Sesame and flaxseed oil: nutritional quality and effects on serum lipids and glucose in rats

Óleo de linhaça e gergelim: qualidade nutricional e efeitos sobre lipídios e glicose séricos em ratos

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Abstract

This study evaluated the nutritional value of sesame and flaxseed oils and their effects on the lipid and glucose profile of rats fed diets containing different fat combinations. Fatty acid composition, refractive index, and iodine and saponification values were analyzed to characterize the oils. In the biological assay, Wistar rats were fed different diets, whose fat composition consisted of varying combinations of flaxseed oil, sesame oil, and animal fat. The primary constituents of the sesame oil were oleic (28.6%), linoleic (28.4%), and lauric acid (14.6%); for the flaxseed oil they were alpha-linolenic (39.90%), oleic (17.97%) and linoleic acid (12.25%). The iodine and saponification values of the oils were within the reference range. Rats fed flaxseed oil-based diets had the lowest serum cholesterol values, whereas rats fed diets with flaxseed oil + sesame oil + animal fat had the highest glucose levels. HDL levels decreased significantly with flaxseed oil. Sesame and flaxseed oils are sources of polyunsaturated fatty acids (PUFA), and the flaxseed oil-based diet had a hypocholesterolemic effect, whereas sesame oil showed oxidative stability since it contains high levels of monounsaturated and saturated fatty acids.
Keywords: *diets; flaxseed; sesame; lipids; nutritional quality index.*

Resumo

Este estudo avaliou o valor nutricional do óleo de gergelim e de linhaça e seus efeitos sobre o perfil lipídico e glicose de ratos alimentados com dietas contendo diferentes combinações de gordura. Perfil de ácidos graxos, índice de refração, iodo e saponificação foram analisados para caracterizar os óleos. No ensaio biológico, ratos Wistar foram alimentados com diferentes dietas, cuja composição de gordura foi composta de combinações distintas de óleo de linhaça, gergelim e gordura animal. Os principais constituintes do óleo de sésamo foram: oleico (28,6%), linoleico (28,4%) e láurico (14,6%); para o óleo de linhaça, foram: alfalinolênico (39,90%), oleico (17,97%) e linoleico (12,25 %). Valores de iodo e saponificação dos óleos ficaram dentro do intervalo de referência. Ratos alimentados com óleo de linhaça nas dietas tiveram menores valores de colesterol sérico, enquanto que ratos alimentados com óleo de linhaça + óleo de gergelim + gordura animal obtiveram mais altos níveis de glicose. Níveis de HDL diminuíram significativamente com óleo de linhaça. Óleo de gergelim e linhaça são fontes de AGPI, e a dieta contendo óleo de linhaça teve um efeito hipocolesterolêmico, e no óleo de gergelim verificou-se estabilidade oxidativa, uma vez que este contém altos níveis de monoinsaturados e ácidos graxos saturados.
Palavras-chave: *dietas; linhaça; gergelim; lipídios; índice de qualidade nutricional.*

1 Introduction

Abnormal lipid metabolism is a main cause of dyslipidemia, which is a major risk factor for cardiovascular disease, obesity, cholestasis, and overall mortality. It is well known that diet plays an important role in the control of cholesterol homeostasis (MORISE et al., 2004; FERNANDES et al., 2010). It has been reported that vegetable oils have been used as food and for medicinal purposes for hyperlipidemia and that they may be useful adjuncts to reduce the risk of cardiovascular disease and alterations in liver metabolism. Recent studies have demonstrated that ingestion of polyunsaturated fatty acids (PUFA) present in vegetable oils, is inversely related to the incidence of heart disease by decreasing cholesterol and plasma triglyceride levels (GALVÃO et al., 2008).

Arteriosclerotic vascular disease (ASVD) is associated to genetic factors, sex, age, smoking, sedentary lifestyle, overweight, hypertension, dyslipidemia, and diabetes, but this and other cardiovascular disorders can be prevented by controlling dietary fat and cholesterol levels. Current recommended fat intakes are based on fat quality rather than amount (RAPOSO, 2010). In this respect, some types of fat such as PUFAs from the omega 3 (ω-3) family have gained importance as functional food.

Omega-3 PUFAs are primarily found in fish, especially in twait shad, salmon, tuna, and anchovies (WHELAN; RUST, 2006). Another important source of PUFA is flaxseed obtained from *Linum usitatissimum* plants (Linaceae family), cropped mainly in Argentina, Brazil, Canada, China, India. and Turkey

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(SAMMOUR, 1999). Flaxseed is a flat, oval-shaped seed (CHUNG; LEI; LI-CHAN, 2005; MARQUES et al., 2011) whose oil contains 53% alpha-linolenic acid (ALA), an essential ω-3 fatty acid (UNITED STATES..., 2006). Flaxseed is also a good source of dietary fiber (20-25%) (VIJAIMOHAN et al., 2006) and lignans (>500 μg/g), which are plant steroids analogous to mammalian estrogen (STODOLNIK et al., 2005).

