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Effects of chia (*Salvia hispanica* L.) on calcium bioavailability and inflammation in *Wistar* rats

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ABSTRACT

Chia is a good source of calcium, however it is not been previously reported its bioavailability associated with an inflammatory condition. Thus, the present study evaluated the effect of chia on calcium bioavailability, inflammation, and oxidative stress in Wistar rats fed a high-fat diet or standard diet for 35 days. Chia consumption resulted in lower calcium balance and calcium absorption and retention rates. In addition, the urinary calcium concentration was lower in groups that were fed chia. The bone resistance of animals feed chia was lower than that in rats fed the standard diet receiving calcium carbonate. Animals that were fed chia showed lower total, very low-density lipoprotein, and low-density lipoprotein cholesterol levels than animalsfed calcium carbonate. Animals fed standard diet showed higher superoxide dismutase plasma concentrations than animals in the high fat calcium carbonate group. PPAR-α protein levels were higher in animals fed chia whereas TNF-α and IL-10 were lower in these animals. NFκB mRNA expression and protein levels were lower in the groups that received chia compared with HFD + CC. Chia intake presented low calcium bioavailability regardless of the type of diet consumed and was able to improved inflammation and the lipid profile in young Wistar rat. Besides this, the consumption of this seed increased the activity of antioxidants enzymes.

1. Introduction

Chia (*Salvia hispanica* L.), is an herbaceous plant with high nutritional and functional value (Ixtaina, Nolasco, & Tomás, 2008) due to high concentrations of essential fatty acids, dietary fiber, phenolic compounds, and proteins (Ayerza & Coates, 2011; Chicco, D'Alessandro, Hein, Oliva, & Lombardo, 2009; Silva et al., 2017; Vuksan et al., 2007). Moreover, the seeds of this plant stand out due to their high concentrations of minerals, including calcium (Silva et al., 2017). Although calcium is present at high concentrations in chia, the bioavailability of this mineral for use in metabolic processes or storage in humans must be evaluated (Jafari & Mcclements, 2017).

The prevalence of calcium deficiency is around 51% (3.5 billion people) worldwide (Kumssa et al., 2015). Calcium intake is currently decreasing across all age groups, resulting in a higher prevalence of osteoporosis and osteomalacia (Greupner, Schneider, & Hahn, 2017). In addition to low calcium intake, the current diets of many populations are characterized by high sugar and fat consumption, which can impair calcium bioavailability. This modification in food consumption is associated with an increased risk of cardiovascular diseases, obesity, and other disorders due to inflammation and oxidative stress (Catrysse & Van Loo, 2017; Cochain & Zernecke, 2017).

Changing in food intake can induce the inflammation, culminates in the activation of specific signaling pathways, thus leading to the production of inflammatory substances. These pathways include NF-κB signaling and the production of inflammatory cytokines (Catrysse, & Loo, 2017). PPAR-α negatively regulates inflammation. PPAR-α ligands can inhibit the action of proinflammatory cytokines by interfering with NF-κB activity in their signaling pathway. In addition to inflammation, PPAR-α plays a role in oxidative stress (Dotson et al., 2016). In addition, the inflammatory process also acts on bone metabolism through the secretion of cytokines by adipose tissue, e.g., tumor necrosis factor alpha (TNF-α), and interleukins (Morettini, Storm, Sacchetti, Cappozzo, & Mazzà, 2015). The high levels of these proinflammatory cytokines

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Table 1
Nutritional composition of experimental diets.

Ingredients (per kg of diet)	Experimental diets			
	SD + CC	SD + C	HFD + CC	HFD + C
Calcium carbonate (mg)	5.00	0.00	5.00	0.00
Chia (g)	0.00	416.80	0.00	416.80
Albumin (g)	217.90	117.60	217.90	117.60
Dextrinized starch (g)	132.00	132.00	132.00	115.10
Sucrose (g)	100.00	100.00	100.00	100.00
Soybean oil (mL)	134.20	0.00	134.20	0.00
Lard (g)	0.00	0.00	200.00	200.00
Microcrystalline cellulose (g)	139.20	0.00	139.20	0.00
Calcium-free mineral mix (g)	35.00	35.00	35.00	35.00
Vitamin mix (g)	10.00	10.00	10.00	10.00
L-cystine (g)	3.00	3.00	3.00	3.00
Choline bitartrate (g)	2.50	2.50	2.50	2.50
Corn starch (g)	221.20	183.10	21.20	0.00
Nutritional composition				
Total calories (kcal)	3700.42[b]	3624.75[b]	4700.42[a]	4624.11[a]
Caloric density (kcal.g^{-1})	3.70[b]	3.62[b]	4.70[a]	4.62[a]
Calcium (g.kg^{-1})*	2.41 ± 0.00[a]	2.53 ± 0.02[a]	2.57 ± 0.04[a]	2.58 ± 0.00[a]

* Analyzed according to the methodology proposed by Gomes (2011). SD + CC: standard diet + calcium carbonate; SD + C: standard diet + chia; HFD + CC: high fat diet + calcium carbonate; HFD + C: high fat diet + chia. Means with different letters in the same row indicate a significant difference ($p < .05$) according to the Newman-Keuls test.

can promote the differentiation into osteoclasts and consequently the process of bone resorption (Wong, Chin, Suhaimi, Ahmad, & Ima-Nirwana, 2016). Studies have shown that ingestion of chia can act beneficially on the pathways of inflammation in animals fed high fat diet or not (Marineli, Lenquiste, Moraes, & Maróstica, 2015; Poudyal et al., 2013; Poudyal, Panchal, Waanders, Ward, & Brown, 2012; Poudyal, Panchal, Ward, Waanders, & Brown, 2012; Rincón-Cervera, Valenzuela, & Hernandez-Rodas, 2017).

