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Extruded sorghum flour (*Sorghum bicolor* L.) modulate adiposity and inflammation in high fat diet-induced obese rats

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ABSTRACT

The aim was to determine the effect of extruded sorghum flour (ESF) on the adiposity and inflammation modulation in obese Wistar rats. Four experimental groups: normal control (AIN-93), obese control (HFD) and two tested groups (replacing 50% of cellulose and 100% of cornstarch by ESF in the HFD - HFDS50; and replacing 100% of cellulose and 100% of cornstarch by ESF in the HFD - HFDS100) were used. The luteolinidin and 5-methoxy-luteliolinidin were the main deoxyanthocianidins identified in ESF. The Lee index, percentage of adiposity, fatty acid synthase gene expression, TNF-α, blood levels of glucose and the adipocyte hypertrophy were lower in the groups treated with ESF when compared to HFD. The groups treated with ESF up-regulated the lipoprotein lipase, peroxisome proliferator activated receptor-γ gene expression and IL-10. ESF has potential as a functional food since it reduced the metabolic risk of obesity associated with adiposity and inflammation.

1. Introduction

Historically, obesity has been a problem in developed countries. However, overweight and obesity are increasing in developing and underdeveloped countries (WHO, 2014). Worldwide, obesity has more than doubled since 1980. In 2014, more than 1.9 billion adults were classified as overweight. Among those adults, over 600 million were obese (WHO, 2016). Obesity is characterized by the excess of fat mass, which accumulates due to an imbalance between energy intake and energy expenditure, as well as the contribution of environmental and genetic factors (WHO, 2016).

The fat mass in obesity is accompanied by hypertrophy and hyperplasia of the adipocyte. The change in adipocyte differentiation depends on the number of preadipocytes in adipocytes, a process known as adipogenesis (Queiroz et al., 2012). The peroxisome proliferator that activates the receptor-γ (PPAR-γ) is a central regulator of adipogenesis, which also activates the transcription of genes involved in insulin signaling, glucose uptake, and absorption and storage of fatty acids (Brown et al., 2003). Thus, PPAR-γ coordinates the expression of specific adipogenic genes such as fatty acid synthase (FAS) and lipoprotein lipase (LPL). FAS is responsible for the synthesis and accumulation of triglycerides during the differentiation of adipocytes. LPL is the most important regulator for the deposition of triglycerides, which is hydrolyzed to very low density lipoproteins (VLDL) and chylomicrons, releasing fatty acids, which are then uptaken by the adipocytes (Ahn, Lee, Kim, & Ha, 2007).

The consumption of natural antioxidants and the adoption of a healthy diet are the best options to reduce the incidence of obesity and others non communicable disease associated (Barbalho et al., 2011a, 2011b). In this context, sorghum (*Sorghum bicolor* L.) has been highlighted as a potential food for prevention and modulation of chronic diseases, which is associated with its content of dietary fiber, lipids, phenolic compounds, tannins and flavonoids such as anthocyanins, flavones and flavanones (Cardoso, Pinheiro, Martino, & Pinheiro Sant'ana, 2015; Moraes et al., 2012). The content of these compounds may vary depending on sorghum processing, such as extrusion cooking,

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oven drying and conventional stove cooking (Afify, El-Beltagi, El-Salam, & Omran, 2012; Cardoso, Pinheiro, Martino, & Pinheiro Sant'ana, 2015).

Isolated sorghum compounds have been investigated in vitro (Devi, Saravanakumar, & Mohandas, 2011) and *in vivo* (Chung et al., 2011; Moraes et al., 2012), demonstrating antioxidant and anti-inflammatory activity. Few studies have investigated the effect of whole sorghum in oxidative stress and inflammation in animals fed with high fat diets (Moraes et al., 2012). To date, no published studies have related the intake of extruded sorghum flour (ESF) by obese animals with the inflammation and adiposity modulation. This study reported the effect of the extruded sorghum flour added to a high fat diet on the modulation of adiposity and subclinical inflammation in obese Wistar rats.

2. Materials and methods

2.1. Standards and reagents

The standards of luteolinidin chloride, luteolin, apigenin, 5-methoxy-luteolinidin, 7-methoxy-apigeninidin, naringenin and eriodictiol (purity from 96.7% to 98.9%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The apigeninidin chloride (purity of 97.2%) was obtained from Chromadex (Santa Ana, CA, USA). High performance liquid chromatography (HPLC) grade reagents (acetic acid, acetone, acetonitrile, ethyl acetate, hexane, isopropyl alcohol, methyl alcohol, and formic acid) were purchased from Tedia (São Paulo, Brazil).

2.2. Raw sorghum and extruded grains/milling

Whole grains of the sorghum genotype SC 319 (origin: Uganda) with brown pericarp and with high levels of dietary fiber and phenolic compounds as tanins (proanthocyanidins) and 3-deoxyanthocyanidins (Cardoso, Pinheiro, Martino, & Pinheiro Sant'ana, 2015) were used. The sorghum seeds were planted at the Embrapa Milho e Sorgo research station, located in Nova Porteirinha, MG, Brazil, at latitude 15°47′S, longitude 43°18′W and 516 m above sea level, in June 2011. The grains were harvested, sorted, packed in polyethylene bags, and sent to Embrapa Agroindústria de Alimentos, Rio de Janeiro, Brazil, where they were processed. The whole sorghum grains were milled in a laboratory disk mill 3600 (Perten Instruments, Huddinge, Sweden) set to aperture 4 between the disks to produce a whole meal flour that was processed in a co-rotating twin-screw Evolum HT 25 (Clextral, Firminy, France) with a screw diameter of 25 mm, length: diameter ratio of 40:1 and ten temperature zones. The screw speed (1.009*g*), screw configuration and temperature profile were kept constant. Whole sorghum flours were fed into the feeding zone by a twinscrew, loss-in-weight gravimetric feeder model GRMD15 (Schenck Process, Darmstadt, Germany) at rate of 9 kg/h and were monitored by Schenck Process Easy Serve software (Schenck Process, Darmstadt, Germany). To compensate moisture differences in the samples and provide a final moisture content of 12%, distilled water was injected between the first and second feeding zones using a plunger metering pump model J-X 8/1 (AILIPU Pump Co. Ltd., China). The samples were collected over 15–20 min. Subsequently, the extrudates were dried in a fan oven at 60 °C and then ground in a 7.5 HP fitted knife-hammer mill with a sieve aperture of 1 mm (Treu, Rio de Janeiro, Brazil) to obtain extruded sorghum flour (ESF) (Vargas-Solorzano, Carvalho, Takeiti, Ascheri, & Queiroz, 2014).

