactive Measurement Module. In each intestinal region, 10 villi and 10 crypts per animal were selected and measured for length in a straight line (µm). The villus height measurements were taken from the upper base of the crypt to the apex of the villus, while the crypts were measured from the lower base to the upper base of the crypt, as described by Sousa et al. [28].

2.2.8. Collagen Concentrate

Samples of *Pectoralis major* muscle were freeze-dried and two grams were used for the analysis of hydroxyproline concentration, using the spectrophotometric methodology described by Della Torre et al. [31]. The contents of hydroxyproline were multiplied by the medium factor to estimate the total collagen concentration in the muscle, as described by Macovescu et al. [32].

2.2.9. Histological Analyses

After 21 days, one bird per experimental unit was selected for the removal of a 2.0 cm long fragment of the breast muscle and skin covering the breast muscles for histological analysis to detect the presence of collagen using the Masson Trichrome staining method (Easypath, code n EP-11-20013).

After collection, the fragments were fixed in a 10% formaldehyde solution for 24 h and then transferred to 70% alcohol. The samples underwent standard histological processing (as previously described); the sections were stained with Masson's Trichrome stain, and the mounting was carried out with colorless stained glass varnish 500 (Acrilex®) [30]. The analyses of the histological sections were performed using a microscope to quantify the presence of collagen in the muscle and skin of the breast of the birds.

Tissue slides that were stained in the present study were examined using a light microscope at 40×, using ToupView software version 1.0 to measure the intensity of collagen fibers staining (blue color). Briefly, five similar fields in all slides from the same organ were subjected to an image color evaluation process, in which a bright blue color was considered a positive result. The evaluation was subjective, considering the degree of blue

staining intensities minimal, medium or moderate. The evaluation was performed in a blind manner on coded histological blades that were examined by a qualified histopathologist.

2.2.10. Economic Analysis

The economic profitability assessment considered the average cost of feed, cost per bird, profit per bird and profitability index, as described by Pasquali et al. [33]. The average cost (BRL) per kilogram of feed was calculated considering the average cost of feed in all phases, according to the prices of ingredients, based on quotations made in 2019 (the year the trial was executed). The prices per kilogram of ingredients used were as follows: corn, BRL 0.80; soybean meal, BRL 1.65; soybean oil, BRL 3.00; limestone, BRL 0.75; dicalcium phosphate, BRL 3.86; common salt, BRL 1.00; DL-methionine, BRL 27.00; L-lysine, BRL 18.00; L-threonine, BRL 15.85; L-arginine, BRL 90.00; vitamin–mineral premix, BRL 25.00; and buriti oil, BRL 40.00. The cost per bird was calculated considering the feed cost (feed intake x feed cost) + the cost of a one-day-old chick (BRL 2.20 per bird) + estimated variable costs (disinfection, vaccines, medicines, electricity, labor and charges, considering BRL 0.30 according to the FAESP system). The profit per bird was estimated from the difference between the gross treatment revenue (the total number of live broilers, in kg, per treatment x the average price of kg live broilers, BRL 5.60 kg^{-1}) and cost per bird. The profitability index was determined by the ratio of the total treatment profit (total revenue–total cost) to the total treatment revenue. The data were calculated by experimental units for statistical analysis.

2.2.11. Statistical Analyses

The data of all variables were screened for outliers and assessed for normality (Cramer–von Mises test) and variance homogeneity (Levene test). After confirming the assumptions, the data underwent univariate analysis of variance using PROC GLM and were compared by the SNK test at a 5% probability. The estimates of the use of BO were established by polynomial regression for the significant variables. The SAS® OnDemand for Academics was used for the analyses (https://www.sas.com/pt_br/software/on-demand-for-academics.html, accessed 8 September 2023)).

3. Results

3.1. Nutritional Composition and Metabolizable Energy of BO

The results of the chemical composition and AME corrected in the dry matter (AME/DM) and natural matter (AME/NM) of BO are described in Table 2, with an average AME value of 6854 + 521 kcal/kg and high concentration of oleic acid (70.62%) and palmitic acid (18.57%).

