Probiotics and Antimicrobial Proteins
https://doi.org/10.1007/s12602-021-09760-0

Antibiotic Followed by a Potential Probiotic Increases Brown Adipose Tissue, Reduces Biometric Measurements, and Changes Intestinal Microbiota Phyla in Obesity

Mariana de Moura e Dias[1] · Sandra Aparecida dos Reis Louzano[1] · Lisiane Lopes da Conceição[1] · Rayssa da Conceição Fernandes[1] · Tiago Antônio de Oliveira Mendes[2] · Solange Silveira Pereira[1] · Leandro Licursi de Oliveira[3] · Maria do Carmo Gouveia Peluzio[1]

Accepted: 22 February 2021

Abstract

The development of adjuvant therapies for obesity treatment is justified by the high prevalence of this disease worldwide, and the relationship between obesity and intestinal microbiota is a promising target for obesity treatment. Therefore, this study aimed at investigating the adjuvant treatment of obesity through the use of potential probiotics and antibiotics, either separately or sequentially. In the first phase of the experiment, animals had diet-induced obesity with consumption of a high saturated fat diet and a fructose solution. After this period, there was a reduction in caloric supply, that is the conventional treatment of obesity, and the animals were divided into 5 experimental groups: control group (G1), obese group (G2), potential probiotic group (G3), antibiotic group (G4), and antibiotic followed by potential probiotic group (G5). The adjuvant treatments lasted 4 weeks and were administered daily, via gavage: Animals in G1 and G2 received distilled water, the G3 obtained *Lactobacillus gasseri* LG-G12, and the G4 received ceftriaxone. The G5 received ceftriaxone for 2 weeks, followed by the offer of *Lactobacillus gasseri* LG-G12 for another 2 weeks. Parameters related to obesity, such as biometric measurements, food consumption, biochemical tests, histological assessments, short-chain fatty acids concentration, and composition of the intestinal microbiota, were analyzed. The treatment with caloric restriction and sequential supply of antibiotics and potential probiotics was able to reduce biometric measures, increase brown adipose tissue, and alter the intestinal microbiota phyla, standing out as a promising treatment for obesity.

Keywords Obesity · Probiotics · Antibiotics · Intestinal microbiota

Introduction

The standard nutritional treatment for obesity is based on the change of eating and living habits, decreasing caloric consumption and increasing energy expenditure [1–4].

Since 2004, a relationship has been observed between the risk for the obesity development and the intestinal microbiota composition [5]. In this scenario, the modulation of this microbiota is suggested to be functional as an adjuvant treatment for obesity [6, 7].

This intestinal microbiota is formed by archaea, fungi, helminths, viruses, and other microorganisms [8]. In healthy adult individuals, this microbiota can encompass more than 100 trillion microorganisms, which are predominantly anaerobic bacteria, belonging mainly to *Firmicutis* and *Bacteroidetes* phyla [9, 10]. This preeminent diversity of intestinal microbiota leads to a lower propensity for alterations within this microbiota by external events [7], i.e., lesser constant imbalances related to the increased risk for obesity development [6, 11–16], as well as another diseases like diabetes [17], non-alcoholic fatty liver disease [18], or polycystic ovary syndrome [19].

✉ Mariana de Moura e Dias
mmouradias@hotmail.com

[1] Department of Nutrition and Health, Universidade Federal de Viçosa, Avenue PH Rolfs S/N, Viçosa, Minas Gerais 36570-900, Brazil

[2] Department of Biochemistry and Molecular Biology, Universidade Federal de Viçosa, Avenue PH Rolfs S/N, Viçosa, Minas Gerais 36570-900, Brazil

[3] Department of General Biology, Universidade Federal de Viçosa, Avenue PH Rolfs S/N, Viçosa, Minas Gerais 36570-900, Brazil

Published online: 05 April 2021

Probiotics are considered potential modulators for the intestinal microbiota composition, and their use in the adjuvant treatment of obesity has been investigated by the scientific community [6, 20–22]. These are live microorganisms which, when consumed in adequate quantities, confer a beneficial upshot in the host health [23]. Synthesized antibiotics are other potential modulators [6, 24–27] which mainly act on inhibiting the growth of specific microorganisms, depending on their mode of action [28] and, in this way, can substantially effect on the gut microbiota composition [7].

Among the different probiotic microorganisms, the genus *Lactobacillus* stands out with a large number of species considered probiotic [21], such as *Lactobacillus gasseri*. It is of human origin and, when administered in adequate amounts, is able to survives to the passage through the intestine [29], having necessary characteristics to be considered a probiotic [30]. In addition your regular consumption can help with weight loss [29, 31–33], being interesting for the treatment of obesity. Mechanisms that explain the capacity to treat the obesity involve modulation of the intestinal microbiota, decreased of adipose tissue inflammation, of systemic inflammation and of intestinal permeability [29, 31–33].