Although the association between low calcium intake and the development of cardiovascular disease has been verified (Soares, Pannu, Calton, Reid, & Hills, 2017), the association between calcium absorption and inflammatory biomarkers of high-fat intake has not been investigated. In addition, consumption of a high-fat diet contributes to the activation of inflammatory pathways, promoting increased oxidative stress, deregulating lipid metabolism, and inducing dyslipidemia, which, in turn can decrease calcium absorption (Ventura et al., 2017). The hypothesis of the present study is that chia, as a source of calcium and bioactive compounds, can modulate bone metabolism, inflammation, and oxidative stress. Thus, the objective of the study was to investigate the bioavailability of calcium in chia and the influence of chia consumption on the blood lipid profile, oxidative stress, and inflammation in young *Wistar* rats fed a standard or high-fat diet.

2. Materials and methods

2.1. Raw materials and preparation of flours

Chia seeds (*Salvia hispanica* L.) grown in the state of Rio Grande do Sul (Brazil) were used for the study. To obtain the flour, the seeds were ground up in three replicates, using a knife mill (Marconi Equipment, Brazil) to a particle size of 850 μm. Subsequently, chia flour was packed in polyethylene aluminum bags and stored in a freezer (−18 °C ± 1 °C) until analysis.

2.2. Determination of calcium content and chemical composition

Calcium content in chia was quantified according to ref. (Gomes, 2011). The calcium concentration was determined by coupled plasma atomic emission spectrometry (instrument model Optima 3300 DV, Perkin Elmer, Massachusetts, USA) with an inducible plasma argon source. Calibration curves were constructed by means of standard solutions of calcium, according to Pires et al. (2015). The analyses were performed in triplicate.

The analyses of moisture, ash, lipids, carbohydrates, proteins, total dietary fiber, calcium and total phenolic compounds were performed as reported previously (Silva et al., 2017) and the data were used to determine the composition of the experimental diets, because we employed the same chia sample for the standard and high-fat diet group (lipids: 32.2%; carbohydrates: 4.6%; proteins: 18.2%; total dietary fiber: 33.4%; calcium: 0.25%; total phenolic: 0.97%). The chemical composition was determined according to AOAC (2012). The total energy value of chia was estimated considering the conversion factors of 4 kcal·g^{-1} for protein or carbohydrate and 9 kcal·g^{-1} for lipids.

2.3. Animals and diets

A controlled experimental study was carried out to assess the bioavailability of calcium by measuring calcium balance, retention, and absorption. Thirty-two male rats (*Rattus norvegicus*, *Wistar, albinus* variation), newly weaned, 21 days old, from the Central Animal Facility of the Center for Biological Sciences and Health at Federal University of Viçosa, Minas Gerais, Brazil, were systematically subdivided into 4 groups with 8 animals each, randomized by body weight. The animals were distributed into individual stainless-steel cages in a controlled temperature environment (22 °C) and automatically controlled light and dark cycles of 12 h. The animals received deionized water and their respective experimental diets ad libitum. The experimental diets were based either on the standard AIN-93G diet (Reeves & Fahey, 1993) or high-fat diet (Research Diets, New Brunswick, NJ) with modifications. The standard diet was composed of 20% protein, 30% fat and 50% carbohydrate. The high fat diet was prepared in the following proportions: 64% fat, 16% protein and 20% carbohydrate. The amount of chia offered in the diets, which was based on composition, provided 50% of the recommended amount (0.0025 kg of calcium per 1 kg of diet) (Bryk et al., 2016). The other ingredients were added in sufficient quantities to provide the planned amounts of lipids, proteins, carbohydrates, fiber, and calories (Table 1).

The experimental groups received one of the following four diets: standard diet + calcium carbonate (SD + CC), standard diet + chia (SD + C), high-fat diet + calcium carbonate (HFD + CC), or high-fat diet + chia (HFD + C). Chia served as a source of calcium in the SD + C and HFD + C groups, whereas calcium carbonate was used as

Table 2
Sequencing primers used in the RT-qPCR analysis.

Genes	Oligonucleotide (5′-3′)	
	Forward	Reverse
GAPDH	AGGTTGTCTCCTGTCACTTC	CTGTTGCTGTAGCCATATTC
PPAR-α	CCTGCCTTCCCTGTGAACT	ATCTGCTTCAAGTGGGGAGA
NFκB	ACCGAAGCAGGAGCTATCAA	GCGTACACATTCTGGGGAGT
Zn-SOD1	GAGCAGAAGGCAAGCGGTGAA	CCACATTGCCCAGGTCTG
TNF- α	ACGGCATGGATCTCAAAGAC	AGATAGCAAATCGGCTGACG
IL-10	ACTACCATAGCCACAACGCA	TTTCTGTTTCCTACGGCGCT

GAPDH: glyceraldehyde 3-phosphate dehydrogenase; PPAR-α: peroxisome proliferator-activated receptor alpha; NFκB: factor nuclear kappa B; SOD: superoxide dismutase; TNF-α: tumor Necrosis Factor Alpha; IL-10: interleukin 10.

the source of calcium in the SD + CC and HFD + CC groups. On the 35th day, after 12 h of fasting, the animals were anesthetized with isoflurane (Isoforine, Cristália®) and blood was collected by cardiac puncture. Blood was centrifuged at 4 °C for 600 s (Fanem-204, São Paulo, Brazil) and the plasma was stored at −80 °C. Urine, feces, liver tissue, and the right femur were collected and stored at −80 °C before analysis. Prior to euthanasia, each animal was individually housed in a metabolic cage. Samples of urine and feces were collected during 3 days for determination of the calcium bioavailability.

