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Consumption of virgin coconut oil in *Wistar* rats increases saturated fatty acids in the liver and adipose tissue, as well as adipose tissue inflammation

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ABSTRACT

The recommended amount of intake of virgin coconut oil (VCO) for health benefits has not been defined. This study aims to evaluate the effects of partial dietary substitution of lipid source by VCO in the health of *Wistar* rats. The animals were divided as: G1, 100% of soybean oil; G2, 10.4% of VCO; G3, 50% of VCO; and G4, 95% of VCO. Weight and body measurements were not affected by substitution, as well as morphometry of adipocytes, food intake and hepatic expression of PPAR-α and CPT-1 genes. VCO consumption affected the serum lipid profile in a dose-dependent way, just like the tissue incorporation of saturated fatty acids, the inflammation in adipose tissue, and the antioxidant and antimicrobial effects. Since, it was not possible to establish the best dose of VCO to be consumed, the current recommendations for the consumption of satured fatty acids should be followed.

1. Introduction

Unlike other vegetable oils, virgin coconut oil (*Cocos nucifera L.*) has a high concentration of medium chain fatty acid (MCFA) in the form of medium chain triglycerides (MCT). Furthermore, it is rich in saturated fatty acids (SFA), such as lauric acid (C12:0) (Assunção, Ferreira, Santos, Cabral, & Florêncio, 2009; Marina, Che Man, & Amin, 2009). This unique fatty acid composition of virgin coconut oil results in its distinct digestion, absorption and metabolism compared to other vegetable oils (Marina et al., 2009).

The digestion of MCT begins in the stomach with the action of gastric lipase, an enzyme with high affinity for fatty acids at the sn-3 position of the glycerol molecule. In the duodenum, a high quantity of free fatty acids and diglyceride are produced due to the high water solubility of MCFA. This causes a low secretion of cholecystokinin and bile salts, which consequently reduces the secretion of pancreatic enzymes. However, due to the small chain size of MCFA, its hydrolysis is fast and complete. In addition, about 30% of MCT may not undergo hydrolysis, thus they are absorbed in their entirety and subsequently hydrolyzed by an intestinal lipase (Sáyago-Ayerdi, Vaqueiro, Schultz-Moreira, Bastida, & Sánchez-Muniz, 2008). In the enterocytes, the hydrolysis products are not re-esterified to triglycerides because of the low affinity of acyl-CoA synthetase enzyme for fatty acids with 12 carbon atoms or less. MCT are therefore bound to the albumin and transported to the liver by portal circulation (Mu & Hoy, 2004).

In the liver, MCT are predominantly metabolized by β-oxidation. In this process, carnitine palmitoyl transferase enzyme is not required for intra-mitochondrial fatty acid transport and as a result, MCT are metabolized more rapidly. In addition, acetyl-CoA molecules formed during the oxidation process are preferably metabolized to ketone

Abbreviations: AT, adipose tissue; FCE, food conversion efficiency; G1, Group 1: AIN-93M control diet; G2, Group 2: 10.4% of dietary lipid source is virgin coconut oil; G3, Group 3: 50% of dietary lipid source is virgin coconut oil; G4, Group 4: 95% of dietary lipid source is virgin coconut oil; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LCFA, long chain fatty acids; MCFA, medium chain fatty acids; TG, triglycerides; TNFα, tumor necrosis factor alpha; VCO, virgin coconut oil

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bodies (Ooyama, Kojima, Aoyama, & Takeuchi, 2009). Therefore, MCFAs are a fast source of energy, making them less available for adipose tissue (AT) deposition (Marten, Pfeuffer, & Schrezenmeir, 2006).

Many studies have been carried out to investigate whether the regular consumption of coconut oil alters the body weight (St-Onge, Bourque, Ross, & Parsons, 2003; St-Onge, Ross, Parsons, & Jones, 2003), lipid profile (Assunção et al., 2009; Cardoso, Moreira, Oliveira, Luiz, & Rosa, 2015; Vijayakumar et al., 2016) and inflammatory biomarkers of its consumers (Hamsi et al., 2015). However, to date, the findings of such studies are controversial. In this sense, the results found can be partially attributed to the different doses of coconut oil consumed, as well as the total percentage of lipids and SFA present in the diet prescribed.

Thus, there is a gap in the literature with regard to the dose of coconut oil to be incorporated into a normolipid diet, which is beneficial to the health of its consumers. Accordingly, the purpose of this study is to evaluate the effects of the partial substitution of lipid sources in a isocaloric and normolipid diet with different concentrations of virgin coconut oil in *Wistar* rats, in relation to anthropometric, biochemical and inflammatory parameters, oxidative stress and the composition of the intestinal microbiota.

2. Methodology

2.1. Lipid profile of oils

Organic virgin coconut oil, obtained by the cold press method was supplied by Dr. Orgânico® (Brazil). Soybean oil (Soya®, Brazil) was used as a standard since it is one of the most consumed oils worldwide and is recommended by Reeves, Nielsen, and Fahey (1993) as the main lipid source for the AIN93 diet.

In order to evaluate the fatty acid profile of the oils used in this study, the fatty acids were extracted according to the methodology of Folch, Less, and Stanley (1957), and they were submitted to saponification and esterification according to Hartman and Lago (1973) (Table 1).

2.2. Experimental design

Thirty-two female *Wistar* rats were obtained from the animal facility of the Center for Biological Sciences and Health of Universidade Federal de Viçosa. The animals were kept in individual cages, in a temperature controlled environment (21 ± 3 °C) and 12-hour light/dark cycle. The animals consumed distilled water and experimental diets *ad libitum*.

Table 1
Profile of fatty acids (%) of the test oils.