Obesity is a worldwide public health issue, which has elevated prevalence and socioeconomic impact [34]. This medical condition triggers an increase in early mortality, augmenting the mortality cases as a whole, since obesity is linked to the occurrence of chronic non-communicable diseases, such as cardiovascular disease and diabetes [2, 35].

Considering the complexity of obesity and the urge to improve the treatments already available about this medical condition, this study aimed at investigating innovative alternatives to enhance treatments for obesity and its secondary metabolic changes in an animal model by modifying the composition of the diet, associating it with adjuvant treatment as well as with a potential probiotic and/or antibiotic.

Materials and Methods

Test Products

Lactobacillus gasseri LG-G12 was used as a potential probiotic (Lemma®, São Paulo, Brazil), in the dosage of 10^9 colony forming units (CFU)/day, which is the minimum dose to be offered to have a probiotic claim according to Agência Nacional de Vigilância Sanitária (Anvisa) [30]. The calculation to acquire this quantity was carried out according to information provided by the manufacturer. This potential probiotic was chosen due to the positive effects of different strains of *Lactobacillus gasseri* on controlling excessive weight and body adiposity [29, 31–33, 36] and due to the fact that it has characteristics to be considered a probiotic [30], such as its human origin and its ability to survive intestinal transit [29]. In addition, it is observed that different strains of *Lactobacillus gasseri* decreases systemic inflammation and intestinal permeability [29, 31–33] which are recognized mechanisms made by probiotics in the control of obesity [4, 6, 37].

The selected antibiotic was ceftriaxone disodium heptahydrate (Triaxton, Blau Famacêutica S/A®, Cotia, Brazil) in the dosage of 500 mg/kg of each mice body weight [14]. This antibiotic acts mainly against gram-negative bacteria, which have been linked to an increased risk for obesity development [38]. In addition, ceftriaxone is not absorbed by the body, acting only locally in the small intestine and colon [39, 40].

Experimental Design

The study was approved by the Ethics Committee on Animals Use of Universidade Federal de Viçosa, under protocol 33/2018. The principles recommended by the Nacional Council for the Control of Animal Experimentation were followed [41].

Forty C57BL/6 J mice from the Central Vivarium of the Biological and Health Sciences Center, Universidade Federal de Viçosa, Minas Gerais, Brazil, were used in this experiment.

Throughout the experiment, the animals remained in collective cages (8 animals/box), in an environment with controlled temperature (22 °C ± 2 °C) and a 12-h photo period. The animals had free access to water/fructose (Synth®, Diadema, Brazil) solution and to food, administered according to the pair-feeding scheme.

Obesity induction (phase 01) was achieved by randomly dividing the animals into two groups: obese group ($n = 32$) and control group ($n = 8$). During this 12-week phase, the animals in the obese group received a high-fat diet with 60% of calories from saturated fat [42] and 10% fructose solution (Synth®) [43], which replaced drinking water (0.4 kcal per mL of solution consumed). The control group was fed with AIN-93M [44] diet, and distilled water was provided.

After phase 01, a significant difference ($p < 0.005$) in body mass between the groups was noticed indicating obesity induction. Consequently, the animals in the obese group were redistributed into 4 experimental groups ($n = 8$) for the treatment commencement (phase 02). Phase 02 lasted for 4 weeks, and all animals received a standard AIN-93M diet and distilled water during that period, as well as supplementary adjuvant treatments with potential probiotic and/or antibiotic. This diet modification represents a change in the composition of the animals' diet in order to offer lower caloric input, which represents the standard nutritional treatment for obesity.

The adjuvant treatments were administered daily, via gavage. Animals in the control (G1) and obese (G2) groups

received distilled water, while the animals in the potential probiotic group (G3) obtained 10^9 CFU of *Lactobacillus gasseri* LG-G12 (Lemma®)/day and the animals in the antibiotic group (G4) received 500 mg of ceftriaxone (Blau Famacêutica S/A®)/kg. The group supplied with antibiotics and potential probiotics (G5) received 500 mg of ceftriaxone (Blau Famacêutica S/A®) /kg for 2 weeks, followed by the offer of 10^9 CFU of *Lactobacillus gasseri* LG-G12 (Lemma®) for another 2 weeks.

Throughout the experimental period, animal's body weight was measured weekly and food consumption was recorded every 3 days. Body measurements, including abdominal perimeter (measured immediately before the hind legs) and thoracic perimeter (deliberated immediately after the front legs) were assessed at the end of phases 01 and 02, with the aid of an inelastic measuring tape. The abdominal perimeter/thoracic perimeter ratio, the Lee index, and the body fat percentage were calculated.

After 4 months of experiment, the animals were submitted to 12 h of fasting, anesthetized with isoflurane 3% (Cristália®, Itapira, Brazil) and submitted to total exsanguination. Samples of blood, feces, intestines, and adipose tissue were collected and stored for further analysis.