2.3. Proximate composition and bioactive compounds in extruded sorghum

The proximate composition of the extruded sorghum was determined according to the methods proposed by the AOAC (Association of Official Analytical Chemists, 2005) and the carbohydrate content was estimated by the difference. The main 3-deoxyanthocianidins (luteolinidin – LUT, apigeninidin – APG, 7-methoxy-apigeninidin – 7-MeO-AP, and 5-methoxy-luteliolinidin – 5-MeO-LUT), flavones (luteolin and apigenin) and flavanones (naringenin and eriodictyol) were analyzed in the sorghum extract (1 g) in 10 mL of methanol (60:40, v: v) prepared for determination of unbound phenols. Analysis was performed in a HPLC system (Shimadzu, SCL 10AT VP, Japan) equipped with diode array detector (Shimadzu, SPD-M10A, Japan), high pressure pump (Shimadzu, LC-10AT VP, Japan), auto sampler with loop of 500 µL (Shimadzu, SIL-10AF, Japan), and helium degassing system using the chromatographic conditions described by Cardoso et al. (2014) and Yang, Allred, Geera, Allred, and Awika (2012).

The flavonoids were identified by UV–Vis spectra and retention times of the commercial standards. The standard curves prepared using the commercial phenolic standards were used to quantify the corresponding flavonoids. The 5-Meo-LUT and 7-MeO-AP were quantified using luteolinidin and apigeninidin standards, respectively, along with the appropriate molecular weight correction factor (Dykes, Seitz, Rooney, & Rooney, 2009). The R^2 of the standard curve ranged from 0.9939 to 0.9992, limits of detection from 18.98 to 35.12 ng/mL, and limits of quantification from 94.90 to 175.60 ng/mL. The compounds were expressed in µg/g of sample, as single compounds and as the sum of 3-DXAs, flavones, flavanones and flavonoids.

The proanthocyanidins presented in 1 g of sorghum flour were extracted in 20 mL of 70% acetone in water and stirred for 120 min. The suspension was centrifuged at 2790*g* for 10 min and the supernatant was collected and evaporated until complete dryness by rotary evaporation TE 211 (Tecnal, São Paulo, Brazil). The proanthocyanidins were evaluated by HPLC using a fluorescence detector with detection at 230 nm and emission at 321 nm (Langer, Marshall, Day, & Morgan, 2011). The concentration of proanthocyanidins was expressed in µg of catechin equivalents (corrected for molecular weight)/g sample (µg CatEq/g).

2.4. Experimental diets

The cellulose of the test diets was replaced with 50% and 100% of ESF, as source of dietary fiber, which substituted 100% of cornstarch in the diet. In addition, the other ingredients of the diets (casein, maltodextrin, sacharose and soybean oil) were adjusted to guarantee isocaloric high-fat diets with and without ESF (Table 1).

2.5. Experimental design

Male Wistar rats (*Rattus norvegicus*, Albinus variety, Rodentia class) (n = 32), aged 21 days and weighing 69 ± 5 g were obtained from the Animal Center of Biological and Health Sciences, Federal University of Viçosa (UFV). From the 21st to the 60th day, the animals were distributed in four groups and fed with a commercial diet (Presence/*In Vivo*®) and distilled water *ad libitum*. The animals were housed under controlled temperature conditions (22 °C ± 2 °C) and a 12 h light/ dark cycle with the light phase starting at 7 AM.

At the adult phase (61th day), the animals were kept in individual steel cages and randomly divided into two groups. The normal control group (n = 8) received the AIN-93M diet (Reeves, Nielsen, & Fahey, 1993), and the others (n = 24) were fed with a high-fat diet (Moreira et al. 2017), and distilled water *ad libitum* for 7 weeks, to induce obesity. The average weight of the groups which received AIN-93M and a high-fat diet were 306 ± 22 g and 302 ± 23 g, respectively. At the end of this phase, the rats were fasted for 12 h and blood samples were collected by caudal puncture. The body weight ($p < .05$), glucose ($p < .05$) and triglycerides ($p > .05$) levels were performed in order to calculate the biometric indicators and to confirm the effect of HFD (Fig. 2a).

After 7 weeks the animals fed with HFD were grouped (n = 8/group) as follows: obese control group (HFD); HFDS50 – test group with ESF replacing 50% of cellulose and 100% of cornstarch in the HFD; HFDS100 – test group with ESF replacing 100% of cellulose and 100% of cornstarch in the HFD. There was no statistical difference in body

Table 1
Composition of the experimental diets (g/100 g).

Ingredients	AIN-93M[a]	HFD[b]	HFDS50[c]	HFDS100[d]
Casein	17.07	19.50	17.67	15.84
Maltodextrin	15.50	10	3.10	0
Corn starch	43.50	5.32	0	0
Sacharose	10	34.10	34.10	25.04
Soybean oil (mL)	4	1	0.66	0.32
Lard	0	20	20	20
Cellulose	5	5	2.5	0
Vitamin mix	1	1	1	1
Mineral mix	3.5	3.5	3.5	3.5
Bitartarate choline	0.25	0.25	0.25	0.25
L-cistine	0.18	0.18	0.18	0.18
Cholesterol	0	0.15	0.15	0.15
Butylated hydroxytoluene	0.0008	0.0004	0.0004	0.0004
Extruded sorghum flour	0	0	16.86	33.72
3-DXAS total (μg)[#]	nd	nd	16.92	33.85
Luteolinidin (μg)	nd	nd	4.45	8.90
Apigeninidin (μg)	nd	nd	2.39	4.78
5-Methoxy-luteolinidin (μg)	nd	nd	6.82	13.65
7-Methoxy-apigeninidin (μg)	nd	nd	3.27	6.54
Proanthocyanidin total (mg EC)[*] (mg taninos) (mg Catechin Equivalent)	nd	nd	8.20	16.41
Phenolic compounds (mg GAE)[**] allic acid equivalents	nd	nd	0.008	0.16
Calories (kcal)				
Carbohydrate (%)	72.58	42.42	41.09	39.82
Protein (%)	17.96	16.74	17.12	17.58
Lipids (%)	9.47	40.85	41.79	42.61
Caloric density (kcal·g^{-1})	3.80	4.66	4.56	4.44

[#] Total 3-deoxyanthocyanidins; nd: not determined.
[*] Catechin equivalents.
[**] Gallic acid equivalent.
[a] Normal control group (AIN-93M).
[b] Obese control group (HFD).
[c] Test group with extruded sorghum flour (ESF), replaced 50% of cellulose and 100% of corn starch in the HFD (HFDS50).
[d] Test group with ESF replaced 100% of cellulose and 100% of corn starch in the HFD (HFDS100).

weight, body weight gain, and fasting glucose between HFD groups. The normal control (AIN-93M) was maintained during all experiments. The weight gain and food consumption was monitored weekly, during the 8 weeks.

At the end of the experiment, the animals were fasted for 12 h and euthanized by cardiac puncture after anesthesia with isoflurane (Isoforine, Cristália®). Plasma and serum was obtained from blood centrifuged at 3000*g* for 10 min at 4 °C (Fanem-204, São Paulo, Brazil) in tubes with or without heparin, respectively. Retroperitoneal and epididymal adipose tissue were collected, weighed, and the biometric indicators were calculated. The samples were immediately frozen in liquid nitrogen and stored at −80 °C before analysis. In addition, tissue samples were fixed into 10% buffered formalin for histomorphological analysis.