Fatty acid	Virgin coconut oil (%)	Soy oil (%)
C8:0	5.22	–
C10:0	5.41	–
C12:0	51.64	–
C14:0	19.86	0.04
C16:0	8.82	10.57
C18:0	3.04	4.90
C18:1	5.10	24.10
C18:2	0.70	52.00
C18:3	–	5.86
MCFA	62.27	–
LCFA	37.60	100.00
SFA	94.03	15.55
MUFA	5.12	24.16
PUFA	0.69	58.43

Values expressed as a percentage (%). MCFA: medium chain fatty acids; LCFA: long chain fatty acids; SFA: saturated fatty acids; MUFA: monoinsaturated fatty acids; PUFA: polyinsaturated fatty acids; C8:0: caprylic acid; C10:0: capric acid; C12:0: lauric acid; C14:0: myristic acid; C16:0: palmitic acid; C18:0: stearic acid; C18:1: oleic acid; C18:2: linoleic acid; C18:3: linolenic acid.

Table 2
Centesimal composition of the experimental diets.

Ingredients (g/100 g)	Groups			
	G1	G2	G3	G4
Corn starch	46.57	46.57	46.57	46.57
Casein**	14.00	14.00	14.00	14.00
Dextrinized starch	15.50	15.50	15.50	15.50
Refined sugar	10.00	10.00	10.00	10.00
Soybean oil	4.00	3.58	2.00	0.20
Virgin coconut oil	0.00	0.42	2.17	4.12
Cellulose	5.00	5.00	5.00	5.00
Mineral mix	3.50	3.50	3.50	3.50
Vitamin mix	1.00	1.00	1.00	1.00
L-cystine	0.18	0.18	0.18	0.18
Choline bitartrate	0.25	0.25	0.25	0.25
Total kalocaries	37.66	37.66	37.66	37.66

Diets based on the AIN-93 M recommendation (Reeves et al., 1993).
** Casein contained above 85% protein. g: grams. G1: control group; G2: 10.4% of virgin coconut oil; G3: 50% of virgin coconut oil; G4: 95% of virgin coconut oil.

The animals were randomly divided into four experimental groups (n = 8), which diets differed only in the concentrations in which the test oils (virgin coconut oil and soybean oil) made up the lipid source of the diet, therefore isocaloric and isolipidics diets were offered (Table 2). Thus, the experimental groups were defined as Group 1 (G1): AIN-93 M control diet (Reeves et al., 1993); Group 2 (G2): 10.4% of dietary lipid source is virgin coconut oil; Group 3 (G3): 50% of dietary lipid source is virgin coconut oil; and Group 4 (G4): 95% of dietary lipid source is virgin coconut oil.

In order to establish the quantity of virgin coconut oil to be incorporated into the diet of G2, the average daily intake recommended for humans by the manufacturer was considered, being 15–30 mL/day. For G3, it was established that this group would receive half of each type of oil (coconut and soybean). And for G4, the maximum quantity of coconut oil that can substitute soybean without causing harm to the health of the animals was considered (95%), since the essential fatty acids should be provided by the diet.

During the experimental period, the body weight and food consumption of the animals were checked weekly and these data were utilized for the calculation of food conversion efficiency (FCE).

The abdominal perimeter (just before the hind legs) and thoracic perimeter (just behind the front legs) were measured with an inelastic measuring tape at the beginning and end of the experiment. Also, the snout to anus length was measured and the body mass index (BMI) and Lee index were calculated.

After 10 experimental weeks, the animals were subjected to 12 h of fasting, being anesthetized with isoflurane 3% (Cristália, Brazil) and submitted to total exsanguination. Blood, stool and tissue samples were collected and stored for analysis.

The study was approved by the Ethics Committee on Animals Use of Universidade Federal de Viçosa, under protocol 99/2014. The principles recommended by the National Council for the Control of Animal Experimentation were followed (CONSEA, 2008).

2.3. Histology of the intra-abdominal adipose tissue

Intra-abdominal AT samples were removed and fixed in 1% calcium buffered Carson formalin solution (Carson, Martin, & Lynn, 1973). Subsequently, the samples were dehydrated in increasing concentration of ethanol (70–100%) and embedded in Paraplast Plus (Sigma®, Switzerland). Cross-sections of 5 μm thickness were obtained with a rotating microtome (model CUT 4055, Olympus®, EUA) and subsequently stained with hematoxylin and eosin (Díaz-Villaseñor et al., 2013).

Table 3
Sequence of the primers used to evaluated the gene expression of lipid oxidation markers in hepatic tissue.

Gene	Banc access number	Sequence
PPAR-α	NM_013196.1	Forward: 5′-TCACACAATGCAATCCGTTT-3′ Reverse: 5′-GGCCTTGACCTTGTTCATGT-3′
CPT-1	NM_031559.2	Forward: 5′-GGAGACAGACACCATCCAACATA -3′ Reverse: 5′-AGGTGATGGACTTGTCAAACC-3′
GAPDH	NM_017008.3	Forward: 5′-GTATCGGACGCCTGGTTAC-3′ Reverse: 5′-CTTGCCGTGGGTAGAGTCAT-3′

PPAR-α: peroxisome proliferator-activated receptor alpha; CPT-1: carnitine palmitoyltransferase-1; e GAPDH: glyceraldehyde 3-phosphate dehydrogenase.

Twenty images per animal (80 images per group) were obtained using a Primo Star 2012 microscope (Zeiss®, Germany) with an integrated camera (Aixo ERc5s, Zeiss®, Germany), at a 200X magnification. The number and area of adipocytes were determined according to the methodology of Boqué et al. (2009), with the aid of the Adiposoft software (Adiposoft from CIMA, University of Navarra).

2.4. Gene expression of hepatic lipid oxidation markers

Hepatic gene expression was determined by real-time polymerase chain reaction (PCR) in six animals per group, in duplicate.

Extraction of messenger RNA (mRNA) was performed with Trizol reagent (Invitrogen®, USA), according to the recommendations of the manufacturer. Complementary DNA was obtained with the aid of GoScript™ Reverse Transcription System commercial kit (A5000, Promega®, Brazil). Primers (IDT®, Brazil) for PPAR-α and CPT-1, since they are markers of hepatic lipid oxidation (Table 3).