Evaluation of Biochemical Parameters

Total cholesterol, high-density lipoprotein (HDL-cholesterol), triglycerides, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were determined in serum samples, by a colorimetric enzymatic method, using commercial kits (Bioclin®, Belo Horizonte, Brazil) according to procedures described by the manufacturer. The samples were analyzed in an automatic analyzer (Mindray Medical International Limited, BS 200 model, Shenzhen, China).

The concentrations of low-density lipoprotein (LDL-cholesterol) and very low density lipoprotein (VLDL-cholesterol) were calculated according to equations proposed by Friedwald and collaborators [45].

Histological Analysis

Fragments of abdominal adipose tissue were dehydrated, in increasing gradients of ethanol, and included in Paraplast Plus (Sigma®, Buchs, Switzerland). Subsequently, 5 µm histological fragments were obtained in a rotating microtome (model CUT 4055, Olympus®, Miami, Florida, EUA) and stained with both hematoxylin and eosin.

Intending to measure the area and the number of adipocytes, 10 fields per animal were captured through the 10× objective, directly from the light microscope (Primo Star 2012, Zeiss®, Oberkochen, Germany), using the Aixo ERc5s video camera (Zeiss®). The images were analyzed using Image Pro-Plus® software version 4.5 (Media Cybernetics, Rockville, Maryland, USA). All cells that were complete in the photo field portraying well-defined contours were counted [46], and the diameter of twenty cells per field was measured. Moreover, the cell diameter was measured in replicate in different positions.

Inflammatory infiltrate counting was similarly performed. In this assessment, 10 fields per animal were captured through the 20× objective [47]. The images were also analyzed using Image Pro-Plus® software version 4.5 (Media Cybernetics), seeking to identify the presence of inflammatory infiltrates at the intersections of a grid with 315 points.

Tissue samples were removed, fixed in Carson's formalin [48], dehydrated in an increasing ethanol gradient, and included in hydroxyemethylmethacrylate resin (Historesin, Leica®, Zürich, Switzerland) to carry out the morphometric analysis of the small intestine. Slices with 5 µm were obtained on a rotating microtome RM2265 (Leica®) and stained in a solution of Alcian blue (AB) and Schiff's periodic acid (PAS) [49].

The photomicrographs were captured with a 10× objective directly from the LEICA DM750 light microscope (Leica®) using a LEICA 170HD video camera (Leica®). Analyzes were performed with the aid of Image Pro-Plus® software, version 4.5 (Media Cybernetics) morphometric, with the villus height being measured merely in the villi with well-defined epithelium and visible connective tissue.

Villus width was measured at 3 different points (apical, medial, and basal) in the same villus; the mean of these points was considered as the final outcome. Crypt depth was estimated through the images where the base and the apex (opening) of the crypt were discernable [50].

Determination of Intestinal Permeability

After the end of phase 02, the animals received via gavage 200 µL of a solution containing 13.3 mg of lactulose (Daiichi Sankyo®, Barueri, Brazil) and 10.1 mg of mannitol (Synth®, Diadema, Brazil). All urine excreted in the next 24 h was collected [51] with the aid of metabolic cages. During this period, the animals remained fasting.

The quantification of sugars was performed using high-performance liquid chromatography (detector model RID 10A, Shimadzu®, Tokyo, Japan) at a wave length of 210 nm. For chromatographic separation, 20 µL of sample was injected into a mobile column (Aminex model HPX-87H, Bio-Rad®, São Paulo, Brazil), 300 mm long × 7.8 mm in diameter, with a flow of 1 mL/min and pressure of 54 Kgf. Water was used as a mobile phase.

Determination of Short Chain Fatty Acids

The extraction of short chain fatty acids (acetic, propionic, and butyric), from the caecal content, was carried out

according to methodology proposed by Siegfried and collaborators [52].

Readings were performed on a high-performance liquid chromatograph (Ultimate 3000, Dionex, Thermo Fisher Scientific®, Waltham, Massachusetts, USA). For chromatographic separation, samples were injected in a column (RezexROA-Organic Acid H+ (8%), Phenomenex®, Torrance, California, USA) with a length of 300 mm and a diameter of 7.8 mm. The mobile phase used was 5 mmolar of sulfuric acid, the injection flow was 0.7 mL/min, the injection volume equaled to 20 µL, and 45 °C was set as oven temperature. The detector used was Rid RH01 (Shodex®, Tokyo, Japan).

Determination of Intestinal Microbiota Composition

After phase 02, fecal samples were collected from all experimental groups, forming a pool of feces from each group, which was used in the analysis of the intestinal microbiota composition. The samples were analyzed in triplicate, and the pool of feces was used because the animals are isogenic and live in a controlled environment, which means that they are biological replicates.