All experimental procedures with animals were performed in accordance with the ethical principles for animal experimentation and the study approved by the Ethics Committee of the Federal University of Viçosa (Protocol 06/2014; date of approval: February 25th 2015).

2.6. Biometric data

The waist circumference was measured on the largest zone of the rats abdomen using a centimetre (Novelli et al., 2007). Lee index was calculated by the relationship between the cube root of the body weight and the nose-to-anus length. Obese animals whose Lee indices values were greater than or equal to 30 were used (Novelli et al., 2007). For the calculation of adiposity index, the abdominal and epididymal adipose tissue weights were added, and the result was divided by total body weight, then multiplied by 100. The food efficiency ratio (FER) was calculated by the ratio between body weight gain and total intake of diet.

2.7. Biochemical analyses

The lipid profile (total cholesterol, high density lipoprotein-cholesterol and triacylglycerol), renal function (creatinine, uric acid), hepatic function (aspartate aminotransferase, alanine aminotransferase) and fasting glucose were assessed (Cobas Roche Diagnostic, Basel, Switzerland) in the Laboratory of Clinical Analysis of the Health Division of UFV, Viçosa, MG, Brazil.

The oral glucose tolerance test (OGTT) was performed in the 7th week of the experiment (phase II). After a 12 h fast, a glucose solution at a 2 g·kg^{-1} body weight was administered by gavage. Blood samples were collected by tail vein puncture. Blood glucose levels were measured before the solution administration and after 30, 60, 90 and 120 min using the Accutrend® GCT (Manufacturer Roche Diagnostics, Mannheim, Germany) (Zhang et al., 2012). Further, the area under the curves was also calculated.

2.8. Quantification of cytokines by flow cytometry

The anti and pro-inflammatory cytokines, interleukin-10 (IL-10) and tumor necrosis factor alpha (TNF-α), respectively, were quantified in the plasma of the animals using the Cytometric Beads Array Kit (CBA) from BD® brand (CA, USA). Bead population with distinct fluorescence intensities were combined with a capture antibody specific for each cytokine, and mixed. In the CBA, the cytokine capture beads were mixed with the detection antibody conjugated to the fluorochrome PE and then incubated with the test samples to form the “sandwich”. Test tubes used in the analysis were prepared with 50 μL of sample, 50 μL of beads and mixing with 50 μL of the detection reagent. A standard curve was obtained by performing the same procedure. The tubes were homogenized and incubated for two hours at room temperature in the dark before the addition of 1 mL of wash buffer. After centrifugation, the supernatant was discarded and the samples were vortexed briefly to suspend the bead before being read in the FL3 channel FACSCalibur flow cytometer (BD®). To calculate the results, the CellQuest software (BD®) program was used.

2.9. Expression of genes in the epididymal adipose tissue involved in lipid metabolism

The total RNA extraction was performed in the epididymal adipose tissue using Trizol Reagent (Invitrogen, CA, USA) and a Mirvana™ miRNA Isolation Kit (Ambion® by Life TechnologiesTM), used according to the manufacturer protocols. The concentration and purity of mRNA was evaluated by μDrop plate spectrophotometer Multiskan™ GO (Thermo Scientific, DE, USA), and the integrity was confirmed by electrophoresis agarose gel. For cDNA synthesis, the M-MLV Reverse Transcriptase Kit (Invitrogen) was used. All steps were carried out in RNase-free conditions. Relative quantification of gene expression was performed by RT-qPCR using the equipment AB StepOne Real Time PCR System and the reagent 2X SYBR Green Master Mix (Applied Biosystems, CA, USA). The initial parameters used in the run were 20 s at 95 °C (203°F) and then 40 cycles at 95 °C (3 s), 60 °C (30 s) followed by melting curve analysis. Gene expression was determined by using the delta CT method (Livak & Schmittgen, 2001), with glyceraldehyde 3-phosphate dehydrogenase (GAPDH), 5′-AGGTTGTCTCCTGTCAC TTC-3′ (forward) and 5′-CTGTTGCTGTAGCCATATTC-3′ (reverse) mRNA in tissue sampled as reference, by using these primers, to determine gene expression: FAS (fatty acid synthase): AGCCCCTCAAGT GCACAGTG (forward) and TGCCAATGTGTTTTCCCTGA (reverse); LPL (lipoprotein lipase): CAGCTGGGCCTAACTTTGAG (forward) and CCT CTCTGCAATCACACGAA (reverse); PPAR-γ (peroxisome proliferator-

activated receptor gamma) CATTTCTGCTCCACACTATGAA (forward) and CGGGAAGGACTTTATGTATGAG (reverse). The primers were designed with the Primer3 Plus Program, which meets the requirements for real time polymerase chain reaction (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) (Noratto, Martino, Simbo, Byrne, & Mertens-Talcott, 2015). The qPCR data was analyzed with the ΔΔCT method (Hettinger et al., 2001) and normalized by the mean −ΔΔCT in the obese control rat (HFD) and expressed as $2^{-\Delta\Delta CT}$.

2.10. Epididymal adipose tissue: Histomorphometry and histopathology analysis

The fixed epididymal adipose fragments were embedded in paraffin. Semi-serial 5 mm thick sections were obtained in manual microtome using a stainless steel knife and subsequently were stained with Hematoxylin/Eosin (HE). The histological analysis was carried out on an light microscopy (Nikon Phase Contrast 0,90 Dry®, Japão) and images were captured with a ful-DIGI-PRO 5.0 M digital camera using the Software Micrometrics SE Premium (Accu-Scope®) at 20x magnification. The quantitative analysis of adipocytes diameter, perimeter and area, were conducted by the system ImagePro-Plus® version 4.5, using the mean of one thousand cells for each group (Lu et al., 2014).

2.11. Statistical analysis

The normality of the data through the distribution histogram was then verified. Statistically, significant differences between the groups were calculated using one-way analysis of variance (ANOVA) followed by post hoc Duncan test at 5% significance level. The blood glucose area under the curve (AUC) was calculated using the trapezoidal rule. P-values less than .05 ($p < .05$) were considered statistically significant. The results are shown as mean ± standard deviation and all analyses were performed in SPSS Statistics Software, version 20.0, 2011.

3. Results

3.1. Chemical characterization of extruded sorghum flour

The proximate composition of the sorghum extruded is presented in Table 2. All the investigated 3-DXAs were identified in extruded sorghum. The LUT and 5-MeO-LUT were the main DXAs, comprising approximately 26.0% and 40.0% of total DXAs, respectively (Fig. 1). Although present in grains of sorghum *in natura*, both flavones (apigenin and luteolin) and flavanones (naringenin and eriodctyol) were not identified in extruded sorghum. The extruded sorghum presented proanthocyanidins (486.82 µg Catechin Equivalent / g) with prevalence of the forms with a degree of polymerization greater than 10 (64% of total; Table 2).

Table 2
Proximate composition and bioactive compounds in extruded sorghum.