Polyunsaturated fatty acids from the n-6 (ω-6) family, found in nuts, seeds, and vegetable oils such as corn and soybean oils (INSTITUTE..., 2005), are also important. While ω-3 PUFAs are precursors of 3-series prostanoids and 5-series leukotrienes (associated to anti-inflammatory and antithrombotic properties), ω-6 PUFAs are precursors of 2-series prostanoids and 4-series leukotrienes (associated to pro-inflammatory and prothrombotic activity) (McKENNEY; SICA, 2007).

Sesame seed (*Sesamum indicum* L.), another widely consumed seed, is a good ω-6 source. This Pedaliaceae is cropped in both tropical and subtropical countries. India and China are the major producers accounting for 70% of world production. In Brazil, 13,000 tons of sesame seeds are produced over nearly 20,000 ha, yielding approximately 650 kg/ha (ARRIEL; VIEIRA; FIRMINO, 2005). Sesame oil has advantages over other vegetable oils owing to its high nutritional and therapeutic value. Sesame seeds, which are used in traditional Indian (Ayurvedic) and Chinese medicine, contain 57% highly stable oil (RESHMA et al., 2010). Due to its high oxidative stability, sesame oil is added to margarines, salads, and frying oils (YEN; LAY, 1990). Saturated fatty acid (SFA) content in sesame oil is nearly 14%, comparable to soy and corn oil. Oleic and linoleic (LA) acid levels are approximately 45%, which is close to that found in corn, soy, and cottonseed oil (EMBRAPA, 2001).

The Institute of Medicine (2005) developed Dietary Reference Intakes (DRIs) to evaluate adequate fatty acid intake based on the mean consumption of the American population. It recommends that the adequate daily intake of omega-6 is 17 g for men and 12 g for woman; and for ω-3, these values are 1.6 g for men and 1.1 g for women. The beneficial effects of diets containing long chain ω-3 fatty acids (PUFA) justify the regular inclusion of this ingredient in human diet although the conversion of ALA to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the main therapeutic agents of ω-3 fatty acids, is only around 6% (BURDGE, 2006).

Both flaxseed and sesame seed are nutritional supplements, representing an excellent source of PUFA that can promote cardioprotective effects if consumed daily (CHUNG; LEI; LI-CHAN, 2005). A number of studies in rats have investigated the functional properties of their lipid fraction, but the implications of fatty acid ratio in health have been scarcely investigated. As such, the present study evaluated the nutritional value of sesame and flaxseed oils and their effects on the lipid and glucose profile of rats fed diets with different oil combinations.

2 Materials and methods

2.1 Oil extraction

Flaxseed and sesame seeds were obtained from a commercial dealer in Campo Grande, MS, Midwest Region, Brazil. The seeds were ground using a Turratec mill and passed through a Tamis 60 (drum sieve). The oil fraction of the flaxseed and sesame meals produced was extracted with a solvent mixture containing a 2:1:0.8 ratio of methanol: chlorophorm: distilled water (BLIGH; DYER, 1959). The oil extracted was chemically characterized and used to formulate the experimental diets combined with animal fat obtained from a conventional supplier.

2.2 Animals and experimental design

Wistar rats (*Rattus norvegicus*) weaned at 21 days of age and weighing 79.49 ± 0.50g were separated into 8 groups of 6 animals each. The groups received one of 8 diets designed to support growth, with similar protein and fat content and formulated according to the American Institute of Nutrition (AIN) (REEVES; NIELSEN; FAHEY, 1993). The diets had similar composition except for fat source, which consisted of different proportions of linseed oil, sesame oil, and animal fat. The rats were given *ad libitum* access to food and water. The control group (Group C) was given commercial feed (Nuvilab CR-1). Diet composition was analyzed to ensure that caloric and macronutrient balance was in accordance with AIN-93C.

The fat source of the experimental diets consisted of soybean oil for group G_C (control); animal fat for G_A; flaxseed oil for G_F; sesame oil for G_S; animal fat + flaxseed oil for G_{AF}; animal fat + sesame oil for G_{AS}; flaxseed oil + sesame oil for G_{FS}; and animal fat + flaxseed oil + sesame oil for G_{AFS}. Diet ingredients were sieved to produce homogeneous diets with similar granulometry. The antioxidant TBHQ was used to minimize PUFA oxidation in the diets.

Diets were elaborated according to reference values and contained 200 g caseine, 132 g dextrinized starch, 397 g corn starch, 50 g cellulose, 100 g sucrose, 7 g fat, 2.5 g choline bitartrate, 35 g mineral mixture, 10 g vitamin mixture, and 3 g L-cystine per kilogram.

The experiment lasted 45 days; the first 5 days used to adapt the rats to the diets. Animals were weighed once a week, and food intake was measured daily by calculating the difference between the amount of food provided and the non-consumed portion. Feed efficiency (weight gain/feed intake) was also calculated.

At the end of the experiment, the rats were anesthetized for blood collection and subsequently killed. Blood samples were centrifuged to obtain serum fractions, which were stored at −18 °C until analysis.

The present study was carried out in accordance with regulations and ethical guidelines established by the "*Colégio Brasileiro de Experimentação Animal* (COBEA)", and the experimental protocol was approved by the CEUA/UFMS Ethics Committee for Animal Use (Protocol 272).

