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Nutritional evaluation of chia (*Salvia hispanica*) seeds and oil in broiler diets

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ABSTRACT - Two experiments were carried with broilers from 29 to 42 days of age for the nutritional evaluation of dietary chia. Thus, the nitrogen-corrected apparent metabolizable energy (AMEn) and the apparent metabolizability coefficients of gross energy and ether extract of chia seeds and oil, toasted soybean grain (TSG), and soybean oil were determined in the experiment I, by total excreta collection method, using 120 broilers. Each experimental diet (reference diet [RD] and four diets with the tested feedstuffs) was evaluated in eight replicates of three broilers. The TSG and chia seeds replaced the RD in 250 g/kg, whereas soybean and chia oils replaced the RD in 100 g/kg. Simultaneously, a second experiment was carried subdivided into two trials. In the performance trial, we evaluated the dietary feedstuffs effects on performance, carcass and cut yields, blood parameters, and activity of lipogenic enzymes. The nutrient metabolizability coefficients and AMEn were evaluated in the metabolism trial. The AMEn values of 37.49, 37.35, 15.85, and 8.43 MJ/kg of dry matter were determined for chia oil, soybean oil, TSG, and chia seeds, respectively (experiment I). In the second experiment, the best feed conversion was observed in broilers fed diets containing chia oil and TSG. However, the diet formulated with chia seeds worsened broiler feed conversion, exhibited the smaller energy value and apparent metabolizability coefficient of the ether extract, and increased the activity of the malic enzyme and serum total cholesterol level. There was no difference for glucose-6-phosphate dehydrogenase activity and high-density lipoprotein cholesterol level. In general, chia oil showed to be efficient in replacing soybean oil in broiler diets.

Keywords: blood lipid profile, energy value, lipogenic enzymes, metabolizable energy, nutrient use, performance

1. Introduction

Chia (*Salvia hispanica*) seeds are a plant source known to be richest in omega-3 (ω-3) fatty acids (Coates and Ayerza, 2009; Nitrayová et al., 2014). They are also a good protein source (19 to 27 g/100 g; Weber et al., 1991) and have an excellent amino acid profile (Nitrayová et al., 2014). Moreover, unlike other plant sources of ω-3 such as linseed, chia has no known antinutritional factor for poultry (Azcona et al., 2008) and has antioxidant activity (Taga et al., 1984). However, it has a considerable fiber content (37.5 g/100 g; Marineli et al., 2014), which may influence the nutrient digestibility of poultry feed.

The dietary chia potential to improve the nutritional quality of broiler meat was confirmed by our research group (Mendonça et al., 2020). However, poultry production requires knowledge of energy content of the feedstuffs to provide adequate energy to the birds (Junqueira et al., 2005), in addition to knowledge of the nutrient digestibility and use by poultry to maximize their productive performance. According to Alvarenga et al. (2015), the energy value and dietary nutrient metabolizability can influence broiler performance.

Thus, to formulate more accurate diets, the chemical composition, metabolizability coefficients, and energy values must be constantly determined for new and conventional ingredients such as chia and soybean, respectively. In addition, alternative ingredients to soybean are important, since the soybean price has been increasing and its global supply is limited (Kierończyk et al., 2018).

The energy values and metabolizability coefficients for chia oil and seed were not determined to date, as well as the effects of their use in replacement of soybean on physiological and metabolic parameters of broilers are still little explored (Ayerza et al., 2002; Azcona et al., 2008; Komprda et al., 2013). Therefore, two experiments were carried with broilers from 29 to 42 days of age for the nutritional evaluation of dietary chia. Experiment I was conducted to determine the nitrogen-corrected apparent metabolizable energy (AMEn) and the apparent metabolizability coefficients of gross energy and ether extract of chia seeds and oil, toasted soybean grain (TSG), and soybean oil. The second experiment was carried with the objective of evaluating the effects of dietary feedstuffs on performance, carcass and cut yields, blood parameters, and lipogenic enzyme activity in broilers, besides the apparent metabolizability coefficients of nutrients and the AMEn of the diets.

2. Material and Methods

The experiments with animals were conducted according to the institutional committee on animal use (protocol number 074/16) and were conducted in Lavras, a city located in the south region of the Minas Gerais state, at an altitude of 919 m and geographic coordinates 21°14'43" South latitude and 44°59'59" West longitude.

Chia seeds were purchased from a local market in São Paulo, SP, Brazil, the chia oil in Rio de Janeiro, RJ, Brazil, and TSG in Três Corações, MG, Brazil. Soybean oil and other ingredients were purchased from a local market in Lavras, MG, Brazil. To obtain the TSG, the soybean grains were heated at 170 °C for a period of 1.5 min and subsequently brought to a silo remaining for 30 min (where they reached 150 °C). The grains were then removed and cooled to room temperature and posteriorly grounded (2 mm). Chia seeds were added whole to the feed because, according to Nitrayová et al. (2014), they do not need to be ground to release their nutrients. The same feedstuffs were used in all experiments and their chemical composition and gross energy were determined (Table 1).