Initially, DNA from the 16S RNAr region was extracted from samples in accordance with the methodology adapted from Zhang and collaborators [53]. After extraction, the concentration in Qubit was quantified and the quality was verified through electrophoresis in 1.8% agarose gel. Samples denoting an absorbance ratio at 260/230 wave lengths greater than 1.8 were considered suitable for the sequencing step.

DNA sequencing was performed by Macrogen Company (Macrogen Inc®, Seoul, South Korea) using the Illumina MiSeq sequencer (Illumina, San Diego, CA, USA). The amplicons were obtained by PCR, utilizing specific primers (Bakt 341F and Bakt 805R) for V3 and V4 regions of 16S gene. The raw sequencing data (files in the FASTQ format) were trimmed by the Trimmomatic v 0.36 program [54] with a cut off of Phred Quality by 30.

Trimmed data were processed using the DADA2 package version 1.8 [55] on R platform, version 3.6.1 (https://cran.r-project.org/). The data processing followed all the steps recommended by the DADA2 developers, including the following: (1) loading data into software, (2) trimming data to remove low quality bases, (3) filtering to eliminate sequences that were smaller than 160 nucleotides, (4) removing redundancies and identifying unique sequences, (5) eliminating chimeras and estimating errors in sequenced amplicons, and (6) analyzing the frequency of non-redundant sequences and their taxonomic classification, based on alignments performed with the Silva release 138 database [56].

The output files from DADA2 package were used as input to phyloseq package [57] to analyze the results. Using the phyloseq package Chao1, Shannon and Simpson indices were calculated to compare the biodiversity of the intestinal microbiota. Bacterial communities' composition was analyzed at the phylum, family and gender level.

Statistical Analysis

Data normality was determined using the Kolmogorov-Smirnov test. Comparison in phase 01 was performed by T test for independent samples. Comparisons between the experimental groups, in phase 2, were 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 (StataCorp LLC®, College Station, Texas, USA, 2010) and Scientific Data Analysis and Graphing Software 11.0 (IBM®, Armonk, New York, USA, 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. The figures were made in the GraphPad Prism 5 for Windows Software (GraphPad®, San Diego, CA, USA, 2007).

Results

Biometric Parameters

During the treatment phase, no difference was perceived in body mass gain between the experimental groups ($p = 0.073$; Fig. 1), although lower body mass in G1 was observed when

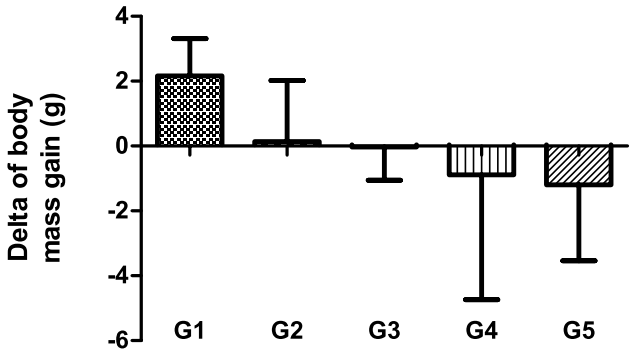

Fig. 1 Difference in mass gain during treatment. **G1** control group, **G2** obese group, **G3** potential probiotic group, **G4** antibiotic group, and **G5** antibiotic followed by potential probiotic group. Results expressed as mean and standard deviation. One way ANOVA

Table 1 Antibiotic followed by potential probiotic group has differences in biometric measurements and somatic indexes at the end of treatment phase

Measurements	G1	G2	G3	G4	G5	*p*
Abdominal perimeter (AP) (cm)	7.00 ± 0.50^{ab}	7.50 ± 0.50^{a}	7.50 ± 0.63^{a}	7.00 ± 0.00^{ab}	6.50 ± 1.00^{b}	0.002§
Thoracic perimeter (AT) (cm)	7.00 ± 0.50^{a}	7.00 ± 0.50^{ab}	7.00 ± 0.50^{a}	6.50 ± 0.50^{ab}	6.00 ± 0.50^{b}	0.000§
AP/TP	1.00 ± 0.04	1.07 ± 0.23	1.07 ± 0.07	1.08 ± 0.01	1.00 ± 0.08	0.050§
Lee index	0.34 ± 0.02^{a}	0.33 ± 0.01^{ab}	0.33 ± 0.01^{ab}	0.33 ± 0.01^{ab}	0.32 ± 0.01^{b}	0.046
Body fat (%)	0.77 ± 0.26	0.70 ± 0.11	0.92 ± 0.10	0.64 ± 0.23	0.81 ± 0.28	0.179
Total adipose tissue (g)	0.99 ± 0.33	0.93 ± 0.12	1.22 ± 0.05	0.88 ± 0.22	1.18 ± 0.32	0.050
Brown adipose tissue (g)	0.27 ± 0.09^{abc}	0.24 ± 0.04^{a}	0.34 ± 0.03^{bc}	0.28 ± 0.07^{abc}	0.37 ± 0.07^{bc}	0.002
Epididymal adipose tissue (g)	0.70 ± 0.20	0.56 ± 0.10	0.71 ± 0.07	0.53 ± 0.23	0.71 ± 0.27	0.243
Abdominal adipose tissue (g)	0.14 ± 0.01^{a}	0.12 ± 0.01^{ab}	0.17 ± 0.04^{ab}	0.08 ± 0.03^{b}	0.10 ± 0.04^{b}	0.000