Body weight gain and food consumption were monitored weekly during the experimental period to determine the feed efficiency ratio (FER) (Silva et al., 2016). The hepatosomatic index and cecal index were calculated as a relation between liver weight or cecum weight and body weight, respectively, multiplied by 100 (Kim, Hong, Jeon, & Kim, 2016).

All the experimental procedures with animals were performed in accordance with Directive 86/609/EEC of November 24, 1986, in compliance with the ethical principles for animal experimentation. The study protocol was approved by the Ethics Committee of the Federal University of Viçosa (Protocol 20/2017; date of approval: July 13th 2017).

2.4. Biochemical analysis

For the determination of biochemical parameters, 0.5 mL of plasma was used. Plasma glucose concentrations, total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), very-low density lipoprotein (VLDL), triacylglycerides (TGL), uric acid, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured by colorimetric methods using commercially available kits (Bioclin®, Belo Horizonte, Brazil). Analyses were conducted on a BS-200 Chemistry Analyzer (Bioclin®).

2.5. Calcium bioavailability

For each animal, calcium intake was estimated according to daily food intake, and calcium absorption, balance, and retention were calculated using equations proposed by Ku, Cho, Choi, and Kim (2015).

Blood plasma and urine calcium levels were measured by colorimetric methods involving commercially available kits (Bioclin®, Belo Horizonte, Brazil). Analyses were performed on a BS-200 Chemistry Analyzer (Bioclin®). The calcium concentrations in feces and the femurs of experimental animals were determined by atomic absorption spectrophotometry (Gomes, 2011).

The size and thickness of the femur were measured by means of a 200-mm digital pachymeter (resolution, 0.01 mm; Model 530–312; Mitutoyo). Maximum tensile strength was determined using a Universal Mechanical Testing Machine (Instron, Norwood, MA USA). Resistance curves were built on a microcomputer in the Blue Hill software.

2.6. Extraction of mRNA from liver tissue and cDNA synthesis

Liver tissue was macerated in liquid nitrogen under RNAse free conditions and the samples were aliquoted for total RNA extraction. Total RNA was extracted with the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). The extracted mRNA was used to synthesize the cDNA with the M-MLV reverse transcription kit (Invitrogen Corp., Grand Island, NY) (Livak & Schmittgen, 2001).

2.7. Determination of gene expression of proteins involved in inflammation by quantitative reverse transcriptase polymerase chain reaction (RT-qPCR)

mRNA Expression levels of genes in the liver that are involved in inflammation processes was analyzed by RT-qPCR. The SYBR Green PCR master mix from Applied Biosystems (Foster City, CA) was employed, and the analyses were performed on the StepOne™ Real-Time PCR System (Thermo Fisher Scientific) by means of the measurement system involving SYBR-Green Fluorescence and Primer Express software (Applied Biosystems, Foster City, CA). Sense and antisense primer sequences were ordered (Choma Biotechnologies) to amplify PPAR-α (ID: 2404316), NFκB (ID: 234230511), Zn-SOD1 (ID: 5615763), TNF-α (ID: 234230513), and IL-10 (ID: 537719). The relative expression levels of mRNA were normalized to the endogenous control:glyceraldehyde 3-phosphate dehydrogenase (GAPDH;Table 2). All the steps were performed under open conditions with RNase.

2.8. NF-κB p65 and PPAR-α quantification

To determine the concentrations of PPAR-α and NF-κB p65 in the liver, hepatic tissue samples were homogenized by means of the NE-PER Nuclear and Cytoplasmic Extraction Kit reagents (Thermo Scientific Fisher, USA). The nuclear fractions of PPAR-α and NF-κB p65 were assessed by immunoassay using the Rat PPAR (Cat # E-EL-R0725-ra; Elabscience, USA) and Rat NF-κB p65 (Cat #E-EL-R0674, Elabscience, USA), ELISA kits, respectively. The microplates that were provided in the ELISA kits were respectively precoated with anti-PPAR-α and anti-NF-κB p65 antibodies. The concentrations of PPAR-α and NF-κB p65 were calculated by comparison to the corresponding standard curves.

2.9. Lipid peroxidation and oxidative stress levels analysis

2.9.1. Homogenate preparation

To obtain a liver homogenate, 200 mg of liver was mixed with 1000 µL of phosphate buffer (50 mM) and 1 mM EDTA (pH 7.4). The sample was macerated and centrifuged at 12,000 × *g* and 4 °C for 10 min, and then the supernatant was removed and stored in an ultra-freezer until analysis.

2.9.2. Malondialdehyde (MDA)

MDA was quantified by the thiobarbituric acid reactive substances (TBARS) method (Kohn & Liversedge, 1944; Pyles, Stejskal, & Einzig, 1993). MDA concentration was calculated via the molar absorptivity coefficient (Buege & Aust, 1978), and the results were expressed in nanomoles of MDA per milligramof protein (MDA/PTN). Total protein in the liver homogenate was quantified by the Bradford method (Bradford, 1976).

2.9.3. Nitric oxide

This analysis was performed in liver tissue and plasma: 50 µL of plasm or homogenate was mixed with solutions A (1% sulfanilamide in 2.5% H_3PO_4) and B (0.1% naphthyl l ethylene diamide dihydrochloride in 2.5% H_3PO_4) in the ratio (1:1) and the microtiter plate was incubated in the dark for 10 min. Absorbance was read on a spectrophotometer (Multiskan Go, Thermo Cientific) at 570 nm and the results were expressed in (µmol NO)//(mg protein) in hepatic tissue and in µM in

plasma (Green et al., 1982).