Table 1 - Chemical components and gross energy of soybean oil, chia oil, toasted soybean grain (TSG), and chia seeds used in the current experiment (g/kg, as-fed basis)[1]

Item	Soybean oil	Chia oil	TSG[2]	Chia seeds
Gross energy (MJ/kg as fed)	39.49	39.23	22.30	24.53
Dry matter	998.5	1000.0	929.6	927.8
Crude protein	-	-	323.2	178.3
Ether extract	-	-	185.3	270.4
Ash	-	-	4.24	3.64
Acid detergent fiber	-	-	155.5	347.5
Neutral detergent fiber	-	-	280.4	561.7
Total dietary fiber	-	-	88.1	308.0

[1] All data are the results of chemical analysis conducted in triplicate.
[2] Samples of TSG were analyzed for KOH soluble protein and urea activity, and these values were 80.75% and 0.03 ΔpH, respectively, which are in accordance with values recommended for whole soybean in broiler feed.

One-day-old broilers were purchased from a commercial hatchery and reared in a conventional broiler shed for 28 days, constituting a single batch. On day 29, the broilers were weighed, homogenized by weight range, and distributed into experiment I (metabolism trial) and experiment II (subdivided in metabolism and performance trials). During the pre-experimental (1 to 28 days of age) and experimental (29 to 42 days of age) periods, water and feed were provided *ad libitum*. The feeds (mashed) were composed based on corn and soybean meal, formulated to meet the broilers' nutritional requirements by the recommendations of Rostagno et al. (2017) (Tables 2 and 3). In the pre-experimental phase, starter (1 to 7 days) and growing (8 to 28 days) diets were provided to the broilers.

Table 2 - Composition and nutrient density of broiler diets (g/kg, as fed basis)

Ingredient (g/kg)	Pre-experimental diet		Experimental diet (29–42 days)				
			Experiment I[1]	Experiment II (performance and metabolism trials)[2]			
	1–7 days	8–28 days	Reference diet	Soybean oil	Chia oil	Toasted soybean grain (TSG)	Chia seeds
Corn	553.3	594.8	651.00	651.00	651.00	644.50	644.50
Soybean meal	381.8	340.6	292.50	292.50	292.50	160.00	160.00
Dicalcium phosphate	19.0	14.6	12.00	12.00	12.00	11.60	11.60
Limestone	9.10	9.1	8.00	8.00	8.00	7.70	7.70
Sodium chloride (NaCl)	5.10	4.8	4.50	4.50	4.50	4.50	4.50
DL-Methionine, 99%	3.65	2.7	2.50	2.50	2.50	2.50	2.50
L-Lysine HCL, 78%	3.10	2.2	2.10	2.10	2.10	2.40	2.40
L-Threonine, 98.5%	1.10	0.7	0.40	0.40	0.40	0.50	0.50
L-Valine, 99%	-	-	0.20	0.20	0.20	0.40	0.40
Mineral supplement[3]	0.50	0.5	0.50	0.50	0.50	0.50	0.50
Vitamin supplement[4]	0.50	0.4	0.40	0.40	0.40	0.50	0.50
Choline chloride, 60%	0.50	0.45	0.40	0.40	0.40	0.40	0.40
Anticoccidial[5]	0.50	0.5	0.50	0.50	0.50	0.50	0.50
Soybean oil	21.8	28.6	25.00	25.00	-	-	-
Chia oil	-	-	-	-	25.00	-	-
TSG	-	-	-	-	-	164.00	-
Chia seed	-	-	-	-	-	-	164.00

[1] Soybean and chia oils replaced reference diet in 100 g/kg; TSG and chia seeds replaced reference diet in 250 g/kg.
[2] Chia oil replaced soybean oil and chia seeds replaced TSG per weight to weight.
[3] Supplemented per kilogram of feed: Zn, 55 mg; Se, 0.18 mg; I, 0.70 mg; Cu, 10 mg; Mn, 78 mg; Fe, 48 mg.
[4] Supplemented per kilogram of feed: folic acid, 0.48 mg; pantothenic acid, 8.70 mg; biotin, 0.018 mg; butylhydroxytoluene (BHT), 1.5 mg; niacin, 11.1 mg; vitamin A, 6000 IU; vitamin B1, 0.8 mg; vitamin E, 12.15 IU; vitamin B12, 8.10 µg; vitamin B2, 3.6 mg; vitamin B6, 1.80 mg; vitamin D3, 1500 IU; vitamin K3, 1.44 mg.
[5] Salinomicin 12%.

2.1. Experiment I (metabolism trial)

Experiment I was carried to determine the AMEn, apparent metabolizability coefficient of ether extract (AMCEE), and apparent metabolizability coefficient of gross energy (AMCGE) of the soybean oil, chia oil, TSG, and chia seeds for broilers.

2.1.1. Animals, installations, experimental design, and diets

One hundred twenty 29-42-day-old male Cobb-500® broilers were used. At 29 days, broilers were weighed, homogenized by weight range (1.534±0.017 kg), and distributed into 40 metabolic cages (50×50×50 cm), located in a metabolic room. Each cage (experimental unit) was composed of a feeding and drinking trough and aluminum trays covered with plastic to collect the excreta under the cages.