2.3 Serum analysis

Total cholesterol and its fractions, triglycerides, and blood glucose were determined in serum samples using enzyme-colorimetric methods with specific kits and spectrophotometric measurements (HAGEN; HAGEN, 1962; FLEG, 1973; CAREY; FELBRUEGGE; WESTGARD, 1974).

2.4 Fatty-acid composition of seed oils

To determine fatty acid composition from sesame seed and flaxseed lipid, the extracted oils were saponified, esterified, and transferred to hexane, according to the Hartman and Lago (1973) method modified by Maia and Rodriguez-Amaya (1993). Fatty acid methyl esters were analyzed using a Shimadzu GC-2010 chromatograph with AOC-5000 autoinjector and flame ionization detector (FID). A Restek Stabilwax-DA fused-silica bonded-phase column (30 m × 0.25 mm; 0.25 μm) was used, with both injector and FID operated at 250 °C. Initial oven temperature of 80 °C was maintained for 3 minutes and then raised to 140 °C at a rate of 10 °C/minutes and to 240 °C at 5 °C/minutes, which was kept for 11 minutes. Methyl ester peaks were identified by comparing their retention times on the column with those of standard fatty acid methyl esters. Quantification was determined using the area correction factor (MAIA; RODRIGUEZ-AMAYA, 1993; HOLLAND et al., 1994).

2.5 Physicochemical characterization of the oils

Seed lipid content was determined by Soxhlet extraction with hexane. The refractive index was obtained using an Abbe direct reading refractometer. To determine iodine value, the oil was placed in an erlenmeyer flask with carbon tetrachloride and Wijs solution. The final solution was titrated with sodium thiosulfate until it turned from black to pink. To determine saponification value, flaxseed and sesame oil were placed in beakers and added with KOH. After the addition of phenolphthalein, the solutions were titrated with HCl until the pink color disappeared. All of the assays were performed in triplicate and according to methods of the American Oil Chemists' Society (1998).

2.6 Nutritional quality index

Nutritional quality, using five different indexes, was evaluated based on the fatty acid composition of the oils. The atherogenic index (AI) (Equation 1) and the thrombogenic index (TI) (Equation 2) considered the monounsaturated acid (MUFA) levels and were based on Ulbricht and Southgate (1991). The hypocholesterolemic: hypercholesterolemic ratio (HH) (Equation 3) was calculated according to Santos, Bessa and Santos (2002). The PUFA:SFA and ω6:ω3 ratios were also calculated.

$$AI = \frac{C_{12:0} + 4 \times C_{14:0} + C_{16:0}}{\sum MUFA + \sum \varpi 6 + \sum \varpi 3} \quad (1)$$

Atherogenicity Index (AI).

$$TI = \frac{C_{14:0} + C_{16:0} + C_{18:0}}{0.5 \times \sum MUFA + 0.5 \times \sum \varpi 6 + 3 \times \sum \varpi 3} \quad (2)$$

Thrombogenicity Index (TI).

$$HH = \frac{C_{18:1Ci39} + C_{18:Z\varpi 6} + C_{20:4\varpi 6} + C_{18:3\varpi 3} + C_{20:5\varpi 3} + C_{22:5\varpi 3} + C_{22:6\varpi 3}}{C_{14:0} + C_{16:0}} \quad (3)$$

Hypocholesterolemic: Hypercholesterolemic ratio (HH).

2.7 Statistical analysis

Data were analyzed using analysis of variance complemented by the Tukey test. Significance level was set at 0.05 and analyzed by one-way analysis of variance (ANOVA).

3 Results and discussion

3.1 Fatty acid composition

Table 1 shows fatty acid composition of the oils. Some studies on sesame oil reported 8.63% myristic acid for cultivar CNPA G2, 9.08% for CNPA G3 and 8.71% for Seridó, but lauric acid was not detected in these varieties (ANTONIASSI et al., 1997). Ünal and Yalçın (2008) found 8.46 ± 0.10% palmitic acid and 4.93 ± 0.10% stearic acid in sesame oil, similar to the values reported in the present study. Embrapa (2001) reports that sesame cultivars CNPA G2, CNPA G3, and CNPA G contain 40.66, 38.53 and 42.27% oleic acid and 44.57, 46.71 and 41.72% linoleic acid (LA), respectively, higher values than those found

Table 1. Fatty acid content (mean ± sd) of sesame oil and flaxseed oil.