G1 = control group, G2 = obese group, G3 = potential probiotic group, G4 = antibiotic group; G5 = antibiotic followed by potential probiotic group. Different letters indicate significant differences ($p < 0.05$). Results expressed as mean and standard deviation for samples with parametric distribution. One-way ANOVA followed by Tukey's post hoc test. § = Results expressed as median ± interquartile range for samples with non-parametric distribution. Kruskal-Wallis test followed by Dunn's post hoc test

Different superscripts letters (a, b, c) on the same line indicate differences between groups ($p < 0.05$)

compared with the other experimental groups (data not shown, $p = 0.005$) at the beginning of that phase.

G5 exhibited lower values of abdominal and thoracic perimeters in comparison to G3, and inferior values of Lee's index when compared with G1 (Table 1). In addition, G5 displayed higher amounts of brown adipose tissue than G1 ($p = 0.046$) and G2 ($p = 0.003$) and lower amounts of abdominal adipose tissue than G1 ($p = 0.034$).

Food and Caloric Consumption

After 4-week treatment, no significant differences were noticed between food consumption ($p = 0.155$) and caloric consumption ($p = 0.154$).

Biochemical Parameters

Lower values of triglycerides and VLDL-cholesterol were observed in G5 when compared with G2 and G4 (Table 2).

Histological Assessment

Differences in abdominal adipose tissue per area ($p = 0.441$), number of adipocytes ($p = 0.098$), and the presence of inflammatory infiltrates ($p = 0.256$) were not found between the different experimental groups.

In the small intestine, G4 showed more elevated villus height ($p = 0.004$) and greater mucosa depth ($p = 0.001$) when compared with G1.

Table 2 Antibiotic followed by potential probiotic group has differences in biochemical parameters

Parameter	G1	G2	G3	G4	G5	*p*
CT (mg/dL)	120.12 ± 9.59	127.42 ± 21.51	136.26 ± 12.34	144.53 ± 55.04	114.07 ± 10.08	0.167
HDL (mg/dL)	64.42 ± 6.02	73.68 ± 5.69	77.46 ± 7.71	81.22 ± 31.27	63.20 ± 5.29	0.153
TG (mg/dL)	76.66 ± 11.20^{ab}	110.56 ± 14.55^{a}	98.13 ± 16.73^{ab}	94.80 ± 44.46^{a}	56.58 ± 5.96^{b}	0.007
LDL (mg/dL)	40.36 ± 7.22	31.62 ± 7.76	39.17 ± 3.37	44.35 ± 16.63	39.56 ± 14.22	0.249
VLDL (mg/dL)	15.33 ± 2.24^{ab}	22.11 ± 2.91^{a}	19.63 ± 3.35^{ab}	18.96 ± 8.89^{a}	11.32 ± 1.19^{b}	0.007
AST (U/L)	185.17 ± 39.61	138.48 ± 10.71	151.01 ± 17.97	156.80 ± 60.08	190.00 ± 41.50	0.059
ALT (U/L)	44.96 ± 4.00	41.80 ± 2.50	46.88 ± 2.00	48.00 ± 32.00	45.33 ± 17.33	0.068§

CT = total cholesterol, HDL = HDL-cholesterol, TG = triglycerides, LDL = LDL-cholesterol, VLDL = VLDL-cholesterol, AST = aspartate aminotransferase, ALT = alanine aminotransferase, G1 = control group, G2 = obese group, G3 = probiotic group, G4 = antibiotic group, G5 = antibiotic followed by potential probiotic group. Different letters on the same line indicate differences between groups. Results expressed as mean and standard deviation. One-way ANOVA followed by Tukey's post hoc test. § = Results expressed as median ± interquartile range for samples with nonparametric distribution. Kruskal-Wallis test followed by Dunn's post hoc test

Different superscripts letters (a, b) on the same line indicate differences between groups

Determination of Intestinal Permeability

Intestinal permeability presented no alterations when exposed to different treatments. This could be proved by the absence of difference in the lactulose/mannitol ratio among all treatments. Nevertheless, G4 excreted higher quantities of lactulose than G1 ($p = 0.014$) and inferior amounts of mannitol to G3 ($p = 0.001$) and G5 ($p = 0.005$).