Table 3 - Chemical composition of pre and experimental diets (g/kg, as fed basis)

Item	Pre-experimental diet		Experimental diet (29–42 days)				
			Experiment I[1]	Experiment II (performance and metabolism trials)[2]			
	1–7 days	8–28 days	Reference diet	Soybean oil	Chia oil	Toasted soybean grain (TSG)	Chia seeds
Metabolizable energy (MJ/kg)[3]	12.37	12.78	12.99	12.99	-	12.99	-
Metabolizable energy (MJ/kg)[4]	-	-	13.00	13.00	13.11	13.33	12.72
Crude protein[3,5]	217.00	201.40	187.50	187.50	187.50	187.30	163.20
Crude protein[6]	-	-	179.90	179.90	179.90	180.70	155.20
Digestible methionine + cystine[3]	9.50	8.20	7.70	7.70	7.70	7.60	-
Digestible lysine[3]	13.30	11.60	10.40	10.40	10.40	10.40	-
Digestible threonine[3]	8.50	7.60	6.80	6.80	6.80	6.80	-
Digestible valine[3]	-	-	8.20	8.20	8.20	8.10	-
Total phosphorus[3,5]	6.90	6.00	6.90	6.90	6.90	6.80	6.10
Available phosphorus[3,5]	4.70	3.80	3.30	3.30	3.30	3.20	3.40
Calcium[3,5]	9.30	8.10	6.90	6.90	6.90	6.80	7.20
Sodium[3,5]	2.20	0.20	2.00	2.00	2.00	2.00	1.90
Acid detergent fiber[6]	-	-	5.23	5.23	5.30	7.01	10.16
Neutral detergent fiber[6]	-	-	17.12	17.12	17.03	19.96	23.81
Total dietary fiber[6]	28.1	26.8	26.80	26.80	26.80	34.20	51.00

[1] Soybean and chia oils replaced reference diet in 100 g/kg; TSG and chia seeds replaced reference diet in 250 g/kg.
[2] Chia oil replaced soybean oil and chia seeds replaced toasted soybean grain per weight to weight.
[3] Calculated according to Rostagno et al. (2017).
[4] Determined in *in vivo* assay. Tabulated mean values are based on six replicate cages per treatment, in which cage is the experimental unit with three broilers.
[5] Composition of chia seeds calculated with values reported by Jin et al. (2012).
[6] All data are the results of chemical analysis conducted in triplicate.

Continuous light was provided for 24 h daily throughout the experimental period. Maximum and minimum temperatures were recorded daily at 16:00 h, using a thermometer located approximately at the broilers' height. The minimum and maximum temperatures in the metabolic room during the experimental period were 22.4±0.82 and 32.5±0.13 °C, respectively, for 29 to 35-day-old broilers. In the period from 36 to 42 days, the minimum temperature was 22.6±0.86 °C and the maximum was 31.1±1.08 °C.

The experimental design was completely randomized with five diets evaluated in eight replicates of three broilers, totalizing 120 broilers. The experimental diets were constituted of a reference diet (RD) formulated to the broilers' requirements as recommended by Rostagno et al. (2017) and four diets containing the evaluated feedstuffs (chia seeds an oil, TSG, and soybean oil) (Tables 2 and 3). The TSG and chia seeds replaced the RD in 250 g/kg, whereas soybean and chia oils replaced the RD in 100 g/kg.

2.1.2. Total excreta collection method and evaluated parameters

Feed and leftovers were weighed on the 36th and 38th days of age of the broilers for the calculation of feed intake (FI) during a period of three days of excreta collection (Rodrigues et al., 2005). The total excreta collection was performed daily at 08:00 h. The excreta from each experimental unit were collected in a labeled plastic bag and stored in a freezer at −5 °C until the last day of the collection to avoid fermentation. At this time, the contents were weighed and homogenized, and 300 g of representative samples of each experimental unit were pre-dried in an oven at 55 °C during 72 h. After pre-drying, the excreta were ground and stored at room temperature until laboratory analysis.

The evaluated feedstuffs, experimental diets, and excreta were subjected to the analyses of dry matter (DM), nitrogen (N), and ether extract (EE) (methods numbers 934.01, 990.03, and 920.39, respectively; AOAC, 2007). Gross energy (GE) was determined in a bomb calorimeter (model C200, IKA®, Stauten, Germany). Neutral detergent fiber (aNDF, without sodium sulfite) and acid detergent fiber (ADF) were analyzed according to Van Soest et al. (1991). In chia seeds and TSG, the total dietary fiber was calculated as the sum of soluble and insoluble dietary fiber (method number 991.43; AOAC, 2007) (Table 1).

Based on the laboratory results, the apparent metabolizable energy (AME) values were calculated using the equations proposed by Matterson et al. (1965), corrected for N retention (AMEn):

$$\text{AME of RD or TD} = (\text{GE ingested} - \text{GE excreted})/\text{DM ingested} \quad (1)$$

$$\text{AME of ingredient} = \text{AME of RD} + [(\text{AME of TD} - \text{AME of RD})/(\text{g ingredient/g feed})] \quad (2)$$

$$\text{AMEn} = [\text{GE ingested} - (\text{GE excreted} \pm 8.22 \times \text{NB})]/\text{DM ingested} \quad (3)$$

$$\text{AMEn of ingredient} = \text{AMEn of RD} + [(\text{AMEn of TD} - \text{AMEn of RD})/(\text{g ingredient/g feed})] \quad (4)$$

in which RD = reference diet, TD = test diet, and NB = nitrogen balance (N ingested − N excreted).

