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ORIGINAL PAPER

Chia Seed Shows Good Protein Quality, Hypoglycemic Effect and Improves the Lipid Profile and Liver and Intestinal Morphology of *Wistar* Rats

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Abstract Chia has been consumed by the world population due to its high fiber, lipids and proteins content. The objective was to evaluate the protein quality of chia untreated (seed and flour) and heat treated (90 °C/20 min), their influence on glucose and lipid homeostasis and integrity of liver and intestinal morphology of *Wistar* rats. 36 male rats, weanling, divided into six groups which received control diet (casein), free protein diet (aproteic) and four diet tests (chia seed; chia seed with heat treatment; chia flour and chia flour with heat treatment) for 14 days were used. The protein efficiency ratio (PER), net protein ratio (NPR) and true digestibility (TD) were evaluated. The biochemical variables and liver and intestinal morphologies of animals were determined. The values of PER, NPR and TD did not differ among the animals that were fed with chia and were lower than the control group. The animals that were fed with chia showed lower concentrations of glucose; triacylglycerides, low-density lipoprotein cholesterol and very low-density lipoprotein and higher high-density lipoprotein cholesterol than the control group. The liver weight of animals that were fed with chia was lower than the control group. Crypt depth and thickness of intestinal muscle layers were higher in groups that were fed with chia. The consumption of chia has shown good digestibility, hypoglycemic effect, improved lipid and glycemic profiles and reduced fat deposition in liver of animals, and also promoted changes in intestinal tissue that enhanced its functionality.

Keywords Protein digestibility · Heat treatment · Chia flour · Chia seed · Lipid · Glucose

Abbreviations

ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase
FER	food efficiency ratio
HDL	high-density lipoprotein cholesterol
LDL	low-density lipoprotein cholesterol
NPR	net protein ratio
PER	protein efficiency ratio
TC	total cholesterol
TD	true digestibility
TGL	triacylglycerides
VLDL	very-low density lipoprotein

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Introduction

Chia (*Salvia hispanica* L.) is an oilseed that stands out because of its high nutritional value [1, 2]. The chemical composition and nutritional value of chia seed vary according to species, climate conditions, place of cultivation and soil [3] and its main components are lipids, dietary fibers and proteins [4]. Chia contains high concentration of proteins [3, 5, 6] which are complex organic compounds essential to the human body. The quality of chia protein refers to its ability to meet the nutritional needs of the organism by means of essential amino acids and nonessential nitrogen, for protein synthesis [7]. The heat treatment and grinding applied to chia seeds for flour production can raise the nutritional quality of food, due to denaturation of its proteins and therefore increasing digestibility [8, 9]. The difference in protein digestibility of chia may be due to the structural modification process which involves heat treating and grinding [9–11].

The presence of lipids, fiber, phenolic compounds and peptides confer to chia an antioxidant activity [12, 13]. In addition, these compounds are related to hypoglycemic effect in humans [14]. However, so far, studies that evaluated the effect of chia in intestinal and liver morphologies have not been found. There are *in vitro* studies evaluating the protein quality of chia with or without heat treatment [2, 15, 16]. Nevertheless, these studies evaluate apparent digestibility, being necessary to evaluate the true digestibility, which is made in *in vivo* studies; however, *in vivo* studies are non-existent. Since chia has a high protein concentration, it is of great interest to determine the utilization of this *in vivo* nutrient. The objective of this study was to evaluate the protein quality of chia untreated (seed and flour) and heat treated; the influence of the chia intake on the homeostasis of lipids and glucose, as well as the food action in the liver and intestinal integrities of *Wistar* rats for a short period of time.

Materials and Methods

Raw Materials and Preparation of Flours

Chia seeds (*Salvia hispanica* L.) were purchased and came from the state of Rio Grande do Sul, Brazil, and were used along with four treatments, namely: chia seed untreated, chia seed with heat treatment, chia flour untreated and chia flour with heat treatment. To obtain flour, the seeds were grounded in three repetitions, using a knife mill with a particle size of 850 μm. For the heat treatment, the binomial time-temperature was based on *in vitro* study [16] with modifications in the heat exposure time. Chia seed and flours (522 g each) were exposed to 90 °C in an oven with air circulation (New Ética®, model 400/6ND, Brazil) for 20 min. Subsequently, seeds and flours were packed in polyethylene bags covered with foil and stored in a freezer (−18 ± 1 °C) until the time of analysis.