Concentration of Short-Chain Fatty Acids

G4 portrayed a lower cecal concentration of acetic acid than the other groups ($p = 0.000$) while G5 had a higher concentration of butyric acid than G2 and G4 ($p = 0.002$). No difference was observed among groups for propionic acid ($p = 0.073$).

Analysis of Intestinal Microbiota

Intestinal microbiota composition did not differ between experimental groups according to the diversity indexes ($p > 0.05$; Fig. 2). However, despite of the apparent microbiota homogeneity, certain taxa exhibited variations in their frequency: The *Bacteroidetes* phylum was smaller in G2 than other groups ($p < 0.05$), and G4 presented higher frequency within this phylum than G5 ($p = 0.012$). For the *Firmicutis* phylum, G2 showed values inferior to G1 ($p < 0.001$), G4 ($p < 0.001$), and G5 ($p = 0.033$), while G4 had more elevated frequency than G3 ($p = 0.005$). Regarding the *Clostridiales* order, minor values were observed in G2 in comparison with the other groups ($p < 0.05$) and higher values in G4 compared with G5 ($p = 0.012$). Moreover, G2 presented lower levels of *Bacteroidetes* genus than G1, G3, and G4 ($p < 0.001$), while G5 had less *Bacteroidetes* than G2 ($p = 0.005$) and G4 ($p = 0.012$; Fig. 2).

Discussion

Obesity is a complex and multifactorial disease which has a treatment based on caloric deficit for loss of body mass. Thus, additional strategies may be required to satisfactorily enhance this treatment. Therefore, the present study provided an adjuvant treatment with *Lactobacillus gasseri* LG-G12 (Lemma®) and ceftriaxone (Blau Famacêutica S/A®) separately (potential probiotic and antibiotic groups, respectively) and sequentially (antibiotic followed by potential probiotic group) in addition to the standard nutritional treatments with caloric restriction.

Regarding body mass, other studies have also found no significant change in weight gain during four weeks treatment, which may indicate that body weight is a parameter that needs a longer period of intervention time to be altered. Kong and collaborators [22] pointed out a lack of difference in body mass, between groups fed a normal diet or a high-fat diet, after offering probiotics for 4 weeks. In addition, Vaughn and collaborators [16] noticed accumulation of body fat when samples were exposed to a high-fat diet for 7 days, but no alterations were observed regarding weight gain.

Contrary to expectations, modifying the rodents diet [44] to standard nourishment with lower caloric supply in phase 2 [1–3] may also have contributed to the absence of differences in relation to body mass. High sugar diets, when compared with high fat diets, present more noticeable impacting changes on both obesity-related intestinal microbiota [22] and AIN93 diet, presenting 75.9% of calories from carbohydrates.

The abdominal perimeter reflects the visceral fat content and is associated with total body fat, standing out as an important parameter for the assessment of central obesity [1]. In this context, minor levels of abdominal perimeter value found in G5 may be resulted from the action of ceftriaxone against Gram-negative bacteria [38, 58, 59], which indicates that changes in the intestinal microbiota were responsible for the reduction of adiposity [60]. A similar mechanism was described by Choi and collaborators [61], who discussed that the reduction of fat liver and triglyceride levels after a treatment with *Lactobacillus plantarum* LMT1-48, with a consequent diminution of body mass and adipose tissue, is justified by the modulation levels of lipopolysaccharides [61]. Since these are molecules present in Gram-negative bacteria, the modulation of those can trigger a chronic low-grade inflammation [19, 61], highlighting that this process is a classic way of developing obesity [4, 37, 38, 61].

Greater amounts of brown adipose tissue were found in G5, which is a positive outcome since this tissue maintains body temperature above room temperature, produces heat from thermogenesis, increases metabolic rates, and regulates metabolic risk factors [62]. Similar to these results, a study performed by Park and collaborators [63] with the probiotic *Lactobacillus amylovorus* KU4 demonstrated certain protection against obesity by stimulating the production of brown adipose tissue and enhancing the metabolic effects resulted from this process. The increase in terms of body temperature, mitochondrial function, rate of oxygen consumption, expression of uncoupling protein 1 (UCP-1), and in expression of the receptor activated by alpha-type peroxisomal proliferators (PPAR-γ) [63] was also evidenced, reinforcing the importance of the brown adipose tissue in the resistance against obesity.

Obesity leads to changes in lipid metabolism, including increased levels of triglycerides and LDL-cholesterol and reduced HDL-cholesterol [64]. Thus, the lower triglyceride values in G5 suggested that this treatment positively modulates the lipid metabolism and, consequently, obesity [65].