To calculate the AMCEE and AMCGE of the feedstuffs, the following equations were used:

$$\text{AMCEE} = (\text{EE ingested} - \text{EE excreted})/\text{EE ingested} \quad (5)$$

$$\text{AMCGE} = (\text{GE ingested} - \text{GE excreted})/\text{GE ingested} \quad (6)$$

2.2. Experiment II (performance and metabolism trials)

Experiment II was subdivided into two trials in which the same feedstuffs used in experiment I (Table 1) were evaluated. A total of 192 male broilers in the period from 29 to 42 days of age were used in experiment II, of which 120 broilers were used in the performance trial and 72 in the metabolism trial. In the performance trial, broiler performance, carcass and cut yields, serum lipid profile, and lipogenic enzyme activity were evaluated. The metabolism trial was carried simultaneously with the performance trial, but in a metabolic room. In the metabolism trial, the AMEn, AMCEE, AMCGE, and apparent metabolizability coefficient of dry matter (AMCDM) and crude protein (AMCCP) of the experimental diets were determined.

2.2.1. Performance trial

2.2.1.1. Animals, installations, experimental design and diets

On day 29, the broilers were weighted, homogenized by weight range (1.684±0.034 kg), and distributed into 24 boxes in a performance shed. Each box (experimental unit) had dimensions of 2.0×1.5 m, had its floor covered with wood shavings, and was equipped with a pendular drinker and feeder. Continuous light was provided for 24 h daily throughout the experimental period. The maximum and minimum temperatures were recorded daily at 16:00 h, using a thermometer located approximately at the broilers' height. The minimum and maximum temperatures in the shed during the experimental period from 29 to 35-day-old broilers were 20.0±0.51 and 32.8±1.10 °C, respectively. In the period from 36 to 42 days old, the minimum temperature was 20.0±1.08 °C and the maximum was 31.4±1.28°C.

The experimental design was completely randomized corresponding to four dietary treatments, evaluated in five replicates (boxes) of six broilers, totalizing 120 broilers. The dietary treatments were constituted of diets containing chia (seed and oil) in replacement of soybean (grain and oil). Initially, the RD containing corn, soybean meal, and soybean oil (25 g/kg) was formulated according to nutritional requirements proposed by Rostagno et al. (2017). Subsequently, an isonutrient diet without soybean oil and containing TSG (164 g/kg) was formulated. Afterward, the soybean grain/oil was replaced on a weight-to-weight basis in the formulation by chia seeds/oil, constituting the other experimental diets. Thus, the TSG and chia seeds were included in the diet at 164 g/kg, whereas the soybean and chia oils were included in the diet at 25 g/kg. The experimental diets (Tables 2 and 3) were supplied *ad libitum* to the broilers during all experimental period.

2.2.1.2. Evaluated parameters

Feed intake, weight gain (WG), and fed conversion ratio (FCR) were evaluated in the experimental period. To calculate the WG, broilers were weighed on the 29th and 42nd days of age. Feed intake was determined by weighing feed and leftovers in the feeders on days 29 and 42. The FCR was calculated by dividing the FI by WG.

At the end of the performance trial (42 days-old), after all broilers were weighed to evaluate performance, broilers were fasted for 12 h. After fasting, three broilers per replicate were randomly selected, weighed for subsequent yield calculations, and slaughtered via cervical dislocation, followed by bleeding and blood sampling (6 mL in a tube without anticoagulant). After slaughter, broilers were scalded, plucked, and eviscerated, as per the experimental treatment and replicate distributions, then weighed, individually packed in plastic bags, and cooled at 5 °C for 24 h to calculate the carcass and cut yields and abdominal fat percentages.

Carcass yield was measured 24 h postmortem. The clean carcass weight (without the head and feet) without going through the chiller as well as its relation to the fasted animal's live weight were used to calculate the yield. After recording the carcass weight, the carcasses were divided into cuts (breast, thigh, drumstick, back, and wings and neck) to determine the cut yield, which was calculated relative to the eviscerated carcass weight. Abdominal fat was determined considering lipid deposition in the region between the bursa of Fabricius and the cloaca, which was then weighed, and its relation to the eviscerated carcass weight was calculated.

At the time of evisceration, liver samples were collected, stored in 2-mL microtubes, immediately frozen in liquid nitrogen, and stored in a freezer (−80 °C) for further analysis of the malic and glucose-6-phosphate dehydrogenase enzyme activities. The collected blood was centrifuged (6500 rpm for 6 min), and the serum was stored at −80 °C for further biochemical analysis.

The collected blood was analyzed for total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol using a microplate reader (model NUNC F, Thermo Fischer Scientific Inc., Kamstrup, Denmark) and serological kits (Labtest®, Lagoa Santa, Brazil). The very-low-density lipoprotein (VLDL) cholesterol value was determined by calculating the difference between the total cholesterol and HDL cholesterol and LDL cholesterol.

Liver tissue homogenates were prepared in 50 mM Tris-HCl buffer, pH 7.4, at the ratio of 5 mL buffer to 1 g tissue. The tissues were homogenized in an Ultra-Turrax (IKA, Staufen, Baden-Württemberg, Germany) tissue macerator, and then centrifuged at 14000 rpm at 4 °C for 30 min. The supernatant was collected and centrifuged again to obtain a clear extract. The samples were aliquoted and stored at −80 °C for subsequent enzymatic analysis.

The activities of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and the malic enzyme (EC 1.1.1.40) were determined following the methodologies proposed by Bautista et al. (1988) and Spina et al. (1970), respectively. Enzymatic kinetics was monitored using a Multiskan Go microplate reader (Thermo Fisher Scientific, Vantaa, Finland). The total soluble protein content in the liver extract was determined by the method described by Bradford (1976) using bovine serum albumin (BSA) as the standard. The enzymatic activity (IU), defined as µmol of substrate converted into its respective product per minute, was expressed per mg of soluble liver protein.