Table 2. Chemical and energetic composition of buriti oil.

Gross energy (kcal/kg of NM)	8375
Metabolizable energy (kcal/kg of NM)	6854 ± 521
Apparent metabolizable energy (kcal/kg DM)	6924 ± 516
Dry matter	99.88
Crude protein	0.75
Ether extract	99.3
Fatty acids (%)	

Table 2. *Cont.*

C12:0	-
C14:0	1.14
C15:0	-
C16:0	18.57
C16:1 cis9	0.54
C17:0	-
C18:0	2.23
C18:1 cis9	76.2
C18:1 cis11–13	1.53
C18:2 n–6	1.88
C18:3 n–3	1.01
C20:0	0.80
C22:0	-
C24:0	-
Others	2.48

3.2. Performance

The broilers' performance from 1 to 21 days was significantly impacted by the levels of BO ($p \leq 0.05$) (Table 3). The average values of body weight, body weight gain and feed conversion were statistically different. The control treatment showed higher a BW and BWG and better feed conversion.

Table 3. Performance of broilers (1 to 21 days) fed diets with inclusion of buriti oil (BO).

BO%	Variables			
	BW, g/bird	**BWG, g/bird**	**FI, g/bird**	**FC**
0	1070.0 [a]	1025.3 [a]	1362.8	1.32 [a]
1	991.6 [b]	947.3 [b]	1352.6	1.43 [b]
2	1021.6 [b]	977.6 [b]	1364.5	1.39 [b]
Probability	0.0223	0.0217	0.5895	0.0483
Regression	ns	ns	ns	ns
SEM	12.4	12.31	4.86	0.02
CV %	4.23	4.38	1.55	4.76

BW, body weight; BWG, body weight gain; FI, feed intake; FC, feed conversion; ns, not significant. Means with differing superscripts indicate statistically significant differences within treatments across weeks at $p < 0.05$ ($n = 18$).

3.3. Nutrient Digestibility

Table 4 shows data for the apparent metabolizable energy (AME) and digestibility coefficient of nutrients. There was a significant effect of BO inclusion on the AME and GEDC. On the other hand, there was no significant effect of BO on the digestibility coefficient (DC) of nutrients (dry matter, protein, ether extract and mineral matter retention).

There was a significant quadratic effect ($p \leq 0.05$) on the apparent metabolizable energy corrected for nitrogen balance ($AMEn = 3043 + 255BO - 81BO^2$; $R^2 = 1$), with the highest AME value recorded when the birds ingested 1.57% BO. There was a linear effect on the gross energy digestibility coefficient ($GEDC = 84.625 + 0.625BO$; $R^2 = 0.861$), showing that the inclusion of BO in the diet increased the GEDC (Table 3).

Table 4. Apparent metabolizable energy (AME) and digestibility coefficient (DC) of nutrients of broilers (8 to 21 days) fed diets with inclusion of BO.

BO%	Variables					
	AMEn	GEDC	DMDC	CPDC	EEDC	MMR
	(Kcal kg^{-1})			(%)		
0	3043	84.48	83.15	83.21	89.0	66.4
1	3216	85.54	85.54	83.42	88.7	66.3
2	3227	85.73	84.03	84.20	90.7	67.8
Probability	<0.0001	0.1190	0.3062	0.3958	0.6625	0.2979
Regression	Q	L	ns	ns	ns	ns
SEM	22.7	0. 26	15.1	0.30	0.90	0.43
CV (%)	1.40	1.14	1.14	1.53	4.07	2.44

AMEn, AME corrected for nitrogen balance; GEDC, gross energy digestibility coefficient; DMDC, dry matter digestibility coefficient; CPDC, crude protein digestibility coefficient; EEDC, ether extract digestibility coefficient; MMR, mineral matter retention; ns, not significant; Q = quadratic; L = linear (n = 18).

3.4. Carcass and Meat Cuts Yield

There was no significant effect ($p > 0.05$) of the inclusion of BO in the diets on the variables of the carcass and meat cut yield of the broiler chickens at 21 days of age (Table 5).