Fatty acid	Sesame oil (%)	Flaxseed oil (%)
Saturated (SFA)		
Caprylic (C8:0)	3.22 ± 0.27	-
Capric (C10:0)	2.06 ± 0.12	-
Lauric (C12:0)	14.59 ± 0.49	-
Myristic (C14:0)	4.38 ± 0.13	-
Palmitic (C16:0)	7.49 ± 0.04^a	4.72 ± 0.04^b
Heptadecanoic (C17:0)	0.03 ± 0.02	-
Stearic (C18:0)	4.04 ± 0.06^a	4.59 ± 0.05^a
Arachidonic (C20:0)	0.35 ± 0.01^a	0.18 ± 0.02^b
Behenic (C22:0)	0.08 ± 0.01^a	0.17 ± 0.03^b
Lignoceric (C24:0)	0.11 ± 0.01^a	0.25 ± 0.01^b
Total SFA	36.43^a	9.97^b
Monounsaturated (MUFA)		
Oleic (C18:1ω-9)	28.59 ± 0.44^a	17.97 ± 0.09^b
Palmitoleic (C16:1ω-7)	0.08 ± 0.01^a	0.06 ± 0.05^b
Vaccenic (C18:1ω-7)	0.52 ± 0.01^a	0.60 ± 0.01^b
Gadoleic (C20:1n9)	-	0.03 ± 0.05
Total MUFA	28.59^a	18.0^b
Polyunsaturated (PUFA)		
Linoleic (C18:2 ω-6)	28.35 ± 0.46^a	12.25 ± 0.05^b
Alpha-linolenic (C18:3ω-3)	0.29 ± 0.01^a	39.90 ± 0.14^b
Eicosanoic (C20:2ω-6)	0.05 ± 0.01^a	0.09 ± 0.08^b
Total PUFA	28.69^a	52.24^b

Non-detected fatty acids are indicated by a dash (-). Different letters in a same row indicate statistical differences ($p < 0.05$).

in the present study. Uzun et al. (2007) found 45.3% LA and 37.0% oleic acid in sesame oil, but they found no linolenic acid fractions.

Omega-3 and ω-6 fatty acids are eicosanoid precursors that regulate immune and inflammatory functions. Some essential fatty acid (EFA) derivatives, such as dihomo-gamma-linolenic and arachidonic acid, both from the ω-6 series, and EPA, from the ω-3 series, are especially important because they are lipid mediators involved in many physiological functions (KRUMMEL, 2007).

Long chain fatty acids are incorporated into cell membranes in the following order: EPA and DHA (ω-3), arachidonic acid (ω-6), and oleic acid (ω-9). In addition to the effects on membrane stability and fluidity, ω-3 and ω-6 are eicosanoid precursors, lipid-soluble inflammatory mediators that are one of the main routes of fatty acid activity (WAITZBERG; BORGES, 2005). Long chain fatty acids also participate in the mediation of many molecular signals involved in cholesterol metabolism (KÖKTEN et al., 2010).

Earlier studies on flaxseed oil report higher PUFA levels than those obtained in the present study. Epaminondas (2009) found 7.06% palmitic acid and Vijaimohan et al. (2006) found more than 51% ALA, 17% LA and 18% oleic acid. Choo, John and Dufour (2007) analyzed 3 flaxseed varieties and found ALA values ranging from 54 to 59.6%. However, Peiretti and Meineri (2008) found 2.9% palmitic, 0.3% oleic, 5.8% LA, and 32.8% ALA levels in flaxseed oil, lower values than those reported here.

The sum of SFA and MUFA was higher in sesame oil than in flaxseed oil. However, the sum of MUFA and PUFA was higher in flaxseed oil than in sesame oil. This indicates that flaxseed oil is a good source of fatty acids with cardioprotective properties. According to the American Heart Association (2004), an adult should consume 20-23% PUFA and 10% SFA daily. The oils reported in the present study meet these standards and can therefore be added to diet.

The human body usually makes use of dietary fatty acids. However, it is capable of producing SFA and MUFA from glucose and amino acids through enzymatic elongation (addition of 2 carbon units) and desaturation (formation of new double bonds). Desaturation activity is stimulated by insulin and inhibited by glucose, adrenalin, and glucagon (KRIS-ETHERTON, 2006).

The incorporation of essential fatty acids may cause structural and functional changes in the phospholipidic membrane, affecting important biological processes such as the synthesis of inflammatory mediators, including eicosanoids (COSTUNER; KARABABA, 2008).

Recent studies have expressed increasing interest in PUFA as essential components of the human diet. They have been shown to play an essential role in a number of biological systems, combating coronary and degenerative disorders. Besides being structural components of cell membranes, PUFA play an important role in regulatory function, acting as a source of eicosanoids and prostaglandins are the predominant component (RAMADAN et al., 2009). In addition, PUFA are the primary bio-membrane components, including the plasma membrane, endoplasmic reticulum, and mitochondrial membrane (KAITHWAS; MAJUMDAR, 2010).

3.2 Nutritional quality indexes

Nutritional quality of sesame seed and flaxseed lipid fractions evaluated by the different indexes is shown in Table 2.

Diets with a PUFA:SFA ratio below 0.45 are considered inadequate (LONDON, 1984) because of their potential to increase blood cholesterol levels. In the present study, it was found PUFA:SFA ratio of 0.79 in sesame oil and 5.25 in flaxseed oil indicating that these oils have good fatty acid balance.

Determination of the ω6:ω3 ratio is important for human heath since excessive consumption of ω6, accompanied by decreased ingestion of ω3, is a risk factor for cardiovascular disorders. These fatty acids compete for enzymes involved in desaturation reactions and chain elongation. Although these enzymes have greater affinity for fatty acids from the ω-3 series, the conversion of linolenic acid into long-chain PUFA is strongly affected by dietary linolenic acid levels (RAMADAN et al., 2009). Thus, the ω6:ω3 ratio of 97.80 found in sesame oils is within the range of 5:1 to 10:1 recommended by the WHO (WORLD..., 1995). The ratio obtained for flaxseed was 0.31, which is below the recommended level.