2.2.2. Metabolism trial

In this trial, 72 broilers (with a mean weight of 1.514±0.015 kg) were distributed into 24 metabolic cages in a metabolic room where they received the four experimental diets provided in the performance trial. The experimental design was completely randomized corresponding to four dietary treatments, evaluated in six replicates (metabolic cage) of three broilers each. The experimental procedures, metabolic room, facilities, equipment, and room temperature were the same adopted for the experiment I.

In trial II, the variables analyzed and laboratory analyses of the experimental feeds and excreta were the same as those performed in experiment I.

The following formulas were used to calculate the AMCDM and AMCCP:

$$\text{AMCDM} = (\text{DM ingested} - \text{DM excreted})/\text{DM ingested} \quad (7)$$

$$\text{AMCCP} = (\text{CP ingested} - \text{CP excreted})/\text{CP ingested} \quad (8)$$

The formulas described in the methodology of experiment I were used to calculate the AMCEE and AMCGE.

The DM, CP, EE, GE, and N were analyzed for corn, soybean meal, TSG, and chia seeds to calculate the CP. For the oils, the DM and GE were determined. The total dietary fiber content in chia seeds and TSG was also evaluated (Table 1).

2.2.3. Statistical analyses

The statistical model used was:

$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij},$$

in which y_{ij} is the response variable measured in the j-th replicate that received the i-th treatment, μ is the general constant, α_i is the fixed effect of the i-th treatment, and ε_{ij} is the random error term.

Data were analyzed using the Bartlett's and Shapiro-Wilk tests, at the P<0.05 statistical level to test the assumptions of the analysis of variance (variance homogeneity and normality). If any assumptions were unmet, the data were logarithmically transformed for statistical analysis. If both assumptions were met, the data were analyzed by analysis of variance (ANOVA), and the means were compared using the Scott-Knott test at 5% probability, using the statistical program R, version 3.2.5 (R Core Team, 2017).

3. Results

3.1. Experiment I (metabolism trial)

The AMEn values of 37.49, 37.35, 15.85, and 8.43 MJ/kg DM were determined for chia oil, soybean oil, TSG, and chia seeds, respectively (Table 4). The soybean and chia oils provided the highest AMCGE and AMCEE (P<0.001), while the dietary use of chia seeds resulted in the lowest values.

Table 4 - Energy values and apparent metabolizability coefficients of gross energy and ether extract of the feedstuffs used in experimental diets for broilers from 29 to 42 days of age[1]

Variable	Feedstuff				SEM	P-value
	Soybean oil	Chia oil	Toasted soybean grain	Chia seed		
AMEn (MJ/kg)	37.35	37.49	15.85	8.43	0.3700	-
AMCGE (g/kg)	0.944a	0.956a	0.661b	0.329c	0.0148	<0.001
AMCEE (g/kg)	0.925a	0.931a	0.868b	0.537c	0.0090	<0.001

AMCEE - apparent metabolizability coefficient of ether extract; AMCGE - apparent metabolizability coefficients of gross energy; AMEn - nitrogen-corrected apparent metabolizable energy; SEM - standard error of the mean.
[1] Data represent mean values of eight replicates pens per treatment (three broilers per pen).
It is considered as a significant difference when P<0.05.
a-c - Means within rows followed by a different letter are significantly different at the 5% probability level by the Scott-Knott test.

3.2. Experiment II (performance trial)

No mortality occurred during the experimental period. Broilers fed diets containing soybean oil and chia seeds exhibited higher FI (P<0.001) (Table 5). The diets formulated with soybean and chia oils resulted in higher (P<0.001) WG of the broilers. The inclusion of chia oil and TSG in the diet improved (P<0.001) the FCR, while the use of chia seeds worsened it.

The inclusion of soybean and chia oils in the broiler diets increased (P<0.05) the weight of the carcass, breast, thigh, and back (Table 6). However, there were no effects (P>0.05) of the experimental diets on yields of carcass and cuts (breast, thigh, drumstick, wing, and back) neither on abdominal fat deposition.

Broilers that received TSG in the diet exhibited lower (P<0.05) serum triglycerides and VLDL cholesterol (Table 7). There was no difference (P>0.05) between dietary treatments for HDL cholesterol level. The diet formulated with chia seeds increased (P<0.05) the serum total cholesterol level. The highest (P<0.05) LDL cholesterol level was determined in broilers fed diet containing TSG and chia seeds.

There was no difference (P>0.05) between dietary treatments for activity of the glucose-6-phosphate dehydrogenase determined in the liver extract (Table 7). However, broilers fed diet containing chia seeds exhibited higher (P<0.05) malic enzyme activity in the liver extract.