Table 5. Carcass and meat cut yield of broilers (21 days) fed diets with inclusion of BO.

BO (%)	Variables (%)								
	CY	BY	TY	DY	WY	RH	RL	RAF	RG
0	73.9	33.7	13.5	14.8	11.9	0.82	2.98	1.32	3.47
1	73.2	33.9	13.6	14.9	11.8	0.76	3.20	1.34	3.68
2	74.8	33.9	13.4	14.9	11.8	0.79	3.00	1.39	3.46
Probability	0.2307	0.8806	0.7107	0.8845	0.8284	0.5193	0.2716	0.7264	0.2380
Regression	ns	ns	ns	ns	ns	ns	ns	ns	ns
SEM	0.37	0.28	0.08	0.11	0.08	0.02	0.06	0.04	0.06
CV %	2.08	3.69	2.73	3.34	3.06	9.95	8.09	12.31	6.74

CY, carcass yield; BY, breast yield; TY, thigh yield; DY, drumstick yield; WY, wing yield; RH, relative heart weight; RL, relative liver weight; RAF, relative abdominal fat weight; RG, relative gizzard weight; CV, coefficient of variation; ns, not significant (n = 54).

3.5. Meat's Physical Analyses

The levels of inclusion of BO had no significant effect on the variable shear force, cook loss and brightness of the raw and cooked breast. However, there was a significant effect on the red and yellow color parameters of the raw and cooked breast of 21-day-old broilers (Table 6).

An increasing linear effect was observed on the coloration of the red region, in both raw (a*RB = 1.1219 + 0.6156BO; R^2 = 0.95) and cooked breast (a*CB = 1.909 + 0.527BO; R^2 = 0.67), indicating that the increased inclusion of BO increased the red coloration of the meat. There was a quadratic effect on the coloration of the yellow region of the raw breast (b*RB = 12.75 + 3.502BO − 1.3717BO^2; R^2 = 0.99) and a linear effect on the cooked breast (b*CB = 13.616 + 0.812BO; R^2 = 0.94), with the the maximum yellow coloration of the raw breast (RB) recorded with the inclusion of 1.28% BO; however, for the cooked breast (CB), the yellow coloration increased with the inclusion of 2% BO.

Table 6. Shear force (SF), cook loss (CL) and color (L*, a* and b*) values of raw and cooked breast filets of broilers fed diets with inclusion of BO.

BO%	SF	CL	Breast Filet Color					
	Kgf/cm	%	Raw			Cooked		
			L*	a*	b*	L*	a*	b*
0	2.271	27.88	55.0	1.23 ^{a}	12.75 ^{a}	56.44	2.15 ^{b}	13.50
1	2.399	29.46	55.2	1.58 ^{a}	14.88 ^{b}	56.01	2.05 ^{b}	14.66
2	2.523	28.17	53.6	2.42 ^{b}	14.26 ^{b}	55.03	3.17 ^{a}	15.11
Probability	0.7581	0.8015	0.4337	<0.0001	0.0202	0.5844	0.0065	0.0672
Regression	ns	ns	ns	L	Q	ns	L	L
SEM	129.5	0.98	0.57	0.13	0.31	0.55	0.17	0.29
CV (%)	34.37	16.10	5.65	23.73	9.57	5.52	30.63	10.34

Means followed by different letters in the same column significantly differ from each other according to the SNK test at a 5% probability. SF, shear force; CL, cook loss; L*, brightness; a*, red color; b*, yellow color; ANOVA, analysis of variance; ns, not significant; Q = quadratic; L = linear; CV, coefficient of variation (n = 36).

3.6. Blood Parameters

The mean blood parameter values are presented in Table 6. There was a significant linear decreasing effect ($p \leq 0.05$) on lymphocytes, indicating that the increased inclusion of BO reduced the number of lymphocytes in the blood (LYMPH = 11,916.9 − 2364.2BO; R^2 = 0.92). The inclusion of BO did not influence ($p > 0.05$) the other blood parameters of the broilers at 21 days of age (Table 7).