Indexes based on the functional properties of the different fatty acids allow better evaluation of the nutritional quality of foods. Although these indexes have not been applied to evaluate edible oils, they have been used to evaluate fish meat. As such, a number of studies on fish meat were mentioned here for comparison purposes.

The HH index considers specific effects of fatty acids on cholesterol metabolism, and high HH values are desired from a nutritional standpoint. In the present study, HH was 4.82 for sesame oil and 14.85 for flaxseed oil, much higher values than those reported for fish from the Brazilian Pantanal, (1.49-1.84) and considered quite high for an animal product (RAMOS FILHO et al., 2008).

The AI (atherogenic index) and TI (thrombogenic index) indexes are considered cardiovascular disease risk factors. Thus, these indexes must be kept low. AI and TI were lower than 1 for both sesame and flaxseed oil due to the cardioprotective effect of their PUFA. Ramos Filho et al. (2008) also found AI and TI values below 1.0 for Pantanal fish, except for pacu fish, which had a TI of 1.16.

Table 2. Evaluation of sesame and flaxseed oil by nutritional quality indexes.

INDEX	Sesame oil	Flaxseed oil
PUFA:SFA ratio	0.79	5.24
ω6:ω3 ratio	97.80	0.31
atherogenicity index (AI)	0.69	0.07
thrombogenicity index (TI)	0.13	0.07
hypocholesterolemic: hypercholesterolemic ratio (HH)	4.82	14.85

3.3 Physicochemical properties of the oils

Seed lipid content, the refractive index, and iodine and saponification values of sesame and flaxseed oils are shown in Table 3. Total lipid content in flaxseed is within the 30-50 $g.kg^{-1}$, range reported in earlier studies (ROTHENBURG; PEREIRA, 2006; VIJAIMOHAN et al., 2006; MATHEWS et al., 2000). Lipid content in the sesame seeds was higher than that obtained in earlier studies on national and imported seed varieties, which did not exceed 54.7% oil yield (EMBRAPA, 2001; RESHMA et al., 2010; UZUN et al., 2007).

The refractive index was 1.465 ± 0.01 for sesame oil and 1.474 ± 0.01 for flaxseed oil. These values are comparable to the refractive index of cottonseed oil (1.458 to 1.466), rapeseed oil (1.465 to 1.467), corn oil (1.465 to 1.468) and soy oil (1.466 to 1.470) (CORSINI; JORGE, 2006).

The refractive index reflects the relationship between the speed of light in the air and in the oil. This value increases with chain length and fatty acid unsaturation and is associated to the iodine index (ORDOÑEZ-PEREDA, 2005). Flaxseed oil has more units of long-chain fatty acids (over 16 carbons) and unsaturations than sesame oil. Thus, the refractive index of flaxseed oil (1.474 ± 0.01) is higher than that of sesame oil (1.465 ± 0.01).

The reference values proposed by the AOCS (AMERICAN..., 1998) for flaxseed oil are 170 g I_2 kg^{-1} for the iodine index and 196 mg KOH kg^{-1} for the saponification index, higher values than those observed in the present study. Similarly, ANVISA (AGÊNCIA..., 1999) reported an iodine value of 104 g I_2 kg^{-1} and saponification value of 195 mg KOH kg^{-1} for sesame oil, higher values than those found in the present study. Discrepancies among the studies are likely associated to different SFA and UFA levels in the oil samples analyzed, which vary according to the region in which the seeds were produced and inherent climatic, soil, and management conditions.

Since saponification value is related to the length of the fatty acid chain, it therefore decreases with the molecular weight of the fatty acid (CECCHI, 2003). Sesame oil has a higher sum of SFA (36.43%) than flaxseed oil (9.97%), confirming the proportionality of the saponification index, which was also higher for sesame oil.

3.4 Weight evolution

In the 3 first weeks of the experiment, the groups fed the experimental diets had higher weight gain than those in the control group (G_C), which were fed the commercial diet (Table 4, $p < 0.05$). Animals fed experimental diets grew faster, probably because they adapted faster. From the 4th to the 6th week, the rats in the experimental groups continued gaining more weight than the control rats ($p < 0.05$), except for G_{AFS}, whose diet was likely less palatable.

Table 3. Physicochemical characteristics (mean ± sd) of sesame oil and flaxseed oil.

Characteristic	Sesame oil	Flaxseed oil
Lipid Contents ($g.kg^{-1}$)	56.50 ± 0.67^a	39.52 ± 0.36^b
Refractive Index at 40 °C	1.465 ± 0.01^a	1.474 ± 0.01^a
Iodine Value (g I_2 kg^{-1})	90.17 ± 0.75^a	123.19 ± 0.09^b
Saponification Value (mg KOH kg^{-1})	416.78 ± 1.07^a	511.90 ± 1.59^b

Different letters in a same row indicate statistical differences ($p < 0.05$).