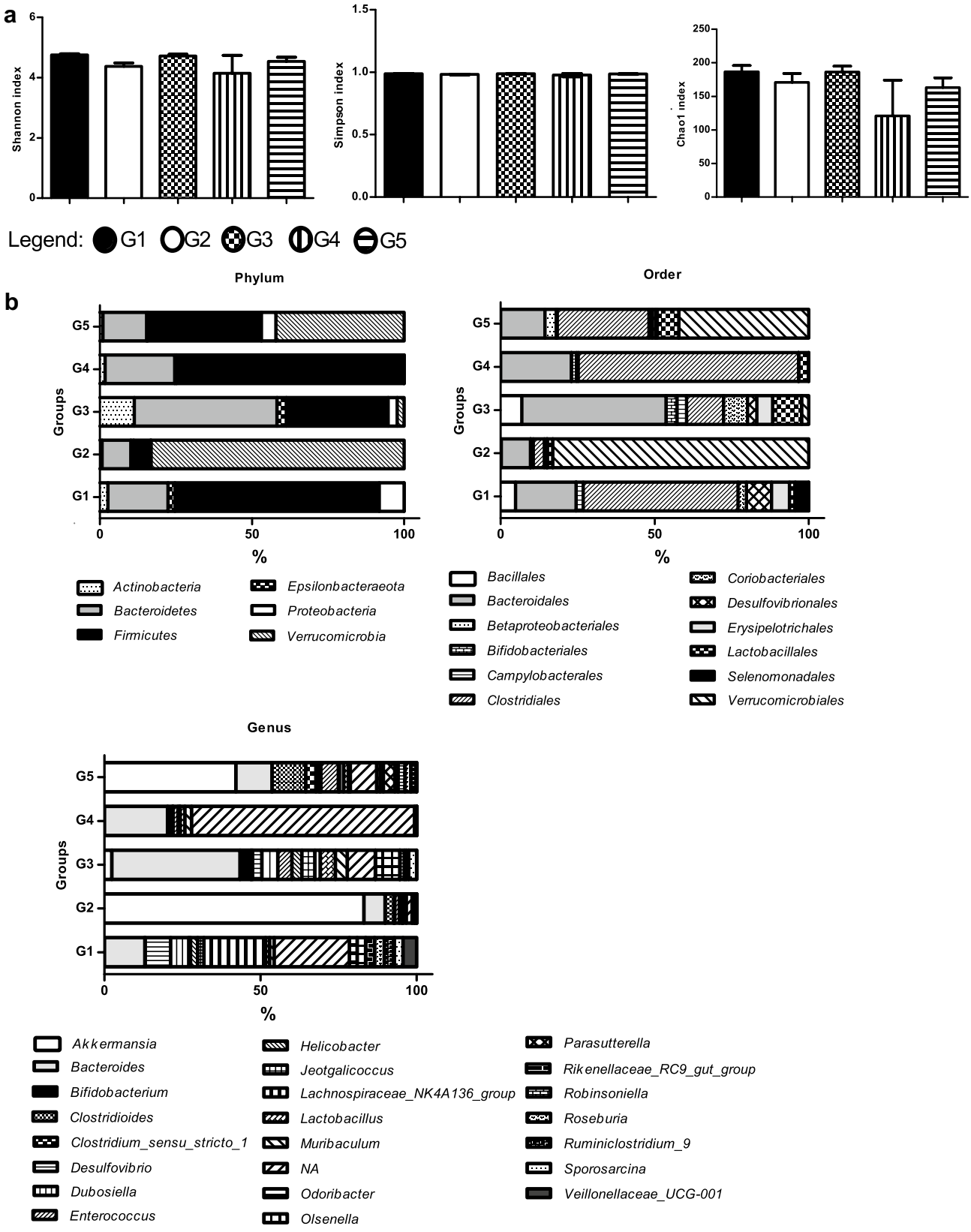

Fig. 2 Composition of intestinal microbiota: Homogeneity in diversity indexes but differences in frequencies. **a** indices of diversity of the intestinal microbiota in the different study groups; **b** structure of theintestinal microbiota at the level of phylum, order, and gender; **G1** control group, **G2** obese group, **G3** potential probiotic group, **G4** antibiotic group and **G5** antibiotic followed by potential probiotic group

In addition, VLDL is a lipoprotein capable of transporting lipids from the liver to the circulation. After VLDL hydrolysis, free fatty acids are released and can be used by tissues or stored in the form of adipose tissue [64]. Therefore, the lower levels of triglycerides in G5 may indicate less availability of free fatty acids to be stored, which is also a remarkable outcome for obesity treatment.

Elevated concentrations of butyric acid found in the current study suggested promotion of intestinal health, since short chain fatty acids (SCFA) are a source of energy for the colonic epithelium, especially butyrate, which has an important role in cell differentiation and, therefore, in intestinal health. SCFA increased production also leads to a decrease in intestinal pH, which prevents the growth of pathogenic bacteria. Moreover, SCFA regulate the adaptive immune system through the activation of regulatory T cells [66], provide a rich energy source for the epithelial cells in the colon, protecting intestinal integrity [37], and induce lipogenesis through activation of sterol regulatory element-binding transcription factor 1 (SREBP-1) [4], which are positive in obesity cases. Thus, there is an urge for regulation of metabolic pathways and health promotion [67].