Proximate composition (g/100 g)			
Carbohydrates	58.16	Lipids	2.30
Proteins	12.20	Total Dietary Fiber	14.59
Ash	1.38	Moisture	11.37
Bioactive compounds			
Total 3-deoxyanthocyanidins (µg/100 g)	100.4 ± 5.9	Total proanthocyanidins µg (Catechin Equivalent/g)	486.82 ± 32.81
Luteolinidin	26.4 ± 0.7	*Monomers*	49.87 ± 3.82
Apigeninidin	14.2 ± 0.6	*Dimers*	55.52 ± 3.75
5-methoxy-luteolinidin	40.5 ± 3.4	*Trimers*	30.17 ± 2.97
7-methoxy-apigeninidin	19.4 ± 1.4	*Oligomers*	
Total Flavones (µg/100 g)	nd	*4*	Nd
Luteolin	nd	*5*	Nd
Apigenin	nd	*6*	35.5 ± 3.18
Total Flavanones (µg/100 g)	nd	*7*	Nd
Naringenin	nd	*8*	Nd
Eriodictyol	nd	*9*	315.67 ± 19.08
		Polymers	486.82 ± 32.81

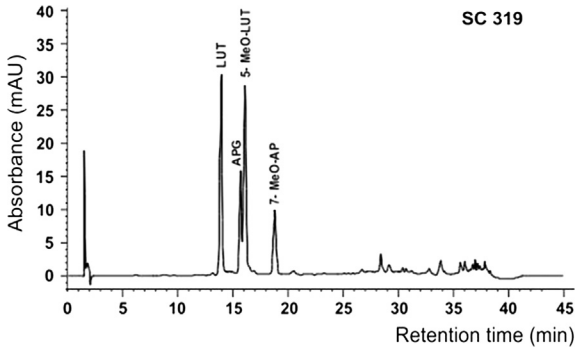

Fig. 1. 3-Deoxyanthocyanidins (3-DXA) profile in extruded sorghum (SC 319 genotype). LUT: luteolinidin, APG: apigeninidin, 7-MeO-AP: 7-methoxy-apigeninidin, and 5-MeO-LUT: 5-methoxy-luteliolinidin.

3.2. Effect of the diets on body weight, food intake, biometric indicators and biochemical variables

The normal control group (AIN-93M) presented higher food consumption (Fig. 2b) ($p < .05$) and a lower food efficiency ratio (Fig. 2c) compared to the obese control group (HFD) and the groups fed with extruded sorghum flour (HFDS50 and HFDS100). The protein and dietary fiber intake was not different among groups. Furthermore, the lipid content of the control diet was lower and the carbohydrate content was higher (AIN-93M; Fig. 2d). In addition, the groups fed with HFD added with extruded sorghum flour had different concentrations of proanthocyanidins, phenolic compounds (Fig. 2e), and 3-deoxyanthocyanidins (Fig. 2f).

The weight of the animals after obesity induction was higher in the groups treated with HFD (Fig. 3a), confirming the induction of obesity. At the end of the experiment, the body mass remained different in the groups fed with HFD, HFDS50 and HFDS100 compared to the normal control group. The weight gain (Fig. 3b), abdominal circumference (Fig. 3c), percentage of adiposity (Fig. 3d), total body fat, epididymal adipose tissue (Fig. 3e) and Lee Index (Fig. 3f) were higher in the obese group (HFD) compared to the normal control group. However, adiposity, adipose tissue (epididymal and total) and Lee index were lower in the group HFDS100 compared to the obese control group ($p < .05$).

Means and standard deviations of biochemical variables of all groups are expressed in Table 3. The plasma concentration of total cholesterol (TC), creatinine, aspartate aminotransferase (AST) and triacylglycerol plasma did not differ among the experimental groups ($p > .05$). The obese control group showed higher fasting plasma glucose ($p > .05$) than the normal control group (Table 3). However, the groups receiving sorghum flour showed no difference among the obese and normal control groups. It was observed as a trend in the reduction of HDL-C in the obese control group ($p = .09$), but the HFDS100 group reversed this effect by increasing the HDL-C level. However, no change ($p > .05$) in the triacylglycerol/HDL ratio was observed in the experimental groups. Uric acid and alanine aminotransferase (ALT) were the highest ($p < .05$) in the obese control group, while the groups treated with sorghum (HDF50 and HFD100) and AIN-93M decreased ($p < .05$) these concentrations.

Fig. 4a shows the results of the OGTTs performed after 8 weeks of high-fat feeding. The results of the oral glucose tolerance test showed that the obese control group (HFD) presented the highest blood glucose at 60 min of glucose administration.