Chemical Composition of Chia Flour

For the determination of chemical composition, 15 g of chia flour were used. The determination of ash, protein, lipids, moisture and total dietary fiber was performed according to the methodology proposed by AOAC [17]. The concentration of carbohydrates was calculated by difference.

Determination of Phenolic Compounds and Phytates

For the determination of phenolic compounds and phytates, 3 g of chia flour were used. The total phenolic compounds in the flours were determined using the Folin-Ciocalteu method [18]. The concentration of phytate was determined by spectrophotometry according to Latta and Eskin [19] method with modifications [20].

Evaluation of Protein Quality of Chia Seeds

Determination of the True Digestibility (TD), Food Efficiency Ratio (FER), Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR)

The true digestibility was calculated according to Bender and Doell [21]. The FER was calculated from the ratio between the weight gain and the total dietary intake by animals. The PER was determined by using the equation proposed by Hegsted [22]. The NPR was determined by using the equation proposed by Bender and Doell [21].

Biological Assay

Preparation and Composition of Diets

The composition of the experimental diets was based on the AIN-93G diet [23], taking into account the chemical composition of chia flour (Table 1). The chia flour was used as a protein source to replace the casein in the diet tests. The amount of chia used to supply 100 % of the protein recommendation for rodents, also provided 100 % of the recommendation of lipids and cellulose, not being added in the AIN-93G diet tests. All diets were formulated to be isocaloric and isoproteic with protein percentage of 9.48 % (Table 2).

Experimental Animals

Thirty-six male rats (*Rattus norvegicus*, *Wistar*, *albinus* variation), newly weaned, and with 21 days of life were systematically divided into six groups with six animals each. The animals were distributed in individual metabolic stainless steel cages in controlled temperature environment (22 °C) and automatically controlled light and dark cycles of 12 h. The animals received distilled water and their respective experimental diets *ad libitum*. The experimental groups received the following diets: positive control (casein); negative control (aproteic); chia seed untreated; chia seed heat treated (90 °C for 20 min); chia flour untreated and chia flour with heat treatment (90 °C for 20 min). After 28 days and after 12 h fasting, the animals were anesthetized with isoflurane (Isoforine®, Cristália, Itapira, Brazil) and then were euthanized by cardiac puncture.

Table 1 Nutritional composition of chia flour, dry basis

Compounds	Concentration (g 100 g^{-1})
Moisture	7.14 ± 0.26
Ash	4.56 ± 0.04
Lipids	32.16 ± 0.29
Protein	18.18 ± 1.20
Total dietary fiber	33.37 ± 0.26
Soluble fiber	2.89 ± 0.09
Insoluble fiber	30.47 ± 0.35
Carbohydrates	4.59 ± 0.34
Phenolic compounds	0.97 ± 0.07
Phytate	0.96 ± 0.11

The study was approved by the Ethics Committee on Animal Research of the Federal University of Viçosa, Brazil (Protocol 97/2014).

Biochemical Analysis

For the determination of biochemical analysis, 0.5 mL of plasma was used. Plasma glucose concentrations, total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) and 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 following the manufacturer's instructions (Bioclin®, Belo Horizonte, Brazil). Analyses were performed on a Cobas Mira Plus device.

Histological Analysis

Semi-serial histological sections of fragments of the liver and cecum with 3 μm thickness were obtained in automatic microtome (Reichert-Jung®, Germany) and were stained by Toluidine Blue technique. The slides were examined under an Olympus CX31 light microscope. It was used ImageJ software (Java) through grid containing 336 points. Points about nucleus, cytoplasm, fat vesicles and hepatocytes were counted. To measure crypt depth and thickness of the circular and longitudinal muscle layers, 20 random fields *per* animal were selected [24] and were obtained by using the ImagePro-Plus® software version 4.5 (Media Cybernetics, Rockville, USA).