Table 7. Blood parameters of broilers fed diets with inclusion of BO.

BO%	Variables								
	PCV	TPP	RBC	LEUC	HET	LYMPH	EOS	MON	HL
	(%)	(g/dL)	($\times 10^6$/µL)	(n/µL)					
0	28.00	3.36	1.48	28,333	13,825	12,295	740	1916	1.17
1	27.83	3.23	1.51	23,500	13,243	8796	797	928	1.59
2	28.83	3.33	1.55	21,333	11,313	7566	850	1745	1.59
Probability	0.6374	0.7503	0.9236	0.3260	0.6297	0.1098	0.9800	0.2887	0.3370
Regression	ns	ns	ns	ns	ns	L	ns	ns	ns
SEM	0.43	0.07	0.07	1905	1064	961	213	268	0.13
CV (%)	6.82	9.48	20.86	32.73	36.44	39.24	108.7	71.82	37.28

PCV, packed cell volume; TPP, total plasma protein; RBC, erythrocyte concentration, LEUC, leucocytes concentration; HET, heterophil; LYMPH, lymphocytes; EOS, eosinophils; MON, monocytes; HT, heterophil/lymphocyte ratio; ns, not significant; L = linear; CV, coefficient of variation (n = 18).

3.7. Intestinal Morphometry

Morphometric analysis of the duodenal morphology of the broilers revealed differences among the control and experimental groups with inclusion of BO regarding the chickens' tissue structure on the 21st day of the feeding trial, as shown in Table 8. The duodenal crypt depth of broilers increased with the inclusion of BO (CD = 169.04 + 11.334BO), and the opposite occurred for the villi–crypt ratio (VCR = 10.02 − 0.607BO). The point of the minimal villi width was observed with 1.04% BO (VW = 192.76 − 49.18BO + 23.54BO^2). In the jejunum, an effect was observed only on crypt depth (CD = 187.17 − 5.955BO). The broilers fed diets with BO had reduced crypt depth (CD).

Table 8. Morphometric (μm) parameters of intestine (duodenum and jejunum) of broilers fed diets with inclusion of BO.

BO%	VH	VW	CD	CW	VCR	MW
	Duodenum (μm)					
0	1688	193	167	58	10.0	267
1	1722	167	184	55	9.4	265
2	1653	189	190	60	8.8	267
Probability	0.8678	0.0533	0.0632	0.1005	0.1717	0.9737
Regression	ns	Q	L	ns	L	ns
SEM	47.03	4.65	4.26	1.13	0.26	4.20
CV (%)	11.51	7.98	8.24	7.01	9.88	7.12
	Jejunum (μm)					
0	1817	177	188	58	9.72	272
1	1815	172	180	52	10.1	283
2	1796	183	176	56	10.4	279
Probability	0.9676	0.3797	0.1232	0.3903	0.5867	0.8684
Regression	ns	ns	L	ns	ns	ns
SEM	32.30	3.32	2.44	1.93	0.23	7.41
CV (%)	7.24	7.23	4.93	14.39	8.95	11.32

VH, villi height; VW, villi width; CD, crypt depth; CW, crypt width; VCR, villi–crypt ratio, MW, muscular width; ns, not significant; Q, quadratic; L = linear; CV, coefficient of variation (n = 18).

3.8. Collagen Concentration and Histological Analyses

There was a significant effect of the treatments on the concentration of total collagen (TC) in the muscle ($p \leq 0.05$), which increased as the inclusion of BO increased (TC = 35.93 + 4.677BO), showing average values of 34.96, 43.61 and 44.13 mg/g of DM, respectively, for the inclusion of 0, 1 and 2% BO in the diet.

It was possible to observe the proliferation of collagen in the slides with samples from the skin of the breast of the birds (Figure 1) by the intensity of blue coloration (Masson's Trichrome stain). An analysis of the skin samples from chickens fed diets supplemented with BO revealed an increase in the proportion of connective tissue, which was proportional to the level of BO inclusion in the diet. Collagen proliferation exhibited three degrees of intensity: minimal, medium and moderate. In animals fed a control diet without BO, the intensity was minimal. In animals fed a control diet supplemented with 1% BO, the intensity was medium, while in animals fed a control diet supplemented with 2% BO, the intensity was moderate. These results are illustrated in Figure 1.