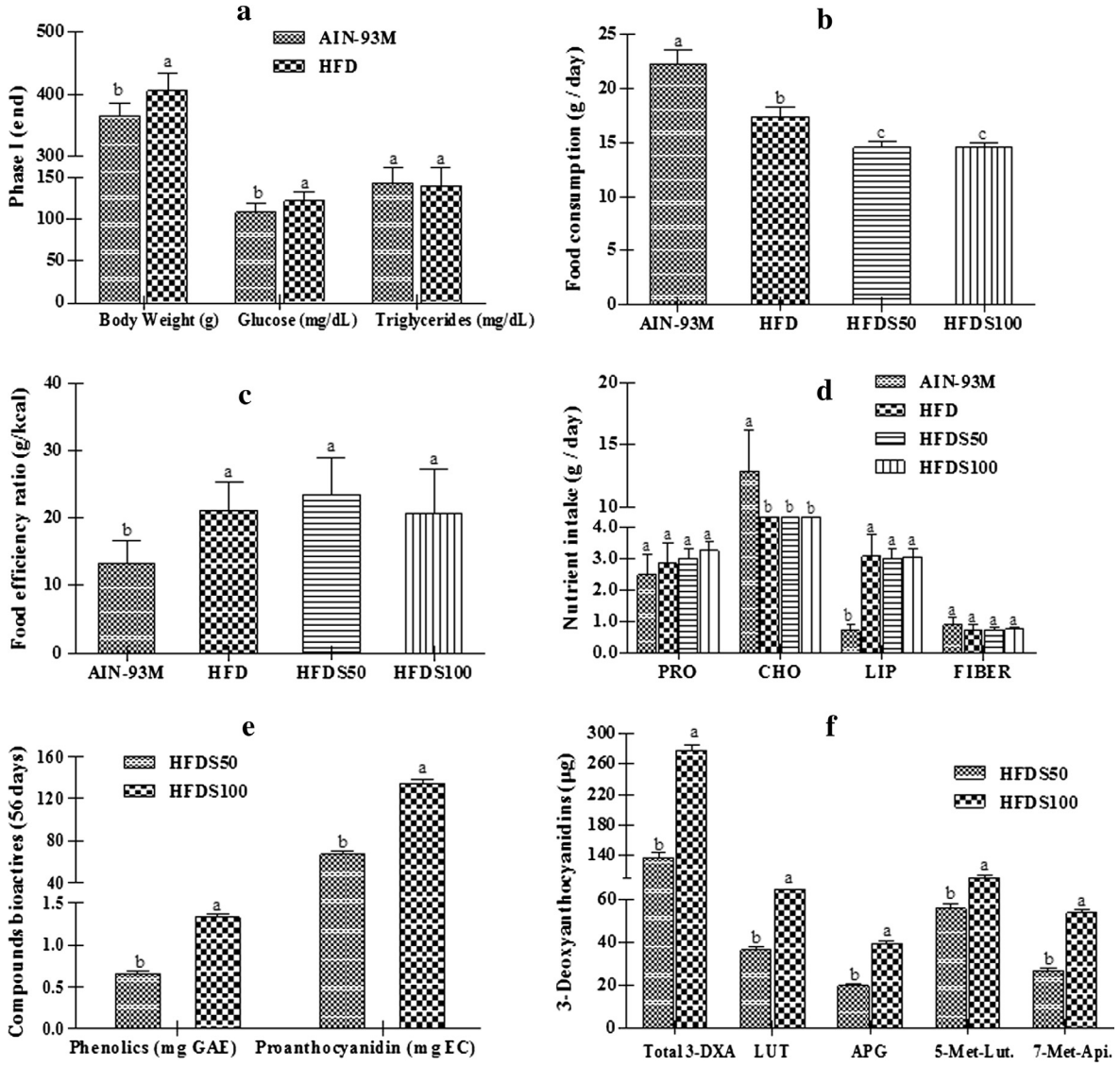

Fig. 2. Body weight, glucose, triglycerides of phase 1 end (a), food consumption (b), food efficiency (c), nutrients intake (d) and bioactive compounds (e: Phenolics and Proanthocyanidin), and 3-Deoxyanthocyanidins (f) of the obese rats treated with extruded sorghum flour as a component of the high fat diet over eight weeks. Data are expressed as mean ± DP (n = 8). Means followed by the same letter do not differ by Duncan test at 5% probability. Normal control group (AIN-93M), obese control group (HFD), test group with extruded sorghum flour (ESF), replaced 50% of cellulose and 100% of corn starch in the HFD (HFDS50) and test group ESF with replaced 100% of cellulose and 100% of corn starch in the HFD (HFDS100).

The oral glucose tolerance tests of the HFD group showed significantly greater area under the curve (AUC; Fig. 4b) compared to the AIN-93M ($p < .05$) and the AUC of sorghum groups did not differ to the AIN-93M group ($p > .05$). Therefore, both HDF50 and HFD100 groups were effective to improve the glucose tolerance.

3.3. Gene expression levels in adiposity tissue and cytokines in the plasma

PPAR-γ gene expression (Fig. 4c) did not differ ($p > .05$) between the obese control group (HFD) and HFDS50 group, however it was high for the AIN-93M and HDFS100 groups (7.71 and 1.61 fold, respectively; $p < .05$) when compared to the HFD group. Moreover, the adipogenic genes expression were down-regulated in epididymal adipose tissue by AIN-93M, HFDS50 and HFDS100 groups, decreasing significantly the gene expression of FAS (Fig. 4d) to 0.45, 0.16 and 0.08 fold (respectively) in relation to the obese control group. The LPL gene expression (Fig. 4e) increased ($p < .05$) to 2.64 fold (HFDS100 group), 2.02 fold (HFDS50), and 2.03 fold (normal control group; $p < .05$) compared to the obese control group.

The obese control group increased pro-inflammatory cytokine TNF-α concentration and the groups treated with sorghum, HFDS50 and HFDS100 decreased ($p < .05$) the protein concentration in a dependent dose manner (Fig. 4f). However, the protein concentration of the anti-inflammatory cytokine (IL-10) decreased ($p < .05$) in the obese control group and increased in the HFDS50, HFDS100 and AIN-93M (normal control) groups (Fig. 4 g).

3.4. Histological study of adipose samples

In Fig. 5, the measure of the epididymal adipose tissue photomicrographs (Fig. 5a), area (Fig. 5b), diameter (Fig. 5c), and perimeter (Fig. 5d) of epididymal adipose tissue cells is presented. The obese control group presented higher adipocyte diameter, perimeter and area compared to the normal control group ($p < .05$). Moreover, ESF (HFD50 and HFD100) reduced the adipocytes diameter compared to the obese control.