Each reaction had a final volume of 20 µL, comprising 10 µL aliquot of universal iTaq SYBR Green supermix (Bio Rad®, USA), 2 µL aliquot of primer at 200 nM/µL concentration and 1 µL aliquot of cDNA at 500 ng/µL concentration. The rest of the volume was filled with sterile water.

The reaction program was 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95° C for 10 sec and 60 °C for 30 sec, 95 °C for 15 sec, 55 °C for 15 sec and 95 °C for 15 sec, where fluorescence was detected after the last cycle. The relative amounts of mRNA were assessed according to the ΔΔct method.

2.5. Biochemical analysis

Serum concentration of triglycerides (TG), total cholesterol, HDL-cholesterol (HDL-c) and glucose were measured by enzymatic colorimetric methods employing commercial kits (Bioclin®, Brazil) and BS200 automatic analyzer (Minray®, China). The concentration of LDL cholesterol (LDL-c) was calculated according to the equation of Friedewald, Levy, and Fredrickson (1972).

2.6. Determination of tissue and fecal fatty acid profiles

Fatty acids from the tissues (liver, intra-abdominal AT, mesenteric AT and perirenal AT) and feces were extracted according to Folch et al. (1957), and saponified and esterified according to Hartman and Lago (1973).

Determination of fatty acid profile was carried out in a gas chromatograph (CG-17 Class, Shimadzu®, Japan) with a SP-2560 (biscianopropil plysiloxane SP-2560) fused silica capillary column whose length and diameter are 30 m and 0.25 mm, respectively. The initial temperature was 150 °C, with a heating rate of 4 °C per minute until the temperature reached 220 °C. The temperatures of the injector and detector were maintained at 200 °C and 240 °C, respectively.

The identification and quantification of the fatty acids were done by comparing their retention time with that of the standard mixture of fatty acids (FAME – Supelco TM C4:0 to C24:0, Sigma-Aldrich®, USA).

2.7. Determination of pro-inflammatory cytokines

Intra-abdominal AT samples (100 mg/animal) were homogenized with phosphate buffer (50 mM, 0.05% Tween-20 and 5 mg/mL aprotinin) and centrifuged at 8000*g* for 5 min at 4° C. The supernatant was analyzed for the quantification of cytokines.

The concentrations of the pro-inflammatory cytokines interleukin-1β (IL-1β), interleukin-12 (IL-12), interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNFα) were determined simultaneously using RECYT-65 k-04 kit (Millipore®, Germany) and Luminex xMAP technology.

2.8. Determination of the concentration of antioxidant enzymes and thiobarbituric acid reactive substances

Hepatic concentrations of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione-S-transferase (GST) were determined according to the adapted methodology of Aebi (1984), Dieterich, Bieligk, Beulich, Hansenfuss, and Prestle (2000) and Habig, Pabst, and Jacoby (1976), respectively. The samples were run in a spectrophotometer (Multiskan GO, Thermo Scientific®, Finland) and the data were expressed in units (U)/mg of protein.

Hepatic concentration of thiobarbituric acid reactive substances (TBARS) was evaluated according to an adapted methodology of Buege and Aust (1978). This method is based on the ability of malondialdehyde to react with thiobarbituric acid (Merck®, Germany) under heat and acidic conditions, to form a pink product that can be measured by spectrophotometry (Multiskan GO, Thermo Scientific®, Finland) at 535mn. The data were expressed in units (U)/mg of protein.

The concentration of protein present in the hepatic tissue samples was determined according to the adapted methodology of Lowry, Rosebrough, Farr, and Randall (1951).

2.9. Characterization of the intestinal microbiota composition

Characterization of the intestinal microbiota composition in the cecal content samples was performed by Flow Cytometry and Fluorescent *In Situ* Hybridization (Flow-FISH), adapted from Collado, Isolauri, Laitinen, and Salminen (2008) and Grzeskowiak et al. (2012).

It was sought to identify the total population of bacteria and four bacterial groups/genera commonly present in the human intestinal microbiota. For this purpose, specific 16S rRNA dual-labeled probes (Alpha DNA®, Canada) for each bacterial group were used.

The samples were read on a flow cytometer (BD FACS Verse, BD®, USA), with a detection of 50.000 events per second, at a low flow rate. Data analysis was performed with the aid of the FlowJo software, version vx 0.7 (FlowJo, 2014), identifying the Eubacteria populations and bacterial groups/genera of interest. The results were expressed in percentage (qualitative) and numerical (quantitative) values.

2.10. Statistical analysis

Data normality was determined using the Kolmogorov-Smirnov test. Comparison between the experimental groups was performed by the variance test (*one way* ANOVA), followed by Tukey's multiple comparisons; or employing the Kruskal Wallis test followed by Dunn's multiple comparison test.

Statistical analyses were performed with the Package Statistical System 20.0 for Windows Evaluation Version software (SPSS, 2010) and Scientific Data Analysis and Graphing Software 11.0 (SigmaPlot, 2008), assuming $p < 0.05$. The results were expressed according to the normality of the variables, i.e., mean ± standard deviation and median ± interquartile range.

3. Results

3.1. Body weight, body measurements and food consumption

Body weight, food consumption, FCE and liver weight were similar among the experimental groups. Furthermore, no differences were found between the groups with regard to body measurements (abdominal and thoracic perimeters, variation in abdominal and thoracic perimeters during the experiment, and ratio of abdominal to thoracic perimeter), BMI and Lee index ($p > 0.05$, Table S1).

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jff.2018.07.036.

3.2. Number and area of adipocytes

The consumption of virgin coconut oil, regardless of concentration, did not affect the number and area of adipocytes ($p > 0.05$; Fig. S1) of the different experimental groups.

3.3. Hepatic gene expression of lipid oxidation markers

The hepatic expression of PPAR-α and CPT-1 genes was similar among the experimental groups ($p > 0.05$; Fig. S2).

3.4. Biochemical parameters

Group G2 presented elevated serum TG concentration when compared to G1 and G4 ($p = 0.004$). In contrast, total cholesterol, HDL-c, LDL-c, and glucose concentrations were similar among the experimental groups ($p > 0.05$, Table S2).