3.5 Blood parameters

Consuming diets enriched with animal fat increases the risk of cardiovascular diseases causing hyperlipidemia and ASVD in addition to augmenting LDL-cholesterol levels over time (ONODY et al., 2003). Table 5 shows that glucose levels were higher in G_{AFS} than in G_C, G_A, G_{AF}, G_S, and G_{AS} ($p > 0.05$), and that G_F and G_{FS} had intermediary values ($p < 0.05$).

Marques et al. (2011) reported that the combination of flaxseed and flaxseed oil in rat diet produces lower glucose levels (160.8 ± 33.6 $mg.dL^{-1}$) than that of diets containing only roasted flaxseed (180.4 ± 35.4 $mg.dL^{-1}$) or flaxseed oil (185.2 ± 65.7 $mg.dL^{-1}$), probably because flaxseed provides a high content of soluble and insoluble fibers that contributes to decreased glucose and triglyceride levels, while flaxseed oil confers cardioprotective benefits. Other *in vivo* beneficial effects of flaxseed are promoted by lignans associated to its fiber matrix. Lignans, which participate in the inactivation of free radicals from fatty acids and reactive oxygen species, have an indirect *in vivo* effect on endogenous antioxidant systems such as glutathione (FERNANDES et al., 2010).

Baba et al. (2000) reported that rats fed sesame oil-based diets exhibited higher glucose levels (120.09 ± 5.00 $mg.dL^{-1}$) than those fed diets based on soybean oil (109 ± 6.08 $mg.dL^{-1}$) and rapeseed oil (96.9 ± 6.19 $mg.dL^{-1}$). Ramesh (2011) showed that the glycemic index of rats treated with sesame oil-based diet for 42 days decreased from 80.15 ± 4.32 to 76.18 ± 3.68 $mg.dL^{-1}$ in normoglycemic rats and from 242.85 ± 7.49 to 222.02 ± 8.27 $mg.dL^{-1}$ in diabetic rats.

Cholesterol levels were lower in G_F than in G_S and G_{AS} ($P < 0.05$), and the other groups showed intermediary values ($p > 0.05$). Omega-3 fatty acids found in flaxseed oil contribute to increase cholesterol excretion via bile, thus depleting the liver cholesterol pool and increasing the synthesis of free cholesterol (MORISE et al., 2004). In addition, diets containing ALA

Table 4. Mean weight gain, food intake and feed efficiency (±sd; n = 6) in rats fed experimental diets based on different fat sources for 45 days.

Group	Weight gain (g)	Food intake (g)	Feed efficiency (g)
G_C	$208.60^b \pm 6.69$	$14.87^b \pm 8.99$	14.03
G_A	$258.07^a \pm 17.17$	$15.88^a \pm 3.44$	16.25
G_F	$273.52^a \pm 11.85$	$15.05^a \pm 14.76$	18.17
G_{AF}	$281.17^a \pm 10.70$	$15.77^a \pm 10.44$	17.83
G_S	$254.67^a \pm 27.97$	$15.09^a \pm 12.54$	16.88
G_{AS}	$260.59^a \pm 11.82$	$15.04^a \pm 11.01$	17.33
G_{FS}	$279.23^a \pm 11.79$	$14.99^a \pm 7.22$	18.63
G_{AFS}	$271.38^a \pm 27.57$	$15.07^a \pm 7.89$	18.01

The diets were based on: G_C = soybean oil; G_A = animal fat; G_F = flaxseed oil; G_{AF} = animal fat + flaxseed oil; G_S = sesame oil; G_{AS} = animal fat + sesame oil; G_{FS} = flaxseed oil + sesame oil; G_{AFS} = animal fat + flaxseed oil + sesame oil. Different letters in a column indicate statistical difference between the groups ($p < 0.05$).

Table 5. Mean values (±sd; n = 10) for blood parameters (in $mg.dL^{-1}$) in rats fed diets with different fat composition for 45 days.