3.9. Economic Analysis

The economic viability data, as analyzed by the methodology from Pasquali et al. [33], can be found in Table 9. Due to the high cost of BO (BRL40.00/kg), the cost per bird increased correspondingly with the level of oil included in the diet. Notably, the gross revenue from birds receiving the inclusion of 2% BO closely mirrored that of the control group without any oil, while the revenue for the 1% treatment was lower. Furthermore, the profit and the profitability index were more favorable for the treatments without BO, demonstrating a decline as the oil inclusion increased.

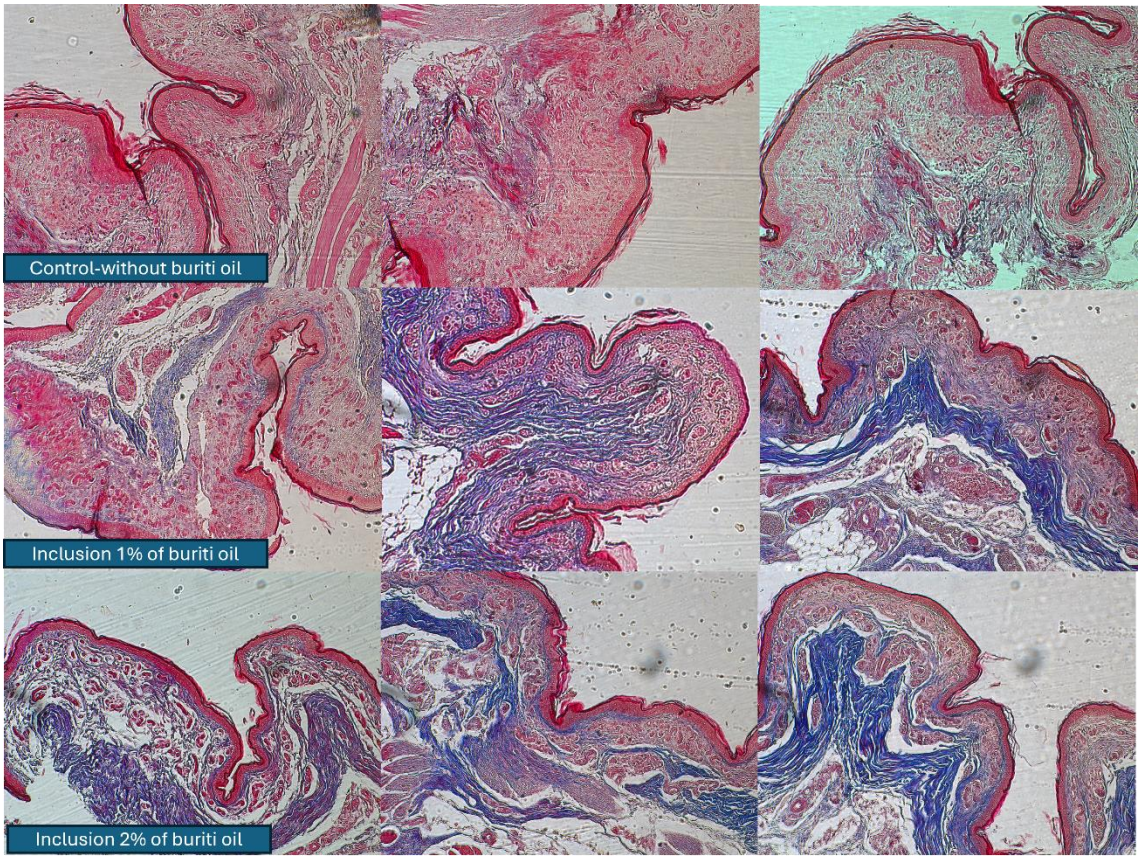

Figure 1. Histological section of skin of broilers according to treatments received.