3.5. Profile of fatty acids of tissues and fecal samples

With regard to the hepatic tissue, group G4 presented higher amounts of capric acid (C10:0) compared to G2 ($p = 0.002$), and myristic acid (C14:0) compared to G2 and G1 ($p < 0.001$). With regard to palmitic acid (C16:0) there was an increase in its concentration in G4 group compared to G1 and G2 groups. Also, the amounts of total SFA ($p = 0.002$) and total MUFA ($p < 0.001$) were higher in groups G3 and G4, compared to the other groups. Hence, higher quantities of total PUFA were observed in groups G1 and G2 compared to the other groups ($p < 0.001$) as a consequence of the higher amount of linoleic acid (C18:2) presented by these groups ($p < 0.001$) (Table 4).

In relation to the fatty acid profile of the adipose tissues (Table 5), the intra-abdominal AT of groups G3 and G4 had higher quantities of C10:0 ($p < 0.001$) when compared to the others. With regard to mesenteric AT, G4 exhibited the highest quantity of C10:0, followed by G3 ($p < 0.001$). For perirenal AT, a similar result ($p < 0.001$) as that of mesenteric AT was found. Furthermore, higher quantities of C14:0 acid were found in the intra-abdominal AT ($p < 0.001$) and perirenal AT ($p = 0.001$) of G3 and G4 when compare to G1. In the mesenteric AT, an increase in C14:0 acid was observed for group G4, followed by G3 ($p < 0.001$) when compared to the other groups. There was also an increase in the amount of C16:0 in the mesenteric and perirenal tissues of G4 compared to G1 and G2 ($p < 0.001$ and $p = 0.004$, respectively), and stearic acid (C18:0) in the intra-abdominal of the G3 and G4 groups compared to G1. In view of these differences the quantities of total SFA in the intra-abdominal AT ($p < 0.001$), mesenteric ($p = 0.008$) and perirenal AT ($p = 0.005$) were higher in G4 compared to G1 (Table 5).

In relation to the MUFAs of the intra-abdominal AT, it was observed that they were lesser ($p = 0.036$) in group G3 when compared to G1. In the perirenal AT, MUFAs increased in G4 compared to G1 and G2 ($p = 0.001$). The amount of total PUFA was reduced in G4 compared to G1 and G2 in all AT evaluated (Table 5).

Regarding the feces, C14:0 fatty acid was lower in the groups that consumed coconut oil (G2 to G4) ($p = 0.001$). The quantity of myristoleic acid (C14:1) increased in G4 ($p < 0.001$) compared to the others. Also, an increase in the quantity of C18:2 in group G4 ($p = 0.010$) was observed when compared to G2 (Table 6).

3.6. Pro-inflammatory cytokines

The animals fed diet containing virgin coconut oil, regardless of concentration, showed an increase in IL-1β concentration ($p = 0.039$) (Fig. 1A). The concentration of IL-12 was higher in the G3 and G4 groups, compared to the other groups ($p = 0.014$) (Fig. 1B). And, the concentrations of IFN-γ and TNF-α were similar among the groups ($p > 0.05$, data not shown).

3.7. Hepatic concentration of antioxidant and TBARS enzymes

The concentration of CAT was higher in the groups that consumed virgin coconut oil (G2 to G4) ($p < 0.001$) compared to the control group (Fig. 2A). The concentration of SOD and GST was similar among the groups ($p > 0.05$, data not shown), and the concentration of TBARS was lower in G3 compared to G1 ($p = 0.021$) (Fig. 2B).

Table 4
Effect of lipidic dietary substitution by virgin coconut oil in liver fatty acids profile (%) of *Wistar* rats.

Fatty acid	Liver				
	G1	G2	G3	G4	*p*
C10:0	0.22 ± 0.01^{ab}	0.08 ± 0.01^{a}	0.23 ± 0.02^{ab}	0.70 ± 0.23^{b}	0.002§
C12:0	0.21 ± 0.04	0.37 ± 0.20	0.43 ± 0.13	0.15 ± 0.03	0.320
C14:0	0.48 ± 0.08^{a}	0.55 ± 0.07^{a}	0.99 ± 0.09^{ab}	1.63 ± 0.21^{b}	< 0.001§
C16:0	23.76 ± 0.63^{a}	23.46 ± 0.58^{a}	27.87 ± 1.21^{ab}	29.30 ± 0.50^{b}	< 0.001§
C18:0	13.83 ± 1.18	14.71 ± 0.82	12.33 ± 1.19	11.06 ± 0.91	0.075
SFA total	38.43 ± 0.84^{a}	39.18 ± 1.37^{a}	42.31 ± 0.52^{b}	40.71 ± 0.53^{b}	0.002
C18:1	20.86 ± 1.78^{a}	20.03 ± 0.84^{a}	27.32 ± 1.45^{b}	32.67 ± 1.40^{c}	< 0.001§
MUFA total	22.81 ± 1.92^{a}	22.23 ± 1.07^{a}	31.24 ± 1.87^{b}	37.84 ± 1.76^{b}	< 0.001§
C18:2	18.20 ± 1.14^{a}	17.40 ± 2.13^{a}	11.21 ± 1.14^{b}	5.34 ± 0.31^{c}	< 0.001
C18:3	0.75 ± 0.08	0.85 ± 0.12	0.42 ± 1.10	0.46 ± 0.07	0.059
PUFA total	37.88 ± 1.60^{a}	37.60 ± 1.83^{a}	26.04 ± 2.11^{b}	18.54 ± 1.53^{c}	< 0.001

Values expressed as mean ± SD for samples with normal distribution and at median ± interquartile range for samples with non-normal distribution (n = 8). Different letters on the same line indicate statistical difference ($p < 0.05$) between groups, according to ANOVA *one way* test followed by Tukey *post hoc* or Kruskal Wallis (§) complemented by Dunn's test; SFA: saturated fatty acids; MUFA: monoinsaturated fatty acids; PUFA: polyinsaturated fatty acids; C8:0: caprylic acid; C10:0: capric acid; C12:0: lauric acid; C14:0: myristic acid; C16:0: palmitic acid; C18:0: stearic acid; C18:1: oleic acid; C18:2: linoleic acid; C18:3: linolenic acid. G1: control group; G2: 10.4% of virgin coconut oil; G3: 50% of virgin coconut oil; G4: 95% of virgin coconut oil.