2.9.4. Superoxide dismutase (SOD)

The quantification of SOD was performed in relative units, and one unit was defined as the amount of SOD enzyme that inhibits the pyrogallol oxidation rate by 50%. The analysis was carried out on a spectrophotometer (Multiskan GO, Thermo Scientific) at 570 nm, and the results were expressed as units of SOD activity per milligram of protein (Marklund, 1985).

2.9.5. Catalase (CAT)

Catalase was analyzed according to the methodology proposed by Aebi (1984). At 0, 30, and 60 s after the reaction was initiated, the absorbance at 240 nm was determined on a spectrophotometer (T70 + UV/VIS Spectrometer). Enzymatic activity was recorded in micromoles per milliliter of a sample. Catalase activity was calculated according to Lambert Beer's law.

2.9.6. Liver and plasma antioxidant capacity

Aliquots (10 μL) of the liver homogenate or plasma were added to the wells along with 20 μL of the reagent metmyoglobin and 150 μL of the ABTS solution. Then, 10 μL of increasing concentrations of trolox standard were pipetted into the wells, in triplicate, to construct a standard curve. The microtiter plate was incubated at room temperature, and then the absorbance at 405 nm was read by means of a spectrophotometer (Multiskan GO). The values are expressed in mM Trolox equivalents.

2.10. Statistical analysis

The treatments were conducted in a completely randomized design, with eight replicates. The results were subjected to analysis of variance. To determine “F-value” significance, the *t*-test and post hoc Newman-Keuls test were carried out to compare means among the diet groups. The main dispersion was expressed as standard deviation. Statistical analyzes were performed in the SPSS software, version 20.0. Data with a *P*-value < .05 were considered statistically significant.

3. Results

All four diets contained the same amount of calcium ($p > .05$), which was approximately 0.0025 kg per kg of diet. At the beginning of the study, until the third week, the total food intake ($p \leq .05$) was higher in in the rats fed the standard diet (SD + CC and SD + C) than in the rats fed the high-fat diet. By contrast, at the end of the experiment, animals that were fed the standard diet containing chia (SD + C) showed higher ($p \leq .05$) food intake than did the other diet groups (Fig. 1). Consumption of phytic acid and total phenolics was higher ($p < .05$) in animals fed the standard diet containing chia (SD + C) than in animals feed the high-fat diet with chia (HFD + C; Table 3). In the groups without chia, these parameters were not measured.

Animals that were fed with calcium carbonate showed higher ($p \leq .05$) saturated fatty acid intake and lower ($p \leq .05$) polyunsaturated fatty acid intake than did the animals that were fed chia (Table 3). In addition, calcium intake was higher ($p \leq .05$) in the rats fed a standard diet with chia (SD + C) owing to their higher food intake (Table 3). Animals fed the standard and high-fat diets with calcium carbonate (SD + CC and HFD + CC) showed lower ($p \leq .05$) weight gain than did the other groups (Table 3). The food efficiency ratio was higher ($p \leq .05$) in the animals fed the high-fat diet (HFD + CC and HFD + C) when compared to the animals fed a standard diet (SD + C and SD + CC; Table 3).

Liver weight and the hepatosomatic index were higher ($p \leq .05$) in the groups that were fed the high-fat diet (HFD + CC and HFD + C) than in the groups that were fed the standard diet (SD + CC and SD + C). The cecal weight and cecal index did not differ significantly among the four experimental groups ($p > .05$; Table 3).

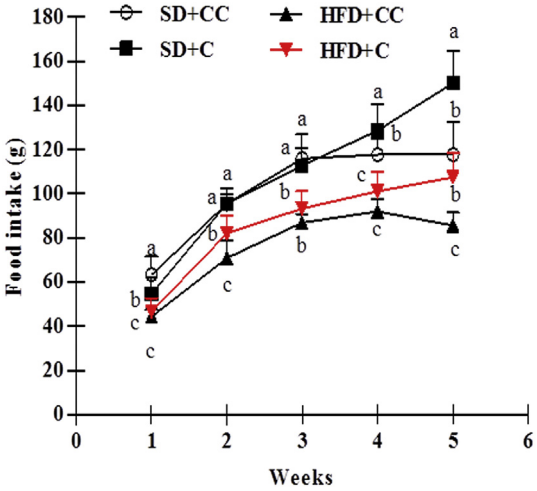

Fig. 1. Food intake of the experimental animals. *Means followed by the same letter in the same graphic did not differed by Newman-Keuls test at 5% of probability. SD + CC: standard diet + calcium carbonate; SD + C: standard diet + chia; HFD + CC: high fat diet + calcium carbonate; HFD + C: high fat diet + chia.

Chia consumption in the rats fed either the standard or high-fat diet led to a lower ($p \leq .05$) calcium balance and calcium absorption and retention rates as compared to the rats fed either the standard or high-fat diet with calcium carbonate. In addition, the urinary calcium concentration was lower ($p \leq .05$) in the groups that were fed chia (SD + C and HFD + C) and in the group fed the high-fat diet with calcium carbonate (HFD + CC). Nonetheless, fecal calcium content was lower ($p \leq .05$) in the animals that were fed calcium carbonate (SD + CC and HFD + CC). The calcium concentration in the femur and blood did not differ among the four groups ($p > .05$) (Fig. 2).

Although bone calcium content did not differ among the experimental groups, the bone resistance of rats fed chia (SD + C and HFD + C) and HFD + CC rats was lower ($p \leq .05$; 31.3-, 51.4-, and 35.8 fold lower, respectively) than that in the rats fed the standard diet with calcium carbonate (SD + CC) (Fig. 2).