Statistical Analysis

The treatments were conducted in a completely randomized design, with six replications. The results were analyzed by analysis of variance. For "F-value" significant, the Duncan test was used to compare means among the experimental groups. Statistical analyzes were performed using the Statistical Analysis System software, version 9.1. *P*-value <0.05 was considered statistically significant.

Table 2 Composition of diet AIN 93-G

Ingredients (g Kg^{-1})	Experimental groups					
	Casein	Aproteic	Chia seed untreated	Chia seed with HT	Chia flour untreated	Chia flour with HT
Casein	117.2	–	–	–	–	–
Chia seed untreated	–	–	522	–	–	–
Chia seed with HT	–	–	–	522	–	–
Chia flour untreated	–	–	–	–	522	–
Chia flour with HT	–	–	–	–	–	522
Dextrinized starch	132	132	132	132	132	132
Sucrose	100	100	100	100	100	100
Soybean oil	168.08	168.08	–	–	–	–
Cellulose	154.5	154.5	–	–	–	–
Mineral mix	35	35	35	35	35	35
Vitamin mix	10	10	10	10	10	10
L-cystine	3	3	3	3	3	3
Choline bitartrate	2.5	2.5	2.5	2.5	2.5	2.5
Starch	277.72	394.92	195.5	195.5	195.5	195.5
Protein (%)	9.38*	–	9.48	9.48	9.48	9.48
Caloric density (kcal g^{-1})	4.08	4.17	3.93	3.93	3.93	3.93

HT heat treatment; * Considering that casein has 80% purity

Results and Discussion

High concentrations of dietary fiber (33.4 %), lipids (32.2 %) and proteins (18.2 %) were found in chia in the present study. Chia showed a concentration of phenolic compounds equal to 0.97 ± 0.07 mg GAE / g sample and phytates corresponding to 0.96 ± 0.11 g/100 g (Table 1). Animals fed with chia showed weight gain, FER, PER, NPR and TD lower ($p < 0.05$) than the control group (casein). The PER values observed in the groups fed with chia ranged from 1.73 to 1.92. However, there was no difference ($p > 0.05$) in the TD among the groups fed with chia. The weight of feces and fecal nitrogen were higher in the group who ate chia compared to the control group ($p < 0.05$) (Table 3). The lower digestibility observed in the groups fed with chia may be associated with a higher concentration of soluble fiber present in the food matrix (2.89 g/100 g) compared to casein (control) and the presence of phenolic compounds (0.97 g/100 g) and phytic acid (0.96 g/100 g) that can act as anti-nutritional factors. The phenolic compounds, phytic acid and dietary fiber can complex with the intestinal contents, preventing access of digestive enzymes and absorption of nutrients; and reducing protein digestibility [25, 26]. Chia presented good protein digestibility regardless of the heat treatment and grinding. The conversion efficiency of chia protein to promote weight gain was about 50 % in relation to casein, as it can be observed by means of PER values. Some authors [16] observed increased protein digestibility *in vitro* from isolated chia when subjected to heat treatment; however, our *in vivo* study contradicts this finding because the protein digestibility, PER and NPR did not differ among the groups fed with chia seed untreated, chia seed with heat treatment, chia flour untreated and chia flour with heat treatment. Similar digestibility values to our study were observed in heat treated soybean (approximately 79 %) and in raw and heat treated flaxseed flour (approximately 78 %) [27, 28]. The PER values observed in the studies above were higher than the ones in the present study. Moraes et al. [29] evaluated the protein quality of sorghum flour and they reported greater digestibility (86 % on average) and lower PER values than in our investigation, which suggests that the amino acid composition of chia is better than sorghum, because a lower digestibility of chia protein was effective to promote weight gain of the animals. The animals fed with different diets containing chia showed blood glucose levels lower ($p < 0.05$) than animals fed with casein (Table 4). This fact may be associated with the increased presence of soluble dietary fiber fraction of chia compared to control diet (casein), which received only cellulose. The soluble dietary fiber increases the viscosity of the intestinal lumen, reducing the contact of glucose with the enterocyte, thus decreasing its absorption [30]. The effect of chia intake in our study was beneficial, because the food promoted greater control of plasma glucose levels in a short period of time (28 days). The groups fed with chia seed or flour, with or without heat treatment, decreased ($p < 0.05$) TGL, LDL, VLDL and increased HDL (Table 4). This can be justified by the supply of chia that satisfied 100 % of the need for fiber and fat of animals. The reduction of HDL was observed in normal rats consuming chia seed oil for 4 weeks [1]. It is known that high concentrations of fatty acids present in the n-3 chia are related to the reduction of VLDL and TGL [31]. Total cholesterol was lower ($p < 0.05$) in the group fed with chia seed with heat treatment compared than in the control one. Thus, chia intake for a short period of time was able to promote improvement in the homeostasis of lipids in animals. Urea and creatinine concentrations did not differ among the experimental groups ($p > 0.05$) and the concentrations of ALT and AST were higher ($p < 0.05$) in all groups that ingested chia (approximately 1.4 and 1.6 times for AST and ALT, respectively) compared to the control group (casein); however, the concentrations of liver enzymes are within normal range for rodents [32]. The weight of the liver, volume of the cytoplasm and the percentage of hepatic fat were lower ($p < 0.05$) in the groups fed with chia than in the control group (casein). The reduction of the volume of hepatocytes in animals fed with different ways of processing chia was directly associated with the decrease in concentration of fat globules in the liver (Online Resource 1). Chia was able to decrease the percentage of liver fat and liver weights due to lower