Table 5
Effect of lipid source substitution in diet for virgin coconut oil on the adipose tissue fatty acid profile (%) of *Wistar* rats.

Fatty acid	Intra-abdominal adipose tissue					Mesenteric adipose tissue	
	G1	G2	G3	G4	*p*	G1	G2
C10:0	0.11 ± 0.03^{a}	0.38 ± 0.03^{a}	2.26 ± 0.24^{b}	3.70 ± 0.37^{b}	< 0.001§	0.08 ± 0.01^{a}	0.50 ± 0.03^{a}
C12:0	–	0.04 ± 0.02	0.04 ± 0.02	0.06 ± 0.01	0.672	0.06 ± 0.01	0.06 ± 0.01
C14:0	1.04 ± 0.03^{a}	1.26 ± 0.04^{a}	2.29 ± 0.47^{b}	4.10 ± 0.25^{b}	< 0.001§	1.05 ± 0.05^{a}	1.43 ± 0.04^{a}
C16:0	24.61 ± 0.40	24.59 ± 1.03	26.19 ± 0.87	26.15 ± 0.50	0.252	24.73 ± 0.41^{a}	25.13 ± 0.61^{a}
C18:0	12.54 ± 6.50^{a}	32.48 ± 7.50^{ab}	45.70 ± 0.62^{b}	41.36 ± 6.59^{b}	0.002§	19.73 ± 8.05	20.74 ± 8.52
SFA total	38.63 ± 6.54^{a}	59.04 ± 7.59^{ab}	76.66 ± 0.95^{b}	75.60 ± 6.28^{b}	< 0.001§	46.01 ± 8.35^{a}	45.65 ± 8.22^{a}
C18:1	30.56 ± 6.71	12.25 ± 7.96	–	–	0.211	22.33 ± 7.93	26.18 ± 7.70
MUFA total	35.43 ± 6.55^{a}	16.91 ± 7.95^{ab}	6.25 ± 0.22^{b}	13.64 ± 6.39^{ab}	0.036§	26.68 ± 7.67	30.75 ± 7.45
C18:2	24.32 ± 1.14^{a}	21.87 ± 1.05^{a}	14.60 ± 0.64^{b}	9.12 ± 2.07^{c}	< 0.001	24.06 ± 1.19^{a}	21.89 ± 1.10^{a}
C18:3	–	0.30 ± 0.12	0.20 ± 0.05	0.13 ± 0.01	0.628	0.46 ± 0.43	–
PUFA total	24.92 ± 1.18^{a}	22.91 ± 1.02^{a}	15.36 ± 0.68^{b}	9.73 ± 0.83^{c}	< 0.001	24.80 ± 1.16^{a}	22.53 ± 1.11^{a}

Fatty acid	Mesenteric adipose tissue			Perirenal adipose tissue				
	G3	G4	*p*	G1	G2	G3	G4	*p*
C10:0	2.46 ± 0.10^{b}	4.60 ± 0.18^{c}	< 0.001	0.10 ± 0.01^{a}	0.60 ± 0.05^{a}	2.50 ± 0.28^{b}	5.11 ± 0.39^{c}	< 0.001§
C12:0	0.03 ± 0.01	0.11 ± 0.06	0.073	0.05 ± 0.02	0.02 ± 0.01	0.10 ± 0.07	0.05 ± 0.01	0.129
C14:0	3.08 ± 0.13^{b}	5.07 ± 0.14^{c}	< 0.001	1.16 ± 0.04^{a}	1.65 ± 0.04^{ab}	3.15 ± 0.24^{b}	3.90 ± 0.85^{b}	0.001
C16:0	27.93 ± 0.52^{b}	30.16 ± 0.92^{b}	< 0.001	20.88 ± 3.00^{a}	23.95 ± 0.68^{ab}	26.82 ± 0.79^{b}	26.99 ± 0.74^{b}	0.004§
C18:0	39.52 ± 6.18	24.49 ± 8.19	0.194	14.22 ± 6.21^{a}	37.25 ± 5.33^{b}	33.80 ± 6.45^{b}	41.21 ± 5.73^{b}	0.006§
SFA total	73.35 ± 6.33^{b}	64.72 ± 7.90^{b}	0.008§	26.42 ± 4.41^{a}	26.51 ± 0.69^{a}	33.89 ± 0.63^{ab}	37.11 ± 3.36^{b}	0.005
C18:1	–	21.37 ± 8.12	0.309	40.28 ± 0.94	–	18.84 ± 11.74	–	0.414
MUFA total	12.21 ± 5.86	28.65 ± 8.06	0.233	45.92 ± 0.78^{a}	47.19 ± 1.05^{a}	49.42 ± 1.81^{ab}	54.68 ± 1.55^{b}	0.001§
C18:2	12.80 ± 0.55^{b}	5.39 ± 0.47^{c}	< 0.001	22.65 ± 3.12^{a}	19.77 ± 2.86^{a}	13.45 ± 0.81a^{b}	4.58 ± 0.83^{b}	0.001§
C18:3	0.40 ± 0.18	2.47 ± 2.39	0.990	–	0.06 ± 0.03	0.18 ± 0.11	0.04 ± 0.01	0.186
PUFA total	13.39 ± 0.59^{b}	5.79 ± 0.57^{c}	< 0.001	23.11 ± 3.13^{a}	20.43 ± 2.85^{a}	14.34 ± 1.16^{ab}	4.85 ± 0.81^{b}	0.001§

Values expressed as mean ± SD for samples with normal distribution and at median ± interquartile range for samples with non-normal distribution (n = 8). Different letters on the same line indicate statistical difference ($p < 0.05$) between groups, according to ANOVA *one way* test followed by Tukey *post hoc* or Kruskal Wallis (§) complemented by Dunn's test. SFA: saturated fatty acids; MUFA: monoinsaturated fatty acids; PUFA: polyinsaturated fatty acids; C8:0: caprylic acid; C10:0: capric acid; C12:0: lauric acid; C14:0: myristic acid; C16:0: palmitic acid; C18:0: stearic acid; C18:1: oleic acid; C18:2: linoleic acid; C18:3: linolenic acid. G1: control group; G2: 10.4% of virgin coconut oil; G3: 50% of virgin coconut oil; G4: 95% of virgin coconut oil.