The rats fed chia (SD + C and HFD + C) showed lower total cholesterol, VLDL, and LDL cholesterol levels ($p < .05$) than did the calcium carbonate (SD + CC and HFD + CC). In contrast, glucose, HDL, AST, urea, and creatinine levels did not differ among the four diet groups ($p > .05$). Nonetheless, the ALT concentration was higher ($p \leq .05$) in the two groups that consumed the high-fat diet (HFD + C and HFD + CC). Triglycerides levels were lower ($p \leq .05$) in the animals that were fed the standard diet with chia (SD + C) than in the animals that were fed the high-fat diet without chia (HFD + CC) (Table 4).

SOD mRNA expression did not differ among experimental groups ($p > .05$). Nevertheless, the animals fed the high-fat diet with chia (HFD + C) manifested a higher ($p \leq .05$) SOD activity in the liver than did the animals fed the high-fat diet without chia (HFD + CC), with values comparable to those of rats fed the standard diet with chia (SD + C) (Table 5). Consumption of the high-fat diet for 35 days did not alter the total antioxidant capacity of the liver and plasma ($p > .05$) in young *Wistar* rats (Table 5). In addition, chia consumption in rats fed the standard or high-fat diet did not improve these parameters. Nevertheless, the plasma catalase concentration was higher ($p \leq .05$) in the rats that were fed chia. The concentration of MDA in the liver and concentration of NO in the liver and plasma did not differ among the different experimental groups ($p > .05$) (Table 5).

PPAR-α mRNA expression (Table 5) was higher ($p \leq .05$) in the rats fed the high-fat diet containing chia (HFD + C) than in the other diet groups. Nonetheless, PPAR-α protein levels were higher ($p \leq .05$) in the

Table 3
Weight gain, FER, indexes and consumption of phytic acid, phenolic compounds, fatty acids and calcium by the experimental animals (n = 8) for 35 days.

Groups	SD + CC	SD + C	HFD + CC	HFD + C
Weight gain (g)	164.40 ± 15.50^{b}	183.20 ± 14.34^{a}	150.76 ± 11.36^{b}	182.14 ± 13.97^{a}
FER	31.82 ± 1.53^{b}	33.88 ± 1.77^{b}	39.67 ± 2.70^{a}	42,31 ± 1.51^{a}
Liver index (g/100 g)	5.66 ± 0.38^{b}	5.20 ± 0.23^{b}	6.35 ± 0.33^{a}	6.14 ± 0.36^{a}
Cecum index (g/100 g)	2.23 ± 0.30^{a}	2.60 ± 0.47^{a}	2.68 ± 0.54^{a}	2.23 ± 0.34^{a}
Phytic acid (g/kg/day)	–	2.21 ± 0.15^{a}	–	1.73 ± 0.13^{b}
Total phenolic intake (mg GAE/kg/day)	–	2.20 ± 0.17^{a}	–	1.75 ± 0.13^{b}
Fatty acids intake (g/kg/day)				
Saturated	86.27 ± 5.03^{a}	6.55 ± 0.58^{c}	63.59 ± 2.89^{b}	5.20 ± 0.19^{c}
Polyunsaturated	26.00 ± 1.52^{c}	60.74 ± 2.13^{a}	19.16 ± 0.87^{d}	50.15 ± 2.85^{b}
n-*3*	0.20 ± 0.01^{c}	14.77 ± 0.98^{a}	0.15 ± 0.01^{c}	11.74 ± 0.65^{b}
n-*6*	3.05 ± 0.14^{c}	4.12 ± 0.27^{a}	2.27 ± 0.11^{d}	3.28 ± 0.18^{b}
Calcium intake (g/kg/day)	1.19 ± 0.07^{b}	1.32 ± 0.01^{a}	0.97 ± 0.04^{c}	1.11 ± 0.08^{b}

SD + CC: standard diet + calcium carbonate; SD + C: standard diet + chia; HFD + CC: high fat diet + calcium carbonate; HFD + C: high fat diet + chia. FER: weight gain/food intake x 100. Indexes: cecum and liver weight/body weight x 100. The phytic acid and total phenolic intake were analyzed by the *t*-test. Means followed by the same letter in the same row did not differ significantly according to the Newman–Keuls test at the 5% threshold of probability.

rats that were fed chia, regardless of the diet type (i.e., in SD + C and HFD + C rats; Table 5). The mRNA expression and the protein levels of NFκB were lower ($p \leq .05$) in the rats fed chia (SD + C and HFD + C) and in the rats fed the standard diet with calcium carbonate (SD + CC; Table 5). Furthermore, mRNA expression of TNF-α and IL-10 were lower ($p \leq .05$) in the animals fed chia (SD + C and HFD + C).

4. Discussion

Chia is a good source of calcium; however, it has not been previously reported its bioavailability associated with inflammatory condition. Thus, the present study focused on the bioavailability of calcium in chia for rats fed a standard or a high-fat diet in part to determine whether a typical Western diet can reduce the bioavailability of calcium. In addition, the study allowed us to test whether chia consumption can improve calcium absorption, the lipid profile, oxidative stress, and inflammatory status in young rats.

The lower food intake and the lower phytic acid and phenolic consumption observed in animals fed the high-fat diet can be attributed to higher energy density and greater satiety during the experimental period, as found other studies (Natal et al., 2017; Silva, Matyelka, et al., 2016). Chia intake reduced the amount of saturated fatty acid and increased the amount of polyunsaturated fatty acids, owing to the composition of unsaturated fatty acids present in chia seeds, e.g., n-3 and n-6 fatty acids (Silva et al., 2017). A significant body weight difference among the groups was observed only in the last week of the experiment. On the other hand, it is known that weight is a less sensitive variable, and that changes in weight are typically observed later than changes in biochemical or cellular markers (Natal et al., 2017).