Table 5 - Performance of broilers fed experimental diets from 29 to 42 days of age[1]

Variable	Experimental diet[2]				SEM	P-value
	Soybean oil	Chia oil	Toasted soybean grain	Chia seeds		
Initial BW (kg/bird)	1.690	1.682	1.679	1.683	0.0052	0.512
Final BW (kg/bird)	3.208a	3.159a	3.078b	3.065b	0.0216	<0.001
FI (kg/bird)	2.905a	2.636b	2.548b	2.984a	0.0401	<0.001
WG (kg/bird)	1.518a	1.477a	1.398b	1.383b	0.0210	<0.001
FCR (kg/kg)	1.914b	1.785c	1.822c	2.138a	0.0286	<0.001

BW - body weight; FI - feed intake; WG - weight gain; FCR - feed conversion ratio; SEM - standard error of the mean.
[1] Data represent mean values of five replicates pens per treatment (six broilers per pen).
[2] In experimental diets, chia oil replaced soybean oil (25 g/kg) and chia seeds replaced toasted soybean grain (164 g/kg) per weight to weight.
It is considered as a significant difference when P<0.05.
a-c - Means within rows followed by a different letter are significantly different at the 5% probability level by the Scott-Knott test.

Table 6 - Carcass yields and abdominal fat of broilers fed experimental diets from 29 to 42 days of age[1]

Variable	Experimental diet[2]				SEM	P-value
	Soybean oil	Chia oil	Toasted soybean grain	Chia seeds		
Body weight (BW, kg)	3.139a	3.119a	3.019b	3.010b	0.0316	0.019
Carcass weight (kg)	2.420a	2.434a	2.350b	2.336b	0.0256	0.034
Carcass yield (kg/100 kg BW)	76.89	78.03	77.83	77.41	0.2830	0.052
Breast weight (kg)	0.991a	1.001a	0.948b	0.934b	0.0163	0.029
Breast yield (kg/100 kg)	40.94	41.04	40.31	40.00	0.4880	0.400
Thigh weight (kg)	0.297a	0.290a	0.280b	0.281b	0.0039	0.022
Thigh yield (kg/100 kg)	12.24	11.89	11.92	12.00	0.1350	0.298
Drumstick weight (kg)	0.325	0.332	0.325	0.322	0.0036	0.287
Drumstick yield (kg/100 kg)	13.54	13.66	13.83	13.81	0.1720	0.587
Wing weight (kg)	0.233	0.235	0.233	0.229	0.0035	0.723
Wing yield (kg/100 kg)	9.62	9.65	9.92	9.82	0.1330	0.386
Back weight (kg)	0.462a	0.469a	0.446b	0.430b	0.0050	0.012
Back yield (kg/100 kg)	19.22	19.29	18.97	18.53	0.1190	0.091
Abdominal fat (g)	31.8	31.3	33.1	33.3	2.7790	0.942
Abdominal fat (g/100 g)	1.31	1.28	1.40	1.43	0.1090	0.753

BW - body weight; SEM - standard error of the mean.
[1] Data represent mean values of five replicates pens per treatment (three broilers per pen).
[2] In experimental diets, chia oil replaced soybean oil (25 g/kg) and chia seeds replaced toasted soybean grain (164 g/kg) per weight to weight.
It is considered as a significant difference when P<0.05.
a-b - Means within rows followed by a different letter are significantly different at the 5% probability level by the Scott-Knott test.

Table 7 - Serum levels of triglycerides (mg/dL), total cholesterol and fractions (mg/dL), and liver enzyme activity (U/mg of soluble protein) of broilers fed experimental diets from 29 to 42 days of age[1]

Variable	Experimental diet[2]				SEM	P-value
	Soybean oil	Chia oil	Toasted soybean grain	Chia seeds		
Triglycerides	47.5a	47.3a	42.3b	47.3a	1.2800	0.019
Total cholesterol	120.2b	119.0b	125.2b	137.0a	3.3100	0.005
VLDL-cholesterol	9.5a	9.5a	8.4b	9.5a	0.2400	0.015
HDL-cholesterol	40.4	44.3	38.1	43.9	2.0300	0.139
LDL-cholesterol	71.9b	65.2b	78.6a	83.7a	3.1600	0.005
G6PD	30.93	32.18	33.00	32.62	2.2660	0.924
Malic enzyme	270.34b	251.82b	300.31b	367.54a	17.9880	0.002

VLDL - very-low-density lipoprotein; HDL - high-density lipoprotein; LDL - low-density lipoprotein; G6PD - G-6-P dehydrogenase; SEM - standard error of the mean.
[1] Data represent mean values of five replicates pens per treatment (three broilers per pen).
[2] In experimental diets, chia oil replaced soybean oil (25 g/kg) and chia seeds replaced toasted soybean grain (164 g/kg) per weight to weight.
It is considered as a significant difference when P<0.05.
a-b - Means within rows followed by a different letter are significantly different at the 5% probability level by the Scott-Knott test.

3.3. Experiment II (metabolism trial)

There was no difference (P>0.05) for AMCDM and AMCCP among the experimental diets offered to the broilers (Table 8). However, the diet formulated with chia seeds exhibited the smaller (P<0.05) AMCEE, AMCGE, and AMEn values.