Table 6
Effect of lipid source substitution in diet for virgin coconut oil on the feces fatty acid profile (%) of *Wistar* rats.

Fatty acid	Feces				
	G1	G2	G3	G4	*p*
C14:0	0.73 ± 0.38[a]	0.36 ± 0.14[b]	0.31 ± 0.22[b]	0.17 ± 0.18[b]	0.001
C16:0	3.54 ± 15.04	3.78 ± 5.02	4.51 ± 1.91	2.27 ± 2.71	0.157[§]
C18:0	7.03 ± 6.59	5.90 ± 6.96	8.06 ± 5.43	2.68 ± 2.53	0.324
SFA total	5.42 ± 30.63	4.77 ± 12.42	7.08 ± 25.60	0.80 ± 2.60	0.080[§]
C14:1	1.31 ± 0.67[a]	1.06 ± 0.56[a]	1.97 ± 0.72[a]	3.25 ± 1.41[b]	< 0.001
C18:1	15.47 ± 8.19	17.57 ± 1.55	15.23 ± 7.25	15.04 ± 7.20	0.880
MUFA total	6.31 ± 23.55	8.91 ± 5.31	11.05 ± 12.38	6.48 ± 5.58	0.220[§]
C18:2	14.95 ± 2.52[abc]	13.24 ± 4.27[b]	17.22 ± 1.82[abc]	18.29 ± 2.38[c]	0.010
C18:3	1.12 ± 0.51	2.47 ± 11.97	2.41 ± 3.39	1.83 ± 2.36	0.264[§]
PUFA total	12.93 ± 4.98	12.56 ± 9.33	18.94 ± 5.70	14.76 ± 3.63	0.062

Values expressed as mean ± SD for samples with normal distribution and at median ± interquartile range for samples with non-normal distribution (n = 8). Different letters on the same line indicate statistical difference ($p < 0.05$) between groups, according to ANOVA *one way* test followed by Tukey *post hoc* or Kruskal Wallis ([§]) complemented by Dunn's test. SFA: saturated fatty acids; MUFA: monoinsaturated fatty acids; PUFA: polyinsaturated fatty acids; C14:0: myristic acid; C16:0: palmitic acid; C18:0: stearic acid; C14:1: myristoleic acid; C18:1: oleic acid; C18:2: linoleic acid; C18:3: linolenic acid. G1: control group; G2: 10.4% of virgin coconut oil; G3: 50% of virgin coconut oil; G4: 95% of virgin coconut oil.

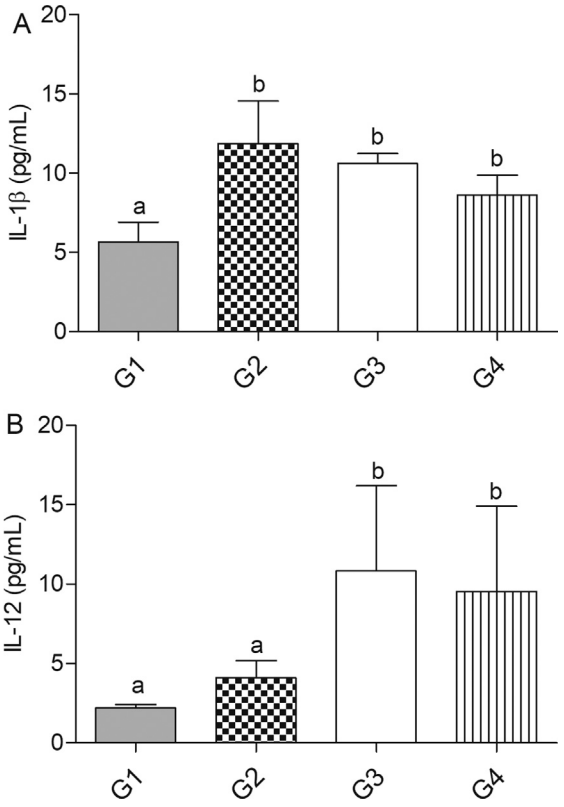

Fig. 1. Concentration of pro-inflammatory cytokines in the intra-abdominal adipose tissue of *Wistar* rats after consumption of different concentrations of virgin coconut oil. A: IL-1β. B: IL-12 concentration. Values are expressed as median ± interquartile range (n = 8). Different letters indicate statistical difference ($p < 0.05$) between the groups, according Kruskal Wallis test complemented by the Dunn's test. G1: control group; G2: 10.4% of virgin coconut oil; G3: 50% of virgin coconut oil; G4: 95% of virgin coconut oil.