Despite the increase in liver weight in our HFD group owing to greater accumulation of fat globules, the levels of the ALT and AST liver enzymes were within the normal range for rodents (Spinelli, Cruz, Godoy, Motta, & Damy, 2014). Nevertheless, ALT concentration was higher in the animals fed the high-fat diet, thus indicating the beginning of a proinflammatory state, which can alter liver homeostasis and cause liver injury (Warner et al., 2017). The cecal weight and cecal index did not differ among the experimental groups, likely because these diets were balanced in terms of macronutrients and dietary fiber. The same result was observed in a another study that evaluated inflammation modulation in adult *Wistar* rats with high-fat diet-induced obesity; in that study, the high-fat diet did not promote differences in cecum weight between test and standard groups (Natal et al., 2016).

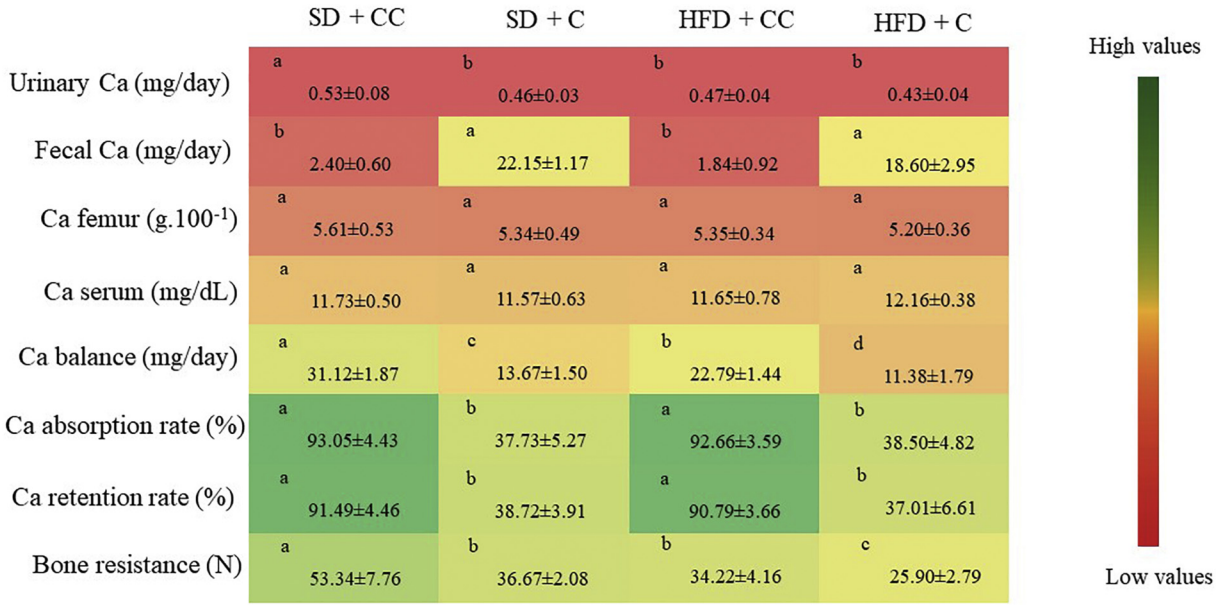

Fig. 2. Effect of chia ingestion in calcium bioavailability in *Wistar* rats (n = 8) for 35 days. SD + CC: standard diet + calcium carbonate; SD + C: standard diet + chia; HFD + CC: high fat diet + calcium carbonate; HFD + C: high fat diet + chia; Ca: calcium, N: newton. Average scores on the lines followed by different letters differ by Newman Keuls test ($p < .05$).

Table 4
Effects of chia consumption for 35 days on the biochemical variables in *Wistar* rats ($n = 8$).

Groups	SD + CC	SD + C	HFD + CC	HFD + C
Glucose ($mg \cdot dL^{-1}$)	188.25 ± 47.35[a]	186.39 ± 10.39[a]	197.63 ± 20.46[a]	190.14 ± 20.01[a]
HDL ($mg \cdot dL^{-1}$)	20.67 ± 2.94[a]	24.57 ± 3.40[a]	21.88 ± 4.36[a]	27.00 ± 4.24[a]
TC ($mg \cdot dL^{-1}$)	56.00 ± 9.73[a]	43.02 ± 8.89[b]	61.03 ± 9.16[a]	46.00 ± 5.87[b]
TGL ($mg \cdot dL^{-1}$)	53.26 ± 7.79[ab]	43.56 ± 8.83[b]	61.53 ± 6.22[a]	52.38 ± 6.20[ab]
AST ($U \cdot L^{-1}$)	154.40 ± 32.03[a]	137.88 ± 9.57[a]	161.83 ± 17.15[a]	155.43 ± 20.12[a]
ALT ($U \cdot L^{-1}$)	45.83 ± 7.39[b]	46.00 ± 6.12[b]	55.67 ± 6.41[a]	55.71 ± 1.80[a]
VLDL ($mg \cdot dL^{-1}$)	10.65 ± 1.56[a]	8.71 ± 1.89[b]	12.31 ± 1.24[a]	10.12 ± 1.47[b]
LDL ($mg \cdot dL^{-1}$)	27.80 ± 5.86[a]	10.34 ± 1.97[b]	27.19 ± 3.90[a]	11.72 ± 2.19[b]
Urea ($mg \cdot dL^{-1}$)	1.90 ± 0.21[a]	1.87 ± 0.47[a]	1.91 ± 0.44[a]	1.92 ± 0.26[a]
Creatinine ($mg \cdot dL^{-1}$)	0.36 ± 0.04[a]	0.35 ± 0.06[a]	0.31 ± 0.03[a]	0.32 ± 0.04[a]