Table 8 - Apparent metabolizability coefficients of nutrients (dry matter, crude protein, ether extract, and gross energy) and energy values on a dry matter (DM) basis of the experimental diets offered to broilers from 29 to 42 days of age[1]

Variable	Experimental diet[2]				SEM	P-value
	Soybean oil	Chia oil	Toasted soybean grain	Chia seeds		
AMCDM	0.797	0.807	0.772	0.781	0.0094	0.066
AMCCP	0.717	0.758	0.716	0.718	0.0147	0.153
AMCEE	0.865a	0.848a	0.832a	0.665b	0.0117	<0.001
AMCGE	0.764a	0.762a	0.754a	0.707b	0.0049	<0.001
AMEn (MJ/kg)	14.62a	14.74a	14.84a	13.76b	0.0950	<0.001

AMCCP - apparent metabolizability coefficients of crude protein; AMCDM - apparent metabolizability coefficient of dry matter; AMCEE - apparent metabolizability coefficient of ether extract; AMCGE - apparent metabolizability coefficients of gross energy; AMEn - nitrogen-corrected apparent metabolizable energy; SEM - standard error of the mean.
[1] Data represent mean values of six replicates pens per treatment (three broilers per pen).
[2] In experimental diets, chia oil replaced soybean oil (25 g/kg) and chia seeds replaced toasted soybean grain (164 g/kg) per weight to weight.
It is considered as a significant difference when P<0.05.
a-b - Means within rows followed by a different letter are significantly different at the 5% level of probability by the Scott-Knott test.

4. Discussion

In experiment II, TSG was totally replaced by chia seeds (164 g/kg) because of the lack of prior knowledge about the AMEn value and utilization of chia nutrients by broilers. Thus, these values and values of chemical composition, of chia, which were determined in Experiment I, enabled determining later that the nutritional levels of the diet with chia seeds in experiment II were below the nutritional requirements recommended by both the NRC (1994) and Brazilian Tables (Rostagno et al., 2017).

The NRC (1994) and Rostagno et al. (2017) recommend, respectively, 14.08 and 13.29 MJ/kg of AMEn for broilers in the rearing period evaluated in the present study. However, the final AMEn of the diet containing chia seeds was 12.72 MJ/kg (Table 3). A similar condition was observed in relation to the CP content of the diet containing chia seeds, because in this diet, the final CP was 15.52%, while the values recommended by the NRC (1994) and Rostagno et al. (2017) are of 20.0 and 19.5%, respectively.

Kamran et al. (2008) observed a linear increase of FI when broilers received diets with reduced contents of CP and metabolizable energy, in all rearing periods. Moreover, according to Leeson et al. (1993), FI is regulated by the dietary energy value. Thus, the lowest metabolizable energy and CP values of the diet containing chia seeds in experiment II may explain the higher FI by the broilers that received this experimental diet, possibly as a mechanism to meet their nutritional requirements. On the other hand, the broilers fed diet containing soybean oil during the experimental period exhibited higher FI, possibly because unlike of the other experimental dietary treatments, they did not have to adapt to the feed, since the diet provided in the pre-experimental phase (from 1 to 28 days of age) also contained soybean oil in its formulation.

According to Alvarenga et al. (2015), dietary energy influences the development and performance of broilers. Therefore, although broilers that received chia seeds in their diet consumed more feed, they did not exhibit higher WG, possibly because they used chia seeds were not ground, reflecting in lower AMCEE, AMCGE, and AMEn values (Table 8). Chia seeds were added whole to the feed because, according to Nitrayová et al. (2014) they do not need to be ground to release their nutrients. However, in the present study, the results suggest that the nutrient use by the broilers could have been maximized if the chia seeds had been ground prior to its inclusion into the feed.

Moreover, chia seeds also have high fiber content. Marineli et al. (2014) determined 37.5% of dietary fiber in the chia seeds. In the present study, the chia seeds exhibited 30.8% of total dietary fiber (Table 1). According to Janssen and Carré (1989), increased dietary fiber content may reduce nutrient and energy use. In the case of chia, this may occur because when the seeds get in contact with water, they gelatinize to form a thick mucilage layer, which acts as a physical barrier changing the passage rate through the gastrointestinal tract and hindering the action of digestive enzymes (Bedford, 2000; Alzueta et al., 2003).

The WG and FCR of the broilers that received dietary chia seeds corroborate the results of Ayerza et al. (2002), who also observed lower WG and FCR in broilers fed diet containing chia. Overall, it was observed that the inclusion of soybean and chia oils in the diet resulted in higher WG than the inclusion of TSG and chia seeds, probably because the AMEn, AMCEE, and AMCGE of the oils were higher than the values determined for TSG and chia seeds (Table 4). Moreover, current broiler lines have high energy requirements; thus, oil must be used in their diets.

Similar to the results of the present study in which the experimental diets did not change the carcass yield, Azcona et al. (2008) also did not observe statistical difference in the carcass yield when the broiler diets were formulated with linseed, canola, chia seeds, or chia flour. However, Azcona et al. (2008) observed that broilers fed diets containing linseed and chia seeds exhibited lower abdominal fat deposition than did those that received the control diet. These authors attributed this difference to changes in the β-oxidation rate of polyunsaturated fatty acids (PUFA) between the evaluated diets, but in the present study, there were no effects of the experimental diets on abdominal fat deposition.

The AMEn values determined for the soybean oil and TSG in experiment I (37.35 and 15.85 MJ/kg DM, respectively) were higher than the values reported by Rostagno et al. (2017) (36.86 MJ/kg DM for soybean oil and 14.59 MJ/kg DM for TSG). Moreover, the AMEn of the TSG determined in this work was also higher than the 13.82 MJ/kg DM cited by the NRC (1994). This underscores the importance of constant updating of the feed table values to contribute with dietary formulations increasingly accurate and efficient.