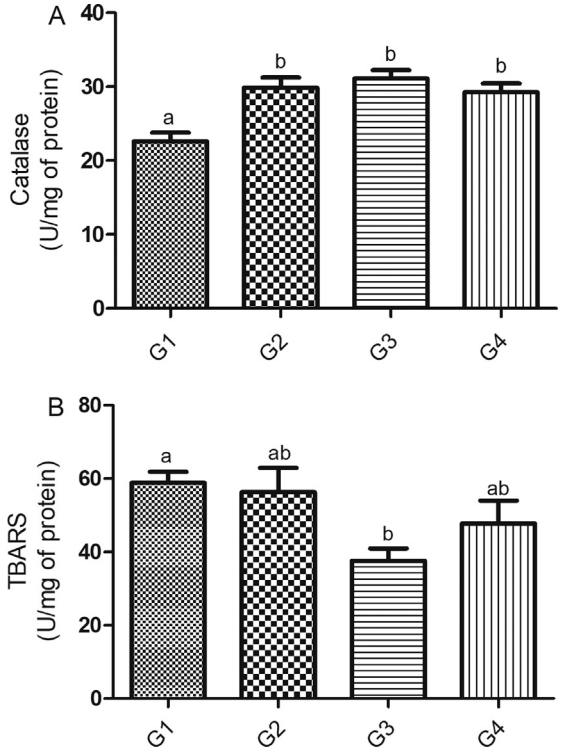

Fig. 2. Effect of consumption of different concentrations of virgin coconut oil in the concentration of antioxidant enzymes in *Wistar* rats. A: Concentration of catalase (CAT). B: Concentration of superoxide dismutase (SOD). Values are expressed as mean ± SD (n = 8). Different letters indicate statistical difference ($p < 0.05$) between groups, according ANOVA test followed by Tukey post hoc. G1: control group; G2: 10.4% of virgin coconut oil; G3: 50% of virgin coconut oil; G4: 95% of virgin coconut oil.

3.8. Characterization of the intestinal microbiota composition

There was a qualitative decrease in the population of *Clostridium histolyticum* in G4 compared to G1 (p = 0.007) and G2 (p = 0.001). As consequence, there was a reduction in the population of total bacteria of G4 compared to G1 (p = 0.018) and G2 (p = 0.046) (Fig. 3).

The quantitative evaluation showed that the population of *Clostridium histolyticum* reduced proportionally according to increase in the dose of virgin coconut oil incorporated into the diet ($p < 0.05$; data not shown).

Further, individually, G1, G2 and G3 showed no differences in the distribution of the four bacterial groups evaluated. However, in G4 there was a reduction in the population of *Clostridium histolyticum* compared to *Bacteroides-Prevotella* (p = 0.006; data not shown).

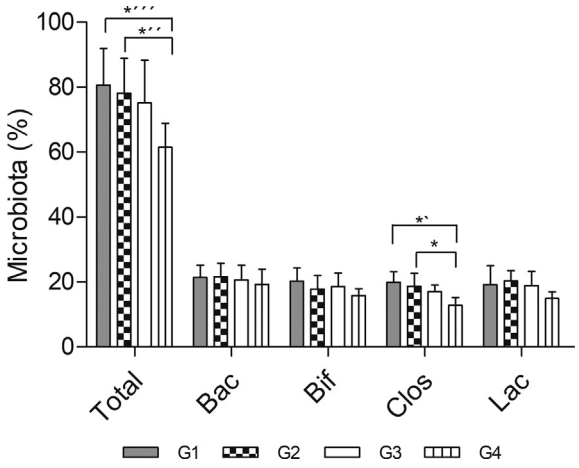

Fig. 3. Percentage of bacterial groups evaluated in the intestinal microbiota of *Wistar* rats fed with different concentrations of virgin coconut oil. Percentage values expressed as mean ± standard deviation. *: p = 0.001; *′: p = 0.007; *″: p = 0.046; *‴: p = 0,018 according to ANOVA test followed by Tukey post hoc. G1: control group; G2: 10.4% of virgin coconut oil; G3: 50% of virgin coconut oil; G4: 95% of virgin coconut oil. (n = 7 for Bac; and n = 6 for other bacterial groups); Bac: *Bacteroides-Prevotella*; Bif: *Bifdobacterium genes*; Clos: *Clostridium histolyticum*; Lac: *Lactobacillus-Enterococcus*; Total: sum of Bac, Bif, Clos and Lac.

4. Discussion

Currently, one of the main appeals for the incorporation of coconut oil into one's diet is centered on weight loss. Such an appeal is based on the fact that the human body prefers to oxidize MCFA instead of LCFA, which would entail a reduced storage of fatty acids in the adipose tissue. Moreover, it has been suggested that MCFA could raise diet-induced thermogenesis, contributing to an increase in the energy expenditure of its consumers (Hill et al., 1989; St-Onge et al., 2003). However, in the present study, it was found that the ingestion of different concentrations of virgin coconut oil did not affect the body weight, body measurements and total number and area of adipocytes of the animals. In addition, the consumption of virgin coconut oil did not affect food intake.

Findings concerning the effect of coconut oil on weight loss and food consumption have been contradictory (Amaral et al., 2016; Deol et al., 2015; Kinsella, Maher, & Clegg, 2017; Panchal, Carnahan, & Brown, 2017). Regarding the present study, it is probable that the experimental time (10 weeks) influenced the final result, since in long-term experiments, adaptations of specific genes can occur, favoring weight gain in the animals (Ferreira et al., 2014), which could have masked the effect of virgin coconut oil. In agreement with this assumption, the present study found that the expression of genes related to hepatic fatty acid oxidation, such as PPAR-α and CPT-1, was similar among the experimental groups. Regarding the number and area of adipocytes, the utilization of normolipid and isocaloric test diets partially justifies the result obtained, since these diets may have not provided a sufficient concentration of macronutrients that influenced the increase or decrease area of adipocytes (Díaz-Villaseñor et al., 2013), and consequently the weight and body measurements of the animals.

Some studies have suggested that the incorporation of coconut oil into a diet would be able to improve the serum lipid profile of its consumers (Assunção et al., 2009; Cardoso et al., 2015; Famurewa et al., 2017; Nevin & Rajamohan, 2004), however, contrary results have also been found (Amaral et al., 2016; Vijayakumar et al., 2016). In the present study, it was observed that the animals that consumed the lowest concentration of virgin coconut oil (10.4% of lipid source) had a higher serum triglyceride concentration compared to the control group (100% of lipid source from soybean oil) and the group that received the highest concentration of virgin coconut oil (95% of the lipid source). This result suggests that depending on the concentration consumed, virgin coconut oil positively or negatively influences serum lipid profile. According to Eyres, Eyres, Chrisholm, and Brown (2016) there is insufficient evidence that coconut oil has a different effect on the serum lipid profile compared to SFA. Thus, the authors conclude that the consumption of coconut oil is not indicated for the reduction of cardiovascular risk, contrary to what has been popularly disseminated.