SD + CC: standard diet + calcium carbonate; SD + C: standard diet + chia; HFD + CC: high fat diet + calcium carbonate; HFD + C: high fat diet + chia; HDL: high-density lipoprotein; TC: total cholesterol; TGL: triacylglyceride; AST: alanine aminotransferase; ALT: aspartate aminotransferase; VLDL: very-low density lipoprotein; LDL: low-density lipoprotein.
Average values in the same row followed by different superscript letters differ significantly according to the Newman–Keuls test ($p < .05$).

Because the body can regulate calcium metabolism via specific mechanisms, calcium concentration in the femur and blood did not differ among the experimental groups. When calcium intake is low there is an increase in calcium absorption and a reduction in its elimination (Fleet, 2017). This fact is supported by the urinary calcium concentration observed in the present study because the calcium content was lower in the rats that were fed the standard diet with chia (SD + C) and the high-fat diet (HFD + C and HFD + CC).

Furthermore, the data of bone resistance allows us to infer that high fat diet was able to reduce the bone resistance and the presence of chia did not improve this effect. In addition, all the evaluated parameters associated with calcium bioavailability were lower in the rats fed chia, thus, showing that the calcium present in chia is not well absorbed, metabolized, and/or utilized by the body when compared to a standard calcium source. The reduction in all the parameters associated with calcium bioavailability can be explained by two mechanisms: 1) the increased fecal calcium excretion observed in the animals that consumed chia and 2) chia is a food of plant origin, rich in phenolic compounds, tannins, and phytate (Silva et al., 2017) and can chelate calcium, forming insoluble molecules, thus reducing its bioavailability (Amalraj & Pius, 2015). It is worth mentioning that the calcium supplied in both the chia and calcium carbonate-containing diets was 50% of the recommended intake for rats. In the present study, we observed that chia consumption was able to improve the rat's lipid homeostasis. The same was observed in other studies (Fortino et al., 2017; Lucero et al., 2017; Poudyal, Panchal, Waanders, et al., 2012, Poudyal, Panchal, Ward, et al., 2012; Sierra, Roco, Alarcon, & Medina, 2015; Silva et al., 2016; Tenore et al., 2018). This result can be attributed to the higher polyunsaturated fatty acid intake by the rats fed chia as compared to the rats that consumed diets without chia. Chia is an oilseed that is rich in polyunsaturated fatty acids, such as n-3, n-6, and n-9 fatty acids, which exert positive effects on the blood lipid profile. It was shown that the high concentrations of n-3 fatty acids present in chia are associated with an improved lipoprotein profile in Wistar rats during a 48-day feeding period (Lucero et al., 2017). In addition, chia seeds contain high concentrations of dietary fiber, including insoluble fiber (Silva et al., 2017) and phenolic compounds, such as caffeic and rosmarinic acids, myricetin, quercetin, and kaempferol (Oliveira-Alves et al., 2017), which also have positive effects on the lipid profile (Natal et al., 2016; Noratto, Martino, Simbo, Byrne, & Mertens-Talcott, 2015; Silva, Dias, et al., 2016; Yang et al., 2011; Yung et al., 2013). In a recent study, our research group demonstrated that chia consumption can reduce glucose, triacylglyceride, LDL cholesterol, and VLDL cholesterol concentrations in the blood of *Wistar* rats fed chia for 28 days (Silva, Dias, et al., 2016). The same result was obtained in a study that evaluated the potential effects of chia seeds on plasma triglyceride levels of healthy subjects with moderate dyslipidemia (Tenore et al., 2018).

Although SOD mRNA expression did not differ among experimental groups, SOD quantification was lower in group that received high fat diet with calcium carbonated suggesting greater generation of free radicals. Chia intake increased the concentration of SOD and catalase, without altering the oxidative stress (MDA and NO) and maintained the total antioxidant activity of plasma and liver, increasing the activity of antioxidants enzymes which have ability to defend the body against the oxidative stress. The same effect was observed by Marineli et al. (2015) and Rincón-Cervera et al. (2016) that conclude chia intake was able to

Table 5
Effects of chia consumption for 35 days on the inflammation and oxidative stress in *Wistar* rats (n = 8).