Table 9. Economic analysis of the use of buriti oil in broiler diets from 1 to 21 days.

BO%	Variables			
	Cost (BRL/bird)	Gross Revenue (BRL/bird)	Profit (BRL/bird)	Profit Index (%)
0	4.43 ^{c}	5.99 ^{a}	1.56 ^{a}	26.11 ^{a}
1	4.91 ^{b}	5.55 ^{b}	0.63 ^{b}	11.18 ^{b}
2	5.56 ^{a}	5.79 ^{ab}	0.32 ^{c}	5.53 ^{c}
Probability	<0.0001	0.0181	<0.0001	<0.0001
SEM	0.104	0.069	0.143	2.33
CV (%)	0.70	3.99	27.43	25.54

Means followed by different letters in the same column significantly differ from each other according to the SNK test, at a 5% probability.

4. Discussion

The AME content obtained for BO in this study was comparable to that reported for other oils commonly used in animal nutrition, such as degummed soybean oil (8403 kcal/kg), corn oil (8755 kcal/kg) and canola oil (8889 kcal/kg) [34].

BO presented a substantial amount of AME (81.84%), since the gross energy of BO was 8375 kcal/kg and the AME obtained was 6854 kcal/kg of NM and 6924 kcal/kg of DM. Compared to the energy value present in the soybean oil, the most used in poultry feed, BO had 958 kcal less of gross energy, with a difference of approximately 10%. This suggests that it can be associated with soybean oil in broiler chicken diets, as it is a natural product and has a better monounsaturated fatty acid profile than soybean oil. According to Almeida et al. [35], the purpose of using one or more sources of combined fat in poultry

diets is both to increase the energy density and the essential fatty acid content in the diets, in addition to improving the growth of the birds and the use of feed energy.

According to Murakami et al. [36], increasing the proportion of polyunsaturated fatty acids of the omega-3 series (Ω3) in the diet can have a positive effect on the nutritional quality of chicken meat and promote a decrease in total lipid levels and cholesterol, an aspect increasingly demanded by consumers. Finally, it is worth noting that Lara el al. [37] reported that it is possible to alter the lipid profile of broiler meat by the means of the type of lipid added to the feed of chickens and consequently improve the quality of the carcass.

The function of oleic acid and polyunsaturated fatty acids is directly related to their immunological, anti-inflammatory potential, as reported by Perini et al. [38], and their indirectly antioxidant potential. BO presents more than 70% oleic acid.

The birds that received the control treatment (without oil) showed better performance parameters compared to those that received diets with BO. This was unexpected, as it had been hypothesized that the functional compounds in BO would improve the birds' performance. However, the metabolizable energy results (Table 3) indicate that the inclusion of BO increased the apparent metabolizable energy (AME) of the diets. Considering that the diets were formulated to meet the birds' nutritional needs, it is plausible that the additional energy provided by the extra-caloric effect of the oil (in the function of functional compounds such as fatty acids and vitamins) exceeded the birds' energy requirements. Consequently, the birds may have redirected some of this excess energy from weight gain processes to metabolize the surplus, which may have negatively impacted their overall performance.

When considering the data obtained in this study and substituting them into the equations of Lara et al. [37], it was found that the animals that ingested BO, in fact, had an average caloric intake above their nutritional requirement, consequently impacting performance. Caravalho et al. [20] studied lower levels (up to 0.8%) of BO and observed a positive response in the weight gain of broilers from 1 to 42 days of age; however, Santis et al. [21] did not observe responses on the performance of birds up to 28 days of age with levels of up to 1.5%. The oil contains substances with anti-inflammatory and antioxidant effects that improve the immune response and contribute to better nutrient utilization, as described by Manhezi et al. [39], favoring better feed conversion.