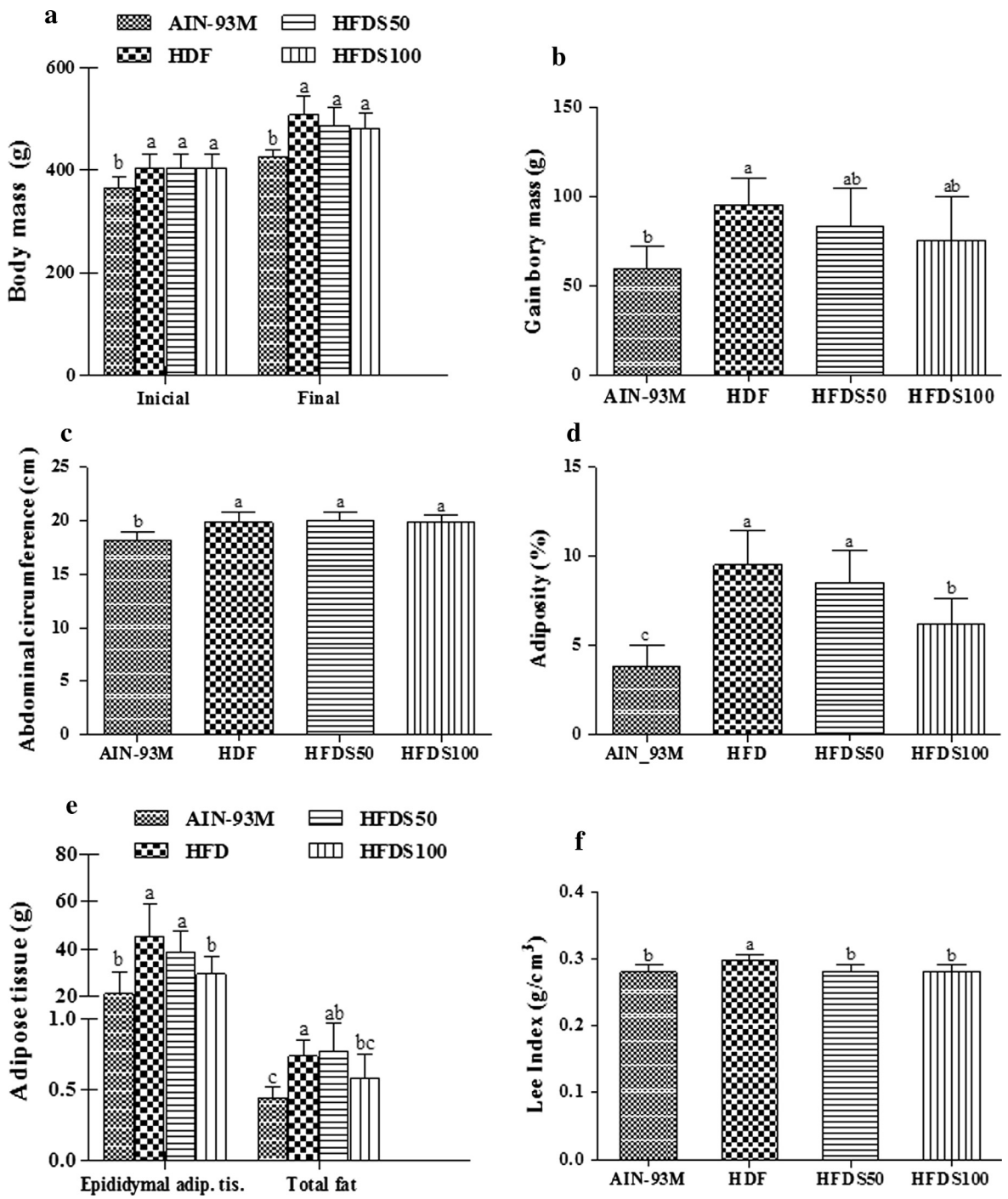

Fig. 3. Biometric measures of the obese rats treated with extruded sorghum flour as a component of the high fat diet over eight weeks. a: Body mass; b: Gain body mass; c: Abdominal circumference; d: Adiposity; e: Adipose tissue; f: Lee index. Data are expressed as mean ± DP (n = 8). Means followed by the same letter do not differ by Duncan test at 5% probability. Normal control group (AIN-93M), obese control group (HFD), test group with extruded sorghum flour (ESF), replaced 50% of cellulose and 100% of corn starch in the HFD (HFDS50) and test group with ESF replaced 100% of cellulose and 100% of corn starch in the HFD (HFDS100).

4. Discussion

This study investigated the potential benefits of SC 319 extruded sorghum flour (with high content of 3-deoxyanthocyanidins, flavones, proanthocyanidins and dietary fiber) on the adiposity and inflammatory development in high fat diet-induced obese rats.

Some studies indicate that the sorghum is a gluten-free cereal that has the highest content of bioactive compounds among cereals, with a wide variety of biologically active metabolites. These have been proven to be effective against diseases such as obesity, diabetes, dyslipidemia, cardiovascular disease, and cancer (Cardoso, Pinheiro, Martino, & Pinheiro Sant'ana, 2015; Moraes et al., 2012; Yang et al., 2012). In our study, the compounds Luteolinidin, Apigeninidin, 5-methoxy-luteolinidin, 7-methoxy-apigeninidin and proanthocyanidins (tannins) were identified and quantified from the EFS (SC319). These compounds may reduce the risk of developing chronic non communicable diseases, such as diabetes, obesity, hypertension, cardiovascular diseases and cancer due to their high antioxidant capacity (Cardoso et al., 2014).

The HFD (obese) groups with and without extruded sorghum flour had a lower food intake, but food efficiency and weight gain were

Table 3
Biochemistry parameter of the obese rats treated with extruded sorghum flour as a component of the high fat diet over eight weeks.

Parâmetros	AIN-93M	HFD	HFDS50	HFDS100	*Reference values
Fasting serum glucose (mg/dL)	114.40 ± 8.20[b]	135.75 ± 8.18[a]	121.13 ± 22.05[ab]	115.5 ± 24.72[ab]	79–144
Total cholesterol (mg/dL)	68.17 ± 8.42[a]	71.10 ± 12.29[a]	73.20 ± 8.61[a]	76.83 ± 5.19[a]	55–79
HDL cholesterol (mg/dL)	23.20 ± 1.3[ab]	21.83 ± 4.62[b]	24.50 ± 3.11[ab]	26.80 ± 1.30[a]	16–54
CT/HDL(mg.dL^{-1})	2.94 ± 0.19[a]	3.02 ± 0.33[a]	2.99 ± 0.34[a]	2.87 ± 0.18[a]	–
Triacylglycerol (mg/dL)	64.40 ± 9.84[a]	74 ± 22.28[a]	71.80 ± 8.04[a]	58.17 ± 5.78[a]	42–160
Creatinine (mg/dL)	0.58 ± 0.10[a]	0.61 ± 0.08[a]	0.65 ± 0.06[a]	0.62 ± 0.04[a]	0.44–0.64
Uric acid (mg/dL)	1.52 ± 0.40[b]	2.45 ± 0.23[a]	1.62 ± 0.18[b]	1.74 ± 0.34[b]	0.9–2.0
AST (mg/dL ^{1})[A]	150.40 ± 47.4[a]	158.40 ± 51.75[a]	158.33 ± 37.38[a]	135.80 ± 36.47[a]	36–58
ALT (mg/dL)[B]	47.83 ± 7.39[b]	224.7 ± 78.77[a]	55 ± 23.39[b]	43.2 ± 21.06[b]	81–180

AST: aspartate aminotransferase; ALT: alanine aminotransferase.
Data are expressed as mean ± SD (n = 8). Means followed by the same letter do not differ by Duncan test at 5% probability. Normal control group (AIN-93M), obese control group (HFD), test group with extruded sorghum flour (ESF), replaced 50% of cellulose and 100% of corn starch in the HFD (HFDS50) and test group with ESF replaced 100% of cellulose and 100% of corn starch in the HFD (HFDS100)
[#]Spinelli, Cruz, Godoy, Motta, and Damy (2014).
* Melo, Dória, Serafini, and Araújo (2012).

similar to the obese control group, demonstrating the higher efficiency of this diet to promote weight gain. The HFDS100 group was efficient in improving the biometry of the obese rats, reducing the adiposity, Lee index, total fat and epididymal adipose tissue, while the HFDS50 only reduced the Lee index. The higher effect of this group in improving the biometry parameter may be attributed to the double intake of bioactive compounds (3-deoxyanthocyanidins, proanthocyanidins and total phenolics) than the HFDS50 group. The higher tannin intake in the HFDS100 group may have favored a higher interaction of the tannin with the protein and dietary starch (Davis & Hoseney, 1979; Hagerman et al., 1998) by reducing the digestibility of these nutrients energy, thus providing reduction of biometric measurements and body weight gain. Moreover, anthocyanins are probably the largest group of phenolic compounds in the human diet. When consumed regularly by humans, these flavonoids have been associated with a reduction in the incidence of diseases such as obesity, diabetes, dyslipidemia, cardiovascular disease, and cancer (Cardoso, Pinheiro, Martino, & Pinheiro Sant'ana, 2015; Mitra & Uddin, 2014).