Groups	SD + CC	SD + C	HFD + CC	HFD + C
SOD (U SOD/mg protein)	5.12 ± 0.23[a]	4.84 ± 0.48[ab]	4.31 ± 0.16[c]	4.60 ± 0.33[b]
TAC (mM trolox)	0.67 ± 0.05[a]	0.71 ± 0.05[a]	0.67 ± 0.07[a]	0.75 ± 0.05[a]
LAC (mM trolox)	0.14 ± 0.00[a]	0.12 ± 0.04[a]	0.13 ± 0.01[a]	0.13 ± 0.04[a]
CAT (μmol/min/mL)	4.59 ± 0.77[ab]	5.54 ± 0.89[a]	3.96 ± 0.48[b]	4.98 ± 0.86[a]
MDA (nmol/mg protein)	3.47 ± 0.18[a]	3.49 ± 0.44[a]	3.27 ± 0.18[a]	3.31 ± 0.52[a]
Hepatic nitric oxide (μmol/mg protein)	$4.72 \times 10^{-6} \pm 2.40 \times 10^{-7}$[a]	$4.95 \times 10^{-6} \pm 4.11 \times 10^{-7}$[a]	$4.14 \times 10^{-6} \pm 3.81 \times 10^{-7}$[a]	$4.57 \times 10^{-6} \pm 1.02 \times x10^{-6}$[a]
Plasma nitric oxide (μmol)	2.82 ± 0.48[a]	3.27 ± 0.65[a]	2.54 ± 0.51[a]	2.92 ± 0.46[a]
mRNA PPAR-α/mRNA GAPDH	1.00 ± 0.00[b]	0.45 ± 0.01[b]	0.40 ± 0.10[b]	1.94 ± 0.59[a]
Hepatic PPAR-α (ng/mL)	30.28 ± 0.38[b]	48.92 ± 0.50[a]	36.47 ± 0.87[b]	58.70 ± 0.59[a]
mRNA NFκB/mRNA GAPDH	1.00 ± 0.00[b]	0.75 ± 0.18[b]	7.40 ± 1.27[a]	2.28 ± 0.51[b]
Hepatic NFκB (ng/mL)	1.80 ± 0.82[b]	1.38 ± 0.12[b]	2.79 ± 0.47[b]	1.96 ± 0.35[a]
mRNA TNF-α/mRNA GAPDH	1.00 ± 0.00[a]	0.76 ± 0.12[b]	1.13 ± 0.10[a]	0.13 ± 0.02[b]
mRNA IL-10/mRNA GAPDH	1.00 ± 0.00[a]	0.44 ± 0.12[b]	1.65 ± 0.51[a]	0.33 ± 0.10[b]

SD + CC: standard diet + calcium carbonate; SD + C: standard diet + chia; HFD + CC: high fat diet + calcium carbonate; HFD + C: high fat diet + chia; MDA: malondialdehyde. SOD: superoxide dismutase; TAC: total antioxidant capacity; LAC: liver antioxidant capacity; CAT: catalase; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; PPAR-α: peroxisome proliferator-activated receptor alpha; NFκB: factor nuclear kappa B; TNF-α: tumor necrosis factor alpha; IL-10: interleukin 10.
Average scores in the same row followed by different superscript letters differ significantly according to the Newman–Keuls test ($p < .05$).

increase SOD concentrations in *Wistar* rats. Plasma catalase concentration was higher in the animals that consumed chia in our study, owing to a positive effect of the compounds present in this food on the activity of the antioxidant enzyme capable of decomposing hydrogen peroxide.

In our study, PPAR-α negatively regulates inflammation since its ligands inhibited pro-inflammatory cytokines, interfering with the NF-κB signaling pathway once NFκB and TNF-α are major transcription factors involved in inflammatory responses (Fountain, Abernathy, Cannon, Joiner, & Hillman, 2017). Chia consumption was able to inhibit the activation of NF-κB and TNF-α, decreasing inflammatory cytokine, as TNF-α, leading to improve in anti-inflammatory body capacity in standard diet and high fat diet that subsequently cause a low-grade inflammatory state (Catrysse & Van Loo, 2017). Thus, based on these results, chia consumption had a beneficial effect on one of the pathways of inflammation in *Wistar* rats, regardless of diet type (standard or high fat). The same effect was observed in other studies that analyzed chia seeds and the omega-3 unsaturated fatty acids resulting from chia consumption in rats feed a high fat diet (Poudyal et al., 2013; Poudyal, Panchal, Waanders, et al., 2012; Poudyal, Panchal, Ward, et al., 2012). This phenomenon could be due the fatty acid,phenolic compounds, and other bioactive compounds present in the food, such as vitamins, minerals, and antioxidant substances (Silva et al., 2017) that increased PPAR-α expression and reduced TNF-α expression in our study, which may, in turn, control secreted factors, gene expression, and cell signaling pathways, thereby reducing inflammation (Natal et al., 2016).

IL-10 mRNA expression diminished in the chia groups because the proinflammatory factors (mRNA expression of NFκB and TNF-α) decreased in these same groups. This finding is only logical because of modulation of the cascade of molecular events leading to a reduction in the production of anti-inflammatory substances, which occurs owing to lower expression of proinflammatory genes in order to maintain a homeostasis in the body (Tunon, Garcia-Mediavilla, Sanchez-Campos, & Gonzalez-Gallego, 2009).

In addition, it is known that inflammation leads to lower bone absorption and enhanced bone resorption because of increased production of proinflammatory cytokines, which downregulates osteoblastogenic pathways while activating osteoclastogenic processes (Wong et al., 2016). In our study, the consumption of the high-fat diet resulted in increased inflammation, reduced the bone resistance, that was not accompanied by a reduction in bone calcium concentration. This finding suggested that long-term inflammation may interfere with calcium bioavailability in animals fed a high-fat diet. Therefore, this food should not be offered as an isolated, bioavailable source of calcium. Finally, the results of the molecular analyses and gene and protein expression assays as well as the evaluated biochemistry parameters were in accordance with the expected results, namely, low bioavailability of calcium in chia and the beneficial effect of chia consumption on the lipid profile and inflammatory processes in young *Wistar* rats fed standard or high fat diet, receiving 50% of calcium requirement on the diet.

5. Conclusions

Chia as a unique source of calcium, showed low calcium bioavailability regardless of the type of diet consumed. Nonetheless, chia consumption reduced inflammatory processes, improved the lipid profile and had no effect on oxidative stress in young *Wistar* rats. We believe that additional longer experimental studies on adult rats are needed to confirm these results.

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Compliance with ethical standards

All the procedures performed in this study involving animals were in accordance with the ethical standards of the Federal University of Viçosa and with the U.K. Animals (Scientific Procedures) Act, 1986.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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