Table 3 Effect of chia ingestion in protein quality index in *Wistar* rats ($n = 6$) for a period of 14 days

Groups	Casein	Chia seed untreated	Chia seed with HT	Chia flour untreated	Chia flour with HT
Weight gain (g)	68.66 ± 4.76^a	19.83 ± 5.49^b	24.00 ± 5.96^b	27.00 ± 8.43^b	25.33 ± 4.13^b
FER	0.34 ± 0.04^a	0.16 ± 0.02^b	0.20 ± 0.05^b	0.18 ± 0.03^b	0.19 ± 0.03^b
PER	3.45 ± 0.49^a	1.73 ± 0.22^b	1.84 ± 0.47^b	1.82 ± 0.32^b	1.92 ± 0.33^b
NPR	4.51 ± 0.60^a	3.22 ± 0.64^b	3.49 ± 0.65^b	3.32 ± 0.32^b	3.52 ± 0.42^b
Fecal dry weight (g)	5.16 ± 0.49^b	6.98 ± 0.41^a	7.55 ± 0.69^a	7.59 ± 1.28^a	7.45 ± 1.11^a
Fecal nitrogen (%)	0.94 ± 0.15^b	2.14 ± 0.12^a	2.20 ± 0.14^a	2.29 ± 0.18^a	2.21 ± 0.14^a
TD (%)	89.42 ± 4.10^a	69.43 ± 6.99^b	67.03 ± 5.34^b	72.23 ± 5.87^b	69.22 ± 4.55^b

FER food efficiency ratio, *PER* protein efficiency ratio, *NPR* net protein ratio, *TD* true digestibility, *HT* heat treatment. Average scores on the lines followed by different letters differ by Duncan test ($p<0.05$)

Table 4 Effect of chia intake on the biochemical variables in *Wistar* rats ($n = 6$) for a period of 28 days