Groups	Glucose	Total cholesterol	HDL-c	LDL- c	VLDL- c	Tryglyceride
G_C	$144.83^{b} \pm 27.23$	$57.00^{ab} \pm 1.67$	$51.17^{a} \pm 2.79$	$5.98^{ab} \pm 0.58$	$5.00^{c} \pm 1.67$	$25.83^{c} \pm 7.83$
G_A	$177.83^{b} \pm 41.92$	$58.00^{ab} \pm 4.98$	$45.17^{abc} \pm 4.88$	$6.43^{a} \pm 1.28$	$11.00^{bc} \pm 3.52$	$54.50^{bc} \pm 16.91$
G_F	$207.17^{ab} \pm 42.52$	$47.33^{b} \pm 5.75$	$39.67^{c} \pm 3.82$	$5.08^{ab} \pm 0.95$	$14.83^{bc} \pm 5.08$	$74.83^{abc} \pm 25.65$
G_{AF}	$172.67^{b} \pm 56.22$	$59.83^{ab} \pm 3.06$	$44.83^{abc} \pm 1.83$	$4.95^{ab} \pm 2.19$	$27.00^{a} \pm 12.18$	$135.17^{a} \pm 61.34$
G_S	$198.33^{b} \pm 36.03$	$63.17^{a} \pm 6.11$	$48.67^{abc} \pm 2.66$	$4.38^{ab} \pm 1.32$	$22.00^{ab} \pm 5.29$	$109.50^{ab} \pm 25.67$
G_{AS}	$191.17^{b} \pm 58.86$	$62.00^{a} \pm 14.14$	$49.00^{ab} \pm 8.58$	$4.50^{ab} \pm 2.40$	$22.17^{ab} \pm 9.66$	$110.50^{ab} \pm 47.79$
G_{FS}	$209.50^{ab} \pm 38.84$	$55.00^{ab} \pm 10.39$	$43.33^{abc} \pm 7.58$	$4.12^{ab} \pm 1.16$	$18.50^{ab} \pm 3.83$	$92.83^{ab} \pm 19.17$
G_{AFS}	$288.00^{a} \pm 65.16$	$54.50^{ab} \pm 5.72$	$41.83^{bc} \pm 2.23$	$3.62^{b} \pm 1.18$	$15.67^{abc} \pm 3.72$	$78.33^{abc} \pm 18.80$

The diets were based on: G_C = soybean oil; G_A = animal fat; G_F = flaxseed oil; G_{AF} = animal fat + flaxseed oil; G_S= sesame oil; G_{AS} = animal fat + sesame oil; G_{FS} = flaxseed oil + sesame oil; G_{AFS} = animal fat + flaxseed oil + sesame oil. Different letters in a column indicate statistical difference between the groups ($p < 0.05$).

decrease fat accumulation in the liver because the acid stimulates the β-oxidation of fatty acids and inhibits their synthesis (RAMADAN et al., 2009; ALMEIDA; BOAVENTURA; GUSMAN-SILVA, 2009). Improving lipid metabolism by enhancing substrate mobilization and β-oxidation in the liver, as well as PUFA rich diets such as those containing flaxseed oil, might promote a cardioprotective effect (BASBAG; TONCER; BASBAG, 2009). On the other hand, animal fat consumption stimulates phospholipid biosynthesis, possibly because it decreases phospholipase activity or increases phospholipid volume triggering an inflammatory process (BASBAG; TONCER; BASBAG, 2009).

HDL-c levels were lower in G_F than in G_C ($p < 0.05$), and the other groups exhibited similar intermediary values ($p < 0.05$). Another study found HDL-c values of 54.0 $mg.dL^{-1}$ in rats fed brown flaxseed oil-based diets, 54.3 $mg.dL^{-1}$ with roasted flaxseed and 51.9 $mg.dL^{-1}$ with crude flaxseed (MARQUES et al., 2011).

LDL-cholesterol levels were higher in G_A than in G_{AFS}, likely because animal fat alone has greater potential to increase this cholesterol fraction than combined with the SFAs and MUFAs of sesame oil. Flaxseed oil action in G_{AFS} was likely diminished by the high MUFA and SFA content. VLDL-c levels in turn were higher in G_{AF} than in G_C and G_{AFS} ($p < 0.05$).

Despite the results obtained, the LDL/HDL ratio calculated to assess risk factors for ASVD (SOCIEDADE…, 2005) was below 1.0 in all treatments. Another study on rats fed flaxseed oil-based diets, HDL-c levels of 21.02 ± 1.58 $mg.dL^{-1}$, LDL-c of 47.59 ± 3.37 $mg.dL^{-1}$ and VLDL of 13.46 ± 1.46 $mg.dL^{-1}$ were found (VIJAIMOHAN et al., 2006).

Other studies demonstrated that the regular consumption of ω-3 PUFA is efficient in reducing total cholesterol, cholesterol fractions, and triglycerides. However, humans with hypertriglyceridemia treated with dietary ω-3 PUFA had a 45% increase in LDL-c levels (89 to 129 $mg.dL^{-1}$), contradicting the cardioprotective effects described for these fats (HARRIS et al., 2008; NAGAO; YANAGITA, 2008).

Fish oil is widely used because of its high ω-3 content, the measurable effects it causes on blood serum as well as its capacity to reduce blood lipoproteins and mediate cellular inflammation. For instance, the consumption of 9 to 12 g^{-1} day of fish oil or 20 to 40 g^{-1} day of flaxseed oil improves lipid profile by decreasing plasma cholesterol (total and fractions) and the levels of inflammatory mediators and platelet aggregation (KAUL et al., 2008). However, 9 to 40 capsules of oil should be consumed daily to provide the health effects reported, which is not practical for consumers since dietary guidelines rarely suggest the consumption of more than 2 capsules per day of any compound. In addition, consumers seldom follow treatments with high doses of oil because the capsules have an unpleasant taste and frequently cause acid eructation (acid belching) (KAUL et al., 2008).