In this study, the addition of BO had no effect on the yield of carcasses and meat cuts. Previous research has reported mixed results; a study by Santis et al. [21] observed that adding up to 1.5% BO reduced carcass yield, whereas Carvalho et al. [20] found no differences in carcass and meat cut yields. It is worth noting that the authors considered the maintenance of the energy level of the rations and, therefore, the variation that occurred was possibly due to the fatty acid profile due to the adjustment between the amounts of soybean oil and BO, increasing the concentrations of oleic acids with the increase in BO. Soybean meal rich in oleic acid was evaluated and found to promote a reduction in animal performance and carcass yield, but it increased the concentration of oleic acid and reduced the concentration of saturated fatty acids in the meat [40].

The results observed in this study for the shear force variable are different from the results observed by Martins [41], who reported that the inclusion of BO (*Maurittia flexuosa* L.) interfered with the texture of the breast meat and that the shear force of the breast meat from chickens fed diets with the inclusion of 2% BO for 3 weeks (21 to 42 days) was 84.7% (1.05 × 1.94 kgf) higher than that found in the meat from chickens fed the diet without the inclusion of BO. In the present study, although not significant, an increase of 5.6% and 11.1% was observed in the shear force of the breast meat from chickens fed 1% and 2% BO, respectively. These results may be related to the age of the animals, since in the present

study, the birds were evaluated at 21 days of age, while Martins [41] evaluated birds at 42 days of age.

According to Wattanachant et al. [42], the tenderness of cooked meat decreases with increasing collagen and cross-linking content (which is associated with meat from older animals and specific muscle types). However, the results found in the present study for shear force were within the normal range for 21-day-old birds, but the concertation of collagen and histology of skin (Figure 1) indicated that there was an increasing tendency in the shear force to be higher with the inclusion of BO in the diets.

For the lightness variable (L*), results similar to those described by Martins [41] were observed. For lightness, the authors observed values of 53.95 to 55.59 for breast meat and 52.63 to 54.58 for thigh meat. Both the results observed by Martins [41] and in the present study were higher than 52, which, according to Schneider [43], are outside the normal range, since L* values are considered normal between 45 and 52. The results of this research for the variables a* and b* show that BO influences the pigmentation of the breast meat of broiler chickens. These results are different from those described by Martins [41], since this author did not observe significant differences ($p < 0.05$) regarding the inclusion of BO in the diet of broiler chickens. According to Pérez-Vendrell et al. [44] and Costa et al. [45], the significant result in the variables a* and b* is explained by the presence of carotenoids that can influence the color of chicken meat and thus increase the intensity of the yellow color represented by the variable b*. Albuquerque et al. [14] observed that BO has a carotenoid concentration above 1700 ppm.

Regarding the blood parameters, similar results were found by Carvalho [46] when evaluating the inclusion of BO levels and its effect on the leukogram, observing an effect only for the variable eosinophils (EOS). The inclusion of 2% BO showed a significant effect.

According to Cardoso et al. [47], there are some factors that influence the leukogram; one of these factors is the experimental conditions offered to the birds during the experimental period. Thus, it is possible that, even without subjecting the birds to a health challenge, antibiotics were not added to the diets; therefore, it is possible that the animals were undergoing some challenge and BO, with its antimicrobial (Batista et al. [48]) and antioxidant (Delbem [49]) effects, possibly presented a lower demand for the animals' immune response, being significant for lymphocytes, but in an absolute way (non-significant), a reduction in leukocytes was also observed. According to Pighinelli [19], BO can improve immunity, as it has a therapeutic effect due to its composition being rich in carotenoids, fatty acids, tocopherol and vitamin E. According to Falcão et al. [50], the possible action of this oil on the immune system of birds may be related to its antimicrobial and anti-inflammatory effects.

Villi height and crypt depth are considered indicators of good intestinal development, reflecting the capacity for the absorption of nutrients, and under normal conditions, the best development occurs when there is a greater villus–crypt ratio [51]. In this perspective, a healthy villus–crypt ratio is one in which the villi are larger and the crypts are shallower, resulting in decreased cell turnover and indicating greater intestinal health.

It can be said that when an injury occurs in the intestinal tissue, BO acts in repairing tissue. According to Santos et al. [52], BO stimulates the cell proliferation process due to its healing function. Therefore, carotenoids, in turn, act to sequester free radicals that are released in cases of injury [14]. BO also acts in the immunological process, as it has polyunsaturated fatty acids that have anti-inflammatory potential and can act by releasing defense cells in situations of aggression to the intestinal mucosa [38].