SCFA also assist in weight control as these fatty acids act as signaling molecules, binding to the G protein-coupled receptor (GPCRs). This link stimulates the production of leptin, which is a hormone related to both satiety and energy metabolism. This link also stimulates the production of hormones that reduce intestinal motility and enhance the absorption of SCFA, which will be used for lipogenesis in the liver. Therefore, the inhibition of this association is a potential therapeutic target to modulate obesity, since this linkage decreases energy extraction from the diet [37, 68].

Caloric restriction and augmented energy expenditure are the first preferences for the treatment of obesity [1–4]. However, in cases of non-responsiveness to this treatment, i.e., in scenarios where weight loss of 5 to 10% is not achieved within 6 months, drugs combined with the use of standard therapy are recommended as alternatives [3, 69].

Drugs for obesity treatment are scarce and not always efficient [3, 37]. Therefore, by consolidating the relationship between obesity and intestinal microbiota [6, 37, 59, 60], as well as the role of antibiotics in modulating this microbiota [26, 27] for the treatment of obesity, especially those that act against Gram-negative bacteria and are not absorbed by body, the focus only on intestinal action is justified [39, 40]. Cephalosporins, which are a third-generation antibiotic, act by decelerating the growth of pathogenic bacteria which results in the restoration of the intestinal microbiome and protection against diseases [58]. Thus, cephalosporins stand out as an interesting option for the treatment of obesity through intestinal modulation.

Contrary to what was expected [20–22, 24–27], the treatments in this study did not lead to changes in the diversity of the intestinal microbiota. However, it is noteworthy that the intestinal microbiota has the ability to rebalance itself after external disturbances [7], which assists in understanding the aforementioned outcomes.

In addition, the treatment with antibiotics followed by potential probiotics was effective in reducing the *Bacteroidetes* phylum and increasing the *Firmicutis* phylum. This indicates a modulation of the intestinal microbiota, altering the classic understanding of obesity, which was usually based on a greater proportion of *Bacteroidetes* in relation to the *Firmicutis* phylum [11, 42, 70].

Using antibiotics followed by potential probiotics for the treatment of obesity is innovative, and the results found in the present study evidenced this treatment as promising. In addition, the results found with this type of treatment have economic potential since an effective treatment of obesity leads to harm reduction with other associated diseases, which reduces the economic costs with drugs or associated treatments, as well as avoids the use of drastic and invasive interventions procedures such as bariatric surgery, which lead to economic and social impact [4, 37].

Hence, further and innovative designs involving the use of antibiotics and potential probiotics sequentially are suggested. Furthermore, studies encompassing a diverse range of antibiotics associated with potential probiotics are interesting whether aiming at different targets and mechanisms of action, as well as at other periods of intervention and distinctive nutritional strategies for weight loss.

The use of antibiotics followed by potential probiotics was positive for the obesity treatment, leading to a reduction in biometric measures and lipid profile, increase in the amount of brown adipose tissue and butyric acid, and resulting in the alteration of the intestinal microbiota phyla. It is understood that these outcomes were acquired resulted from the treatment efficiency in bringing together the characteristics of antibiotic and potential probiotic treatments that targeted Gram negative bacteria and intestinal colonization, respectively. This led to a modulation of intestinal microbiota, which resulted in the improvement of other parameters related to obesity.

We emphasize that due to the potential of the results we found, it is necessary to study this type of sequential treatment in humans, which can clinically confirm the results found by us. So, this article is promising due to its innovative nature, which dwells within the urge for adjuvant treatments for obesity, a worldwide public health issue with critical socioeconomic impacts.

Acknowledgements The authors thank the Laboratório de Nutrição Experimental (DNS/UFV), Laboratório de Microbiologia de Anaeróbios (DMB/UFV) e Laboratório de Análises Clínicas (DNS/UFV), for making equipment and structure available for carrying out the analysis of this work.

Author Contribution MMD participated in all production stages of this article (handling the animals, execution of analysis, analysis of the results and writing of the article); SARL and RCF assisted in the handling of animals, in the execution of analyzes, and in writing of this article; LLC, TAOM, SSP, LLO, and MCGP guided in the choice of the analyzes performed, participated in the analysis of the results, and assisted in the writing of this article. All authors reviewed the manuscript.

Funding The authors received financial support from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG).

Declarations

Ethics Approval The study was approved by the Ethics Committee on Animals Use of Universidade Federal de Viçosa, under protocol 33/2018. The principles recommended by the Nacional Council for the Control of Animal Experimentation were followed [41].

Consent for Publication All authors consent with the publication of this article.

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

Availability of Data and Material All data and materials are available from the corresponding authors on reasonable request.

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