Daily supplementation with 20 to 50 g of flaxseed oil can decrease total cholesterol and LDL-c to normal levels in patients with hypercholesterolemia, and 38 to 40 g of ground flaxseed can reduce lipoprotein A, apolipoprotein A-1, and apolipoprotein B values in postmenopausal women (RODRIGUES-LEVYA et al., 2010). However, overweight adults that consumed 50 g of chia seed (*Salvia hipanica* L.) did not decrease body measures, lipoproteins, serum EPA or DHA levels, but increased ALA levels (SENER et al., 2009).

Sener et al. (2009) reported that rats fed diets with 1% cholesterol supplemented with flaxseed and pumpkin seed (33% p/p), followed by deprivation of ω-3 fatty acids, and then fed diets with 5% flaxseed oil increased adipose tissue formation.

In the present study, rats fed flaxseed oil (G_F) had higher HDL and lower LDL and VLDL levels compared to the findings reported by Morise et al. (2004), who studied the effects of flaxseed oil inclusion in rat diet. They found HDL of 32.8 ± 0.27 $mg.dL^{-1}$, LDL of 4.70 ± 0.05 $mg.dL^{-1}$ and VLDL of 3.00 ± 0.05 $mg.dL^{-1}$.

As with cholesterol, elevated blood triglyceride levels damage vascular endothelial cells, causing cardiovascular disorders (WHELAN; RUST, 2006). Diets based on saturated fats increase triglyceride levels, and despite the increased lipase activity, fat accumulates in the liver (PELLIZZON et al., 2008). In the present study, triglyceride levels were higher in G_{AF} and lower in G_C and G_A ($p < 0.05$). No differences were detected between G_F and G_{AFS} or between G_S, G_{AS}, and G_{FS} ($p > 0.05$). Other studies on the triglyceride levels of Wistar rats as a function of the type of vegetable oil added to their diets reported 81 $mg.dL^{-1}$ for flaxseed oil-based diets, 70 $mg.dL^{-1}$ for rapeseed oil, 66 $mg.dL^{-1}$ for soybean oil, 143 $mg.dL^{-1}$ for olive oil, and 106 $mg.dL^{-1}$ for diets based on sesame oil (MARQUES et al. 2011, BABA et al., 2000).

Serum total cholesterol and LDL levels were lower in healthy adults that consumed flaxseed and sesame oils, but triglyceride levels were unaffected (CUNNANE et al., 1995). Tzang et al. (2009) reported that the consumption of vegetable oils containing PUFAs reduces triglyceride levels, probably because of increased lipase activity. Oxidation of fats and proteins from lipoproteins and cell membranes decreases fat transport causing cell damage and leading to heart disorders (APPOLINÁRIO et al., 2011). LDL transports cholesterol from the liver to peripheral arterial smooth muscle cells, and high LDL levels can cause cholesterol deposition within the arteries of the heart, a risk factor for the development of heart disease (MOREIRA et al., 2010; LUZIA; JORGE, 2011).

Although humans and other animals can synthesize SFA and MUFA, they lack the enzyme that inserts *cis*-double bounds in position 3 and 6 of fatty acids chain to synthesize ALA and LA, respectively. Both acids are part of the same metabolic pathway, competing for Δ^6-desaturase, but they display different mechanisms of action. ALA exerts a major effect on the modulation of lipoproteins, whereas EPA and DHA decrease the synthesis of triglycerides and adiposity (POUDYAL et al., 2011). Moreover, as an essential fatty acid, ALA can be converted into EPA and DHA, and LA is a direct precursor of pro-inflammatory arachidonic acid (AA).

In the course of evolution, the ω-6: ω-3 ratio in human diet was probably similar in primordial times, but changes in eating habits, especially the dietary inclusion of LA-rich vegetable oils such as soybean, corn, sunflower, safflower, and cotton increased this proportion in the Western diet to at least 10:1 (POUDYAL et al., 2011). A reduction in the dietary ω-6: ω-3 ratio can decrease the risk factors for developing metabolic syndrome. The effect of ω-6 fatty acids on cardiovascular disease is still controversial. Some studies attribute the cardioprotective properties of ω-6 fatty acids to their ability to decrease LDL-c levels, while others argue that the pro-inflammatory action of specific eicosanoids derived from AA is harmful. Irrespective of the amount of dietary ω-6 fatty acid, there is growing acceptance that the inclusion of high levels of metabolically more active ALA and EPA, in addition to DHA, is important to reduce the risks of cardiovascular diseases (BROUGHTON; BAYES; CULVER, 2010).

4 Conclusion

The experimental diets supplemented with different fat sources increased weight gain compared with that of the control group. Group G_{AFS} had the highest glucose, and G_F had the lowest total cholesterol levels. HDL-c levels in groups G_C, G_F, G_{AS}, and G_{AFS} changed significantly. LDL-c values were significantly different between G_A and G_{AFS}, and VLDL-c differed between groups G_C, G_{AF}, and G_{AFS}. The triglyceride levels of G_{AF} were higher than those of the other groups.

Flaxseed and sesame seeds have high lipid content and are a source of PUFA (ω-3 and ω-6), suggesting that they have cardioprotective properties. The nutritional quality of the seed oils assessed using AI, AT, HH, PUFA/SFA, and ω6:ω3 indexes indicate that sesame oil and flaxseed oils can be consumed as functional ingredients of human diet.

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