Groups	Casein	Chia seed untreated	Chia seed with HT	Chia flour untreated	Chia flour with HT
Glucose (mg dL^{-1})	172.33 ± 14.22^{a}	118.83 ± 7.31^{b}	128.33 ± 20.66^{b}	127.17 ± 20.72^{b}	127.67 ± 16.77^{b}
HDL (mg dL^{-1})	28.00 ± 4.38^{b}	35.50 ± 3.23^{a}	32.20 ± 3.63^{a}	33.83 ± 3.50^{a}	37.50 ± 4.36^{a}
TC (mg dL^{-1})	63.83 ± 10.34^{a}	58.17 ± 4.79^{ab}	53.60 ± 10.06^{b}	58.67 ± 11.91^{ab}	61.00 ± 10.77^{a}
TGL (mg dL^{-1})	47.50 ± 17.75^{a}	27.33 ± 4.97^{b}	27.33 ± 3.83^{b}	26.34 ± 5.05^{b}	32.50 ± 5.61^{b}
AST (U L^{-1})	98.33 ± 15.96^{b}	155.33 ± 32.21^{a}	116. 50 ± 3.83^{a}	139.33 ± 13.75^{a}	126.33 ± 18.90^{a}
ALT (U L^{-1})	26.50 ± 5.01^{b}	46.17 ± 6.37^{a}	38.33 ± 10.88^{a}	44.00 ± 8.20^{a}	45.50 ± 8.73^{a}
VLDL (mg dL^{-1})	9.50 ± 3.55^{a}	5.47 ± 0.99^{b}	5.47 ± 0.77^{b}	5.27 ± 1.01^{b}	6.50 ± 1.12^{b}
LDL (mg dL^{-1})	26.33 ± 11.13^{a}	17.20 ± 4.79^{b}	13.70 ± 8.47^{b}	19.57 ± 4.59^{b}	17.00 ± 5.93^{b}
Urea (mg dL^{-1})	1.12 ± 0.19^{a}	1.20 ± 0.23^{a}	1.27 ± 0.31^{a}	1.12 ± 0.19^{a}	0.92 ± 0.43^{a}
Creatinin (mg dL^{-1})	0.20 ± 0.04^{a}	0.17 ± 0.06^{a}	0.15 ± 0.02^{a}	0.20 ± 0.04^{a}	0.21 ± 0.09^{a}

HT heat treatment, *HDL* high-density lipoprotein, *TC* total cholesterol, *TGL* triacylglyceride, *AST* alanine aminotaminotransferase, *ALT* aspartate aminotransferase, *VLDL* very-low density lipoprotein, *LDL* low- density lipoprotein. Average scores on the lines followed by different letters differ by Duncan test ($p < 0.05$)

accumulation of lipids in the body, which may have led to increased excretion of fat in the feces, since animals fed with chia showed an increase in fecal weight compared to the control one. Poudyal et al. [33] offered diets containing chia seed for obese mice for eight weeks and observed a reduction of hepatic steatosis in animals. This result may be due to the ability of chia to induce redistribution of lipids in the body, reducing its accumulation in the liver and visceral tissue, thereby exerting a hepatoprotective effect [33]. In our study, normal rats fed with chia for 28 days showed cardioprotective effect that may had a positive effect on insulin sensitivity, reducing plasma glucose levels. Animals receiving the chia flour untreated showed a cecum weight lower than the others ($p < 0.05$). The depth of the crypts and the longitudinal muscle layer thickness, circular and total, were higher in groups fed with chia than in the control group (Online Resource 1). This result is probably due to increased motility of the digestive tract by the intact form that the fiber is found in chia, besides the formation of gel promoted by the soluble fiber fraction, leading to hyperplasia and/or hypertrophy of muscle cells. The same can be explained for increased crypt depth, although cell size, cell turnover and the number of mitoses of intestinal cells had increased [34]. This same effect was observed in studies using soy flour [35] in rats. Thus, chia consumption was able to stimulate the acceleration of intestinal transit, and be beneficial in the prevention of diseases.

The results of our experiment demonstrated that animals fed with chia showed PER, NPR, TD, blood glucose levels, TGL, VLDL, LDL, weight of the liver and percentage of hepatic fat lower than the control group. The thickness of the intestinal muscular layer and HDL were higher in animals fed with chia. The consumption of chia in seed or flour form, with or without heat treatment, for a short period of time, showed good protein digestibility, hypoglycemic effect, improved lipid profile and reduced the deposition of hepatic fat, and promoted changes in the intestinal tissue which favored its functionality.

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Compliance with Ethical Standards All procedures performed in this study involving animals were in accordance with the ethical standards of the Federal University of Viçosa.

Conflict of Interest The authors declare that they have no conflict of interest.

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