The results concerning collagen concentration were consistent with our expectations. This research primarily aimed to evaluate the influence of oil on meat quality and its relationship with collagen content, as consequence of previous studies involving other species, such as lambs, have reported improved meat tenderness associated with the inclusion of

BO [53,54]. Conversely, in our preliminary study (Martins [41], data unpublished), we observed an increase in shear force linked to a prolonged duration of dietary intake with 2% BO included. One plausible explanation for this observation in broilers may be the observed increase in collagen fibers within the muscle. BO is recognized for its beneficial healing properties, attributable to its high content of oleic acid and vitamins A and E [55,56]. Consequently, it may act as an endogenous collagen stimulator, leading to an enhancement in collagen fiber density within the muscle, thereby increasing the shear force of the meat. To substantiate this hypothesis, we evaluated collagen concentration in this study, confirming that buriti oil may indeed function as an endogenous collagen stimulant.

The efficiency of BO in the healing process was demonstrated in a study on its antibacterial and healing activity in the skin wounds of rats, showing its ability to promote an increase in the contraction of wound edges, as well as in the count of fibroblasts and collagen fibers in the group treated with BO compared to the control group [48].These aspects of the oil characterize it very well as an exogenous healing agent; however, it is possible that this property of endogenous collagen stimulation was unknown and was only possible because in the previous studies of Martins [41] an increase in the shear force of meat was observed and the collagen concentration was analyzed in a few samples of meat and skin, with values of approximately 93.7 and 16.2 mg/kg of total collagen (TC) being found in the skin and pectoral muscle, respectively, of chickens fed with 2% BO, in comparison to 90.0 and 13.9 mg/kg of TC in the skin and pectoral muscle of chickens fed diets without buriti (unpublished data), opening perspectives for new studies and the possible classification of BO as a functional or nutraceutical feed. According to the literature, it is possible to state that the concentrations of unsaturated fatty acids present in BO play an important role in tissue regeneration, being important for the formation and deposition of collagen fibers on scars [52].

The results of economic analysis indicated that the incorporation of BO in broiler diets was not economically viable. This evaluation is of particular significance as it represents one of the pioneering studies to investigate the potential application of BO in broiler nutrition.

Our findings validate the potential of buriti oil as a functional food, positively influencing various physiological parameters. Negative performance was supported by a noted increase in metabolizable energy. Carvalho et al. [20] observed enhanced performance with a lower inclusion level (0.45% BO), underscoring the necessity for further research into the potential benefits of incorporating this functional oil into broiler diets.

Buriti is rich in carotenoids, mainly β-carotene, and phenolic compounds such as protocatecuric acid, chlorogenic acid, epicatechin, luteolin and catechin. The fruit may have the potential to be applied in the prevention of inflammatory diseases and as an antimicrobial agent (Silva et al. [56]) and does not indicate any signs of toxicity [57,58]. Moreover, the cultivation of buriti (*Mauritia flexuosa* L.) should be expanded due to the substantial potential of its co-products, which can benefit both human consumption and animal nutrition.

5. Conclusions

The inclusion of BO in broiler feed promotes a nutraceutical effect, improves the use of the diet, increases meat pigmentation, stimulates intestinal cell development, improves the immune response and stimulates collagen synthesis in birds' skin; it can be characterized as a functional feed.

These findings demonstrate that the inclusion of up to 2% BO does not adversely affect the physiological parameters and overall health of birds.

Including 1% and 2% BO in broiler diets from 1 to 21 days of age negatively impacted performance and economic viability.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/poultry4010006/s1. Figure S1: Approval letter from the Ethics Committee; Table S1: The composition of the experimental diet in the pre-starter phase (1–7 days) of broiler chickens; Table S2: The composition of the experimental diet in the starter phase (8 to 21 days) of broiler chickens.

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Informed Consent Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflicts of interest.

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