The fatty acid profile of a tissue reflects the rate of lipid distribution, conversion and deposition in the body (Arterburn, Hall, & Oken, 2006) and is directly related to the profile of fatty acids consumed (Díaz-Villaseñor et al., 2013). Thus, differences in the profile of fatty acids in the liver and adipose tissues (intra-abdominal, perrirenal and mesenteric) were observed in this study, and similar results were reported in previous studies (Díaz-Villaseñor et al., 2013; Leveille, Pardini, & Tillotson, 1967; Raclot & Oudart, 1999; Yli Jama, Haugen, Rebnord, Ringstad, & Pedersen, 2001).

In general, it was observed that the experimental groups that consumed the highest concentrations of virgin coconut oil showed a higher incorporation of capric and myristic fatty acids (C10:0 and C14:0, respectively) in the tissues evaluated. In addition, it was found that higher consumption of virgin coconut oil resulted in greater tissue accumulation of total SFA. As mentioned previously, tissue fatty acid profile is strongly influenced by the diet consumed (Díaz-Villaseñor et al., 2013). Hence, during the assessment of the fatty acid profile of the coconut oil used in this study, it was verified that C14:0 acid is the second most concentrated fatty acid (19.86%), and C10:0 acid is the fourth (5.41%).

The fatty acid profile of a tissue directly influences its physiology, since fatty acids can be used as substrate for various reactions carried out in the organism, and are capable of initiating a cascade of intracellular reactions through interactions with cell surface receptors. The effects associated with the action of fatty acids are not always beneficial to the organism, as in the case of chronic inflammation triggered by SFA (Carlson et al., 2015).

SFA, such as C12:0, that compose in 51.64% of the virgin coconut oil used in this study, is able to activate toll-like receptor 4 of the immune cell and consequently trigger a pro-inflammatory response (Lee et al., 2001; Lee et al., 2003; Lim et al., 2015). Thus, in the present study, the consumption of virgin coconut oil increased the concentration of the pro-inflammatory cytokines IL-1β and IL-12 in the intra-abdominal adipose tissue of the animals, and similar results have been reported in experimental studies with virgin coconut oil (Hamsi et al., 2015) and MCFA (Carlson et al., 2015). This result is of concern, since inflammation in the adipose tissue has been identified as responsible for triggering the development of insulin resistance and consequently diabetes mellitus type 2 (Abranches, Oliveira, Conceição, & Peluzio, 2015).

During the production of virgin coconut oil, some phytochemical compounds such as polyphenols, tocopherols and phytosterols are extracted, which makes this oil a potential antioxidant food (Marina, Che Man, Nazimah, & Amin, 2009). Previous studies have reported the antioxidant effects associated with virgin coconut oil consumption (Arunina & Rajamohan, 2013; Marina et al., 2009; Nevin & Rajamohan, 2006), and in the present study it was observed that the animals that consumed 50% lipid from coconut oil showed a reduction in liver TBARS concentration compared to the others. Moreover, the consumption of virgin coconut oil, independent of its concentration in the diet, caused an increase in the hepatic concentration of the antioxidant enzyme catalase, while the concentration of superoxide dismutase and glutatione-S-transferase was not affected. Thus, it is likely that the antioxidant effect of virgin coconut oil is derived from the phytochemical compounds present in its composition and from its ability to increase the hepatic concentration of catalase (Arunima & Rajamohan, 2013).

In addition to antioxidant potential, coconut oil has antimicrobial potential (Rial, Karelis, Bergeron, & Mounier, 2016), since MCFA would be able to destabilize and lyse the cell membrane of some

microorganisms (Bauer, Williams, Smidt, & Mosenthin, 2006). In this study, a reduction in the population of *Clostridium histolyticum* and total bacteria was observed in the group where 95% source of lipid was supplied by virgin coconut oil. This suggests that the virgin coconut oil presents antimicrobial capacity when consumed in high doses. Such a result is beneficial for its consumers since *Clostridium histolyticum* species is capable of secreting a collagenase-like toxin (Yoshida & Noda, 1965) which could alter the intestinal barrier (Forster, 2008).

5. Conclusion

The incorporation of virgin coconut oil into the diet of the animals did not cause weight loss and/or reduction of body measurements. Such a result would be a consequence of the unchanged expression of hepatic lipid oxidation markers (PPAR-α and CPT-1), as well as food intake.

It is suggested that the effect of coconut oil on serum lipid profile is dose dependent, and further studies are necessary to establish a safe dose for consumption that does not negatively alter this parameter.

The consumption of coconut oil at higher doses (50 or 95% of lipids) increased the incorporation of SFAs into the liver and adipose tissues. This effect may have a negative result on the state of inflammation, which has been extensively associated with an increased risk of developing chronic non-communicable diseases.

Virgin coconut oil seems to have antioxidant and antimicrobial effects, which are beneficial. Thus, like any other food, it has positive and negative effects on the health of its consumers depending on the dose consumed.

Therefore, it is still not possible to establish the best dose of virgin coconut oil to be consumed in order to obtain only health benefits. Thus, current recommendations for the consumption of SFA should be followed, and the dose of virgin coconut oil should not exceed this recommendation in view of the health risks associated with the high consumption of SFA.

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7. Declaration of interest

None.

8. Ethics statement

The study was approved by the Ethics Committee on Animals Use of Universidade Federal de Viçosa, under protocol 99/2014. The principles recommended by the National Council for the Control of Animal Experimentation were followed (CONSEA, 2008).

9. Participation

MMD and NPS work in the study design, in the collection analysis and in the interpretation of data, in the writing of the report and in the decision to submit the article for publication; LLC, SAR, FXV, LLO work in the study design, in the interpretation of data and in the writing of the report; MMSD, COB, SOP, JB, MMGP work in the writing of the report and in the decision to submit the article for publication.

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