Although the gross energy of chia oil was 0.26 MJ/kg DM lower than that in the soybean oil (Table 1), the AMEn determined for chia oil was 0.14 MJ/kg higher than the value determined for soybean oil (Table 4), demonstrating that chia oil is highly used by broilers. In its turn, the AMEn of the TSG for broilers was higher than determined for chia seeds (Table 4). However, unlike chia seeds, TSG was initially ground and then was included in the feed.

According to Kris-Etherton et al. (1988), diets rich in PUFA reduce the cholesterol levels. However, the broilers fed diet containing chia seeds exhibited higher serum total cholesterol level. Besides rich in ω-3 series fatty acids, chia also has considerable amounts of protein and other nutrients (Nitrayová et al., 2014). However, in the present study, the results suggest that chia seeds were not fully digested because they were incorporated whole into the feed. Thus, as a consequence, a variation may have occurred in the quantity and profile of circulating nutrients during the absorptive period, which may have resulted in intensified liver cholesterol biosynthesis. Similarly, in a study conducted with laying hens, Ayerza and Coates (1999) verified that the cholesterol content in the yolk was higher when the diet was formulated with chia seeds. However, further studies are needed to elucidate these results.

The use of chia seeds and oil in broiler diets did not reduce the HDL-cholesterol and triglycerides levels, contrary to what was observed by Ayerza and Coates (2005, 2007) in rats. However, the lipoprotein metabolism in rats is not identical to that observed in broilers, justifying such differences. The readily available PUFA present in chia and soybean oils provided the lowest LDL cholesterol levels in the birds. Weintraub et al. (1988) evaluated the inclusion of saturated fatty acids, ω-6 PUFA, and ω-3 PUFA in human diets and found that both types of PUFA decreased the total cholesterol, triglycerides, VLDL-cholesterol, and LDL cholesterol levels. According to these authors, the ω-6 and ω-3 PUFA present in chylomicrons are more susceptible to lipoprotein lipase action, which contributed to the reduced plasma lipoproteins levels.

Shimomura et al. (1990) reported that the diets rich in PUFA increased the lipoprotein lipase activity in rats. In addition, Beynen and Katan (1985) found that the liver preferentially converts PUFA to ketone bodies rather than to VLDL. In plasma, VLDL is rapidly converted to LDL, which is the main carrier of cholesterol. Thus, with lower VLDL formation, less LDL will be circulating in the plasma. These authors also reported that an increase in sterol excretion occurs in individuals who consume more PUFA, which may have contributed to the lower LDL cholesterol levels determined in the broilers that received soybean or chia oils in the diet.

In birds, lipogenesis occurs mainly in the liver, unlike in pigs where lipid synthesis is highest in adipose tissue (O'Hea and Leveille, 1968; Leveille et al., 1975). Glucose-6-phosphate dehydrogenase and malic enzymes provide reducing equivalents in the form of NADPH for *de novo* lipogenesis in the liver. Therefore, these enzymes can be used as key indicators of lipid biosynthesis (Alvarez et al., 2000). In the present study, only the malic enzyme activity was influenced by diet, in which the highest activity was determined when the broilers received the dietary chia seeds. The activity of the malic enzyme was higher than glucose-6-phosphate dehydrogenase, corroborating O'Hea and Leveille (1968), who reported that the malic enzyme is the primary source of reducing equivalents (NADPH) for lipid synthesis in the liver of birds.

The diet formulated with chia seeds increased the activity of the malic enzyme probably due to its lowest CP level content, because Adams and Davis (2001) and Rosebrough et al. (2008) concluded that the reduced CP content in the diet increase the mRNA expression of the malic enzyme, which can explain the increased activity of malic enzyme in the liver extract of broilers.

These observations may help explain the results obtained in this study since chia seeds were not fully digested by the broilers. Thus, part of the nutrients of the seeds, such as protein, may not have been fully digested, leading to a greater concentration, digestion, and absorption of carbohydrate in relation to the other nutrients in the diet. Moreover, according to Stabile et al. (1998), dietary PUFA inhibit lipogenic enzyme expression in rats, which is consistent with the lower values determined for malic enzyme in broilers fed diets containing soybean oil, chia oil, and TSG, in which nutrient use by broilers was generally higher.

Therefore, the energy values determined for soybean oil, chia oil, TSG, and chia seeds were 37.35, 37.49, 15.85, and 8.43 MJ/kg DM, respectively. The metabolizability of gross energy and ether extract coefficients ranged from 0.32 to 0.96 and from 0.54 to 0.93, respectively, and the oils presented the highest coefficients.

5. Conclusions

Chia oil presents a similar AMEn to soybean oil as well as good metabolizability coefficients of ether extract and gross energy, without compromising the performance and carcass characteristics of broilers.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: N.B.S.N. Mendonça and P.B. Rodrigues. Data curation: N.B.S.N. Mendonça, L.P. Naves and P.B. Rodrigues. Formal analysis: N.B.S.N. Mendonça, S.T. Sobrane Filho, E.M.C. Lima, D.H. Oliveira, F.A. Coelho, F.L. Cruz, L.F. Bernardes and R.H.R. Moreira. Funding acquisition: P.B. Rodrigues. Investigation: N.B.S.N. Mendonça, S.T. Sobrane Filho, E.M.C. Lima and D.H. Oliveira. Supervision: P.B. Rodrigues. Writing – original draft: N.B.S.N. Mendonça and L.P. Naves. Writing – review & editing: L.P. Naves and P.B. Rodrigues.

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