The HFD increased the blood glucose in the highest area under the curve in the obese control group. Some studies had showed that animals fed high-fat diets demonstrated disturbances in glucose metabolism and decreased glucose tolerance (Bais, Singh, & Sharma, 2014; White, Paul, Ashbullby, Herbert, & Depledge, 2013). However, the groups treated with sorghum (HFD50 and HFD100) improved the glucose tolerance promoting hypoglycaemic effect, probably as a function of the phenolic compounds and dietary fiber present in the extruded sorghum flour (Cardoso et al., 2014). The beneficial effect of phenolics is due to the partial inhibition of amylase and α-glucosidase during enzymatic hydrolysis of complex carbohydrates and delays the absorption of glucose, which ultimately controls the postprandial blood glucose levels (Shobana, Usha Kumar, Malleshi, & Ali, 2007). The beneficial effects of dietary fiber is usually attributed either to slowing down gastric emptying or formation of un-absorbable complexes with available carbohydrates in the gut lumen. These two properties might cause delayed absorption of carbohydrates and reduction of absolute quantity absorbed, which may contribute to control the glycemic response (Devi, Vijayabharathi, Sathyabama, Malleshi, & Priyadarisini, 2014).

Natural variation among extruded sorghum varieties contributes to diverse polyphenolic composition. In the present study, LUT and 5-MeO-LUT were the main quantified DXAs. Studies have reported that phenolic compounds in sorghum bran have beneficial health properties, e.g., as antioxidant, anti-inflammatory, and anticancer activity (Awika, Yang, Browing, & Faraj, 2009; Carbonneau, Cisse, Mora-Soumille, & Dangles, 2014; Yang et al., 2012). In our study, it was observed a reduction in TNF-α and an increasing of IL-10 gene expression. This may be due to the synergistic effect of phenolic compounds, proanthocyanidins, tannins and dietary fiber in extruded sorghum flour which may have facilitated the reduction of inflammation. Additionally, an in vitro study, with mononuclear cells from humans, shown that sorghum with high content of 3- deoxyanthocyanidins inhibited the secretion of IL-1β, TNF-α, and nitric oxide, also the sorghum extract with tannin was more effective than the 3-deoxyanthocyanidins to reduce the inflammation (Bralley, Greenspan, Hargrove, & Hartle, 2008; Burdette et al., 2010). Moraes et al. (2012) also observed a TNF-α reduction in the normal adult rats fed with whole sorghum flour as a component of a hyperlipidic diet in sufficient amounts to provide 50% of the dietary fiber consumed.

Sorghum flour can alter the expression of antioxidants and adipogenics genes (PPAR-γ, LPL and FAS). Experiments have shown that PPAR-γ is an important adipogenic regulator (Kennedy, Martinez, Chuang, Lapoint, & Mcintosh, 2009) and interconnected to its role in adipocyte differentiation. Evidence in experimental animals indicates that high-fat diets resulted in reduction of PPAR-γ expression and their target genes (Monsalve, Pyarasani, Delgado-Lopez, & Moore-Carrasco, 2013; Natal et al., 2016). In our study, PPAR-γ gene expression was increased in the AIN-93M and HFS100 groups. The compounds present in the sorghum (HFS100) may have improved the PPAR-γ expression and reduced the inflammatory process involved in the metabolic disorders of obesity (Brown & Plutzky, 2007). However, in the group with substitution of 50% sorghum fiber for cellulose (HFDS50), this increase was not observed. This result is probably due to the insufficient ingestion of antioxidants (3DXA, proanthocyanidin and phenolic compounds) in the HFDS50 group.

TNF-α has been shown to down-regulate LPL expression (Cawthorn & Jaswinder, 2008). In the present study, groups treated with sorghum showed increased LPL gene expression and a decrease of TNF-α, indicating increased lipolysis, which was confirmed by reduction in epididymal adipose tissue. Moreover, the adipogenic gene FAS was increased in the obese control group and in the groups treated with sorghum (HFDS50 and HFDS100), this effect was reversed. This indicates the reduction of lipogenesis, which reduced the diameter and area of adipocyte, leading to a reduction of body weight. In the obese control group, the hypertrophy of adipocytes in epididymal adipose tissue may promote an imbalance in the metabolic homeostasis adipose tissue. Moreover, an increase in the production of adipokines pro-inflammatory, such as TNF-α, and reducing the production of anti-inflammatory adipokines, as IL-10, results in a low grade chronic inflammation (Speretta, Leite, & Duarte, 2014). Studies have been shown that the effect of antioxidant compounds in other foods reduced proinflammatory molecules and increased antiinflammatory markers, such as IL-10 and adiponectin, hence improving the glucose sensitivity and lipid catabolism with LPL increased (Natal et al., 2016). Therefore, the antioxidant compounds present in the sorghum could be effective to modulate both adiposity and inflammation.

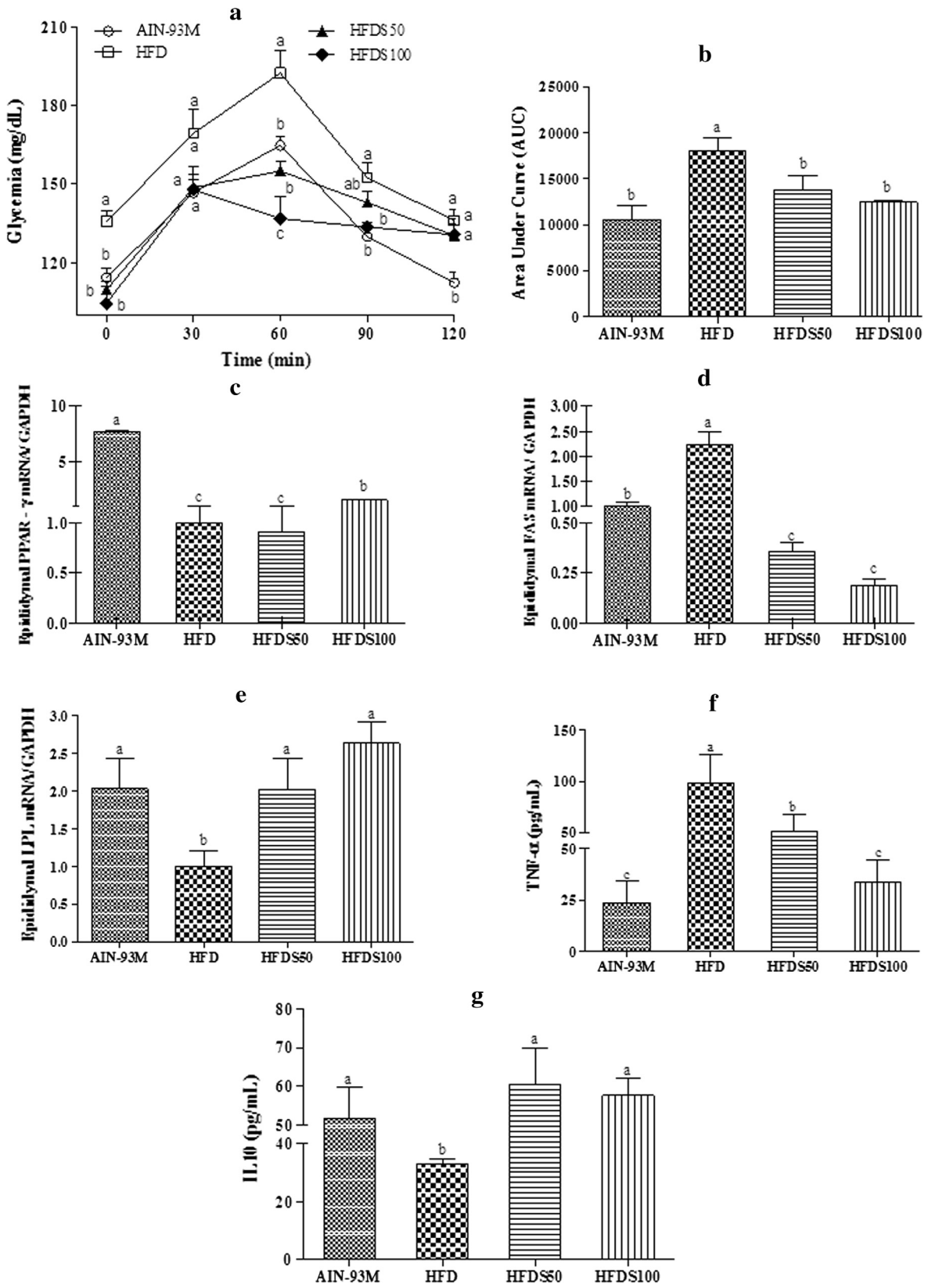

Fig. 4. Blood glucose values at the different oral glucose tolerance test (OGTT) (a), area under the curve (b), adipogenic genes expression in epididymal adipose tissue (c: activator of peroxisome proliferation – PPAR-γ; d: fatty acid synthase – FAS; e: lipoprotein lipase – LPL) and cytokine levels in plasm (f: tumor necrosis factor alpha – TNF-α; and g: interleukin-10 – IL-10) of the obese rats treated with extruded sorghum flour as a component of the high fat diet over eight weeks. Data are expressed as mean ± SD. Means followed by the same letter do not differ by Duncan test at 5% probability. Normal control group (AIN-93M), obese control group (HFD), test group with extruded sorghum flour (ESF), replaced 50% of cellulose and 100% of corn starch in the HFD (HFDS50) and test group with ESF replaced 100% of cellulose and 100% of corn starch in the HFD (HFDS100).

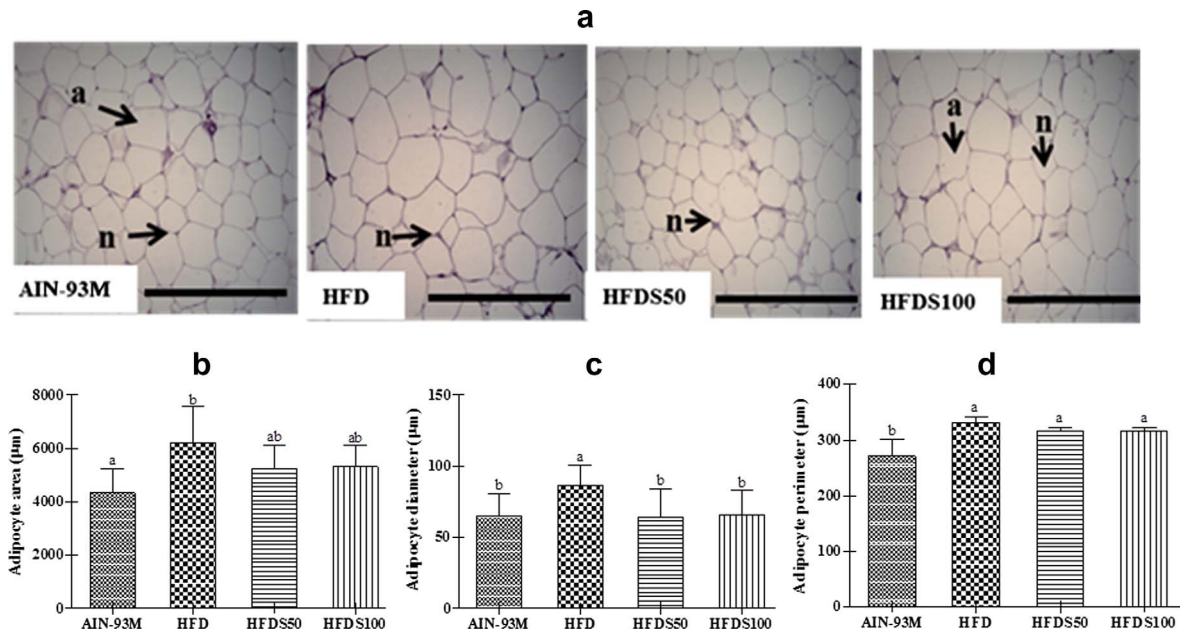

Fig. 5. Photomicrograph of epididymal adipose tissue (a) adipocyte area (b), diameter of adipocytes (c) and adipocyte perimeter (d) of the obese rats treated with extruded sorghum flour as a component of the high fat diet over eight weeks. n: nucleus; a: area. Data are expressed as mean ± SD. Means followed by the same letter do not differ by Duncan test at 5% probability. Normal control group (AIN-93M), obese control group (HFD), test group with extruded sorghum flour (ESF), replaced 50% of cellulose and 100% of corn starch in the HFD (HFDS50) and test group with ESF replaced 100% of cellulose and 100% of corn starch in the HFD (HFDS100). Hematoxylin and eosin. Bar: 50 µm.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are important enzymes involved in liver disease. In our study, we observed an increase in ALT levels in the HFD group, which indicates that the intake of high fat diet and accumulation of liver fat are associated with increased susceptibility to hepatocyte damage (Natal et al., 2016). We also observed a reduction of ALT in HFDS50 and HFDS100 groups that may be attributed to suppression of TNF-α, increased levels of IL-10 and upregulation of LPL (Elmazar, El-Abhar, Schaalan, & Farag, 2013). Alanine aminotransferase is present in high quantities in the liver cells and an increase in the serum levels of this enzyme has been observed in inflammatory liver damage (Kim, Flamm, Di Bisceglie, & Bodenheimer, 2008). Moreover, AST did not differ among groups, which can be attributed to the short, 12-h half-life and the fast normalization in the blood. Furthermore, AST is located in the cytosol and in the mitochondria, while the ALT is located only in the cytosol, which can hinder the detection of AST (Ramaiah, 2007). Concerning the renal function of the rats, the HFDS50 and HFDS100 groups showed decreased uric acid level. It is known that uric acid, in addition to being a biomarker for metabolic disorder, has a direct effect on oxidative stress.

Our findings suggest that the extruded sorghum flour of the SC 319 genotype was able to reduce chronic inflammation, adipogenic genes, hypertrophy of adipose tissue and weight gain. This is probably because it has superior levels of dietary fiber and phenolic compounds such as proanthocyanidins and 3-deoxyanthocyanidins (luteolinidin and 5-methoxy-luteliolinidin).

5. Conclusions

The intake of extruded sorghum flour of the SC 319 genotype in partial and total replacement of dietary fiber and carbohydrate on the HFD of obese rats has potential use as a functional food, since it may reduce the metabolic risk of obesity associated with adiposity and inflammation.

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Conflict of interest

None.

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