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Jaboticaba (*Myrciaria jaboticaba*) powder consumption improves the metabolic profile and regulates gut microbiome composition in high-fat diet-fed mice

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ABSTRACT

The consumption of a high-fat diet can cause metabolic syndrome and induces host gut microbial dysbiosis and non-alcoholic fatty liver disease (NAFLD). We evaluated the effect of polyphenol-rich jaboticaba peel and seed powder (JPSP) on the gut microbial community composition and liver health in a mouse model of NAFLD. Three-month-old C57BL/6 J male mice, received either a control (C, 10% of lipids as energy, n = 16) or high-fat (HF, 50% of lipids as energy, n = 64) diet for nine weeks. The HF mice were randomly subdivided into four groups (n = 16 in each group), three of which (HF-J5, HF-J10, and HF-J15) were supplemented with dietary JPSP for four weeks (5%, 10%, and 15%, respectively). In addition to attenuating weight gain, JPSP consumption improved dyslipidemia and insulin resistance. In a dose-dependent manner, JPSP consumption ameliorated the expression of hepatic lipogenesis genes (AMPK, SREBP-1, HGMCoA, and ABCG8). The effects on the microbial community structure were determined in all JPSP-supplemented groups; however, the HF-J10 and HF-J15 diets led to a drastic depletion in the species of numerous bacterial families (Bifidobacteriaceae, Mogibacteriaceae, Christensenellaceae, Clostridiaceae, Dehalobacteriaceae, Peptococcaceae, Peptostreptococcaceae, and Ruminococcaceae) compared to the HF diet, some of which represented a reversal of increases associated with HF. The Lachnospiraceae and Enterobacteriaceae families and the *Parabacteroides*, *Sutterella*, *Allobaculum*, and *Akkermansia* genera were enriched more in the HF-J10 and HF-J15 groups than in the HF group. In conclusion, JPSP consumption improved obesity-related metabolic profiles and had a strong impact on the microbial community structure, thereby reversing NAFLD and decreasing its severity.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is recognized as the most common chronic liver disease, and its prevalence has increased concomitantly with obesity. NAFLD covers a spectrum of hepatic impairment and remodeling, in which histopathological changes,

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ranging from steatosis to steatohepatitis, can progress to fibrosis, cirrhosis, and hepatocellular carcinoma [1,2]. Hepatic impairment is currently considered a multiple hit disorder owing to different factors contributing to NAFLD progression, such as insulin resistance, inflammation, changes in the gut microbiome composition, and disruption of the gut barrier integrity [2]. Dysbiosis encompasses altered gut microbiota, with the predominance of bacterial phyla that have lipopolysaccharides (LPS) as a component of their outer membrane. The concomitant greater permeability of the gut barrier allows LPS translocation, causing endotoxemia, which is closely related to increased inflammation in the host [3]. These mechanisms are strongly associated with environmental factors, including diet, which is particularly important owing to its potential to act as a regulator of different pathways in the host, such as inflammation, lipogenesis, and thermogenesis [2,4,5].

Plant bioactive compounds have been increasingly explored as a nutritional strategy for NAFLD treatment, owing to the variety of their biological properties [6]. Jaboticaba (*Myrciaria jaboticaba*) is a native Brazilian fruit, the peel and seed of which are rich in phenolic compounds, especially anthocyanins and hydrolyzable tannins [7]. The consumption of jaboticaba peel powder (4% w/w) for six weeks [8] can regulate glucose metabolism by increasing insulin sensitivity and restoring impaired signal transduction through the IR/IRS1/Akt/FoxO pathway. In addition, consuming jaboticaba peel powder (2% w/w) for 12 weeks ameliorates obesity and insulin metabolism, prevents liver steatosis in obese rats, increases glutathione synthesis, and regulates the thiol/disulfide redox balance in diabetic rats [9,10]. Furthermore, jaboticaba peel and seed powder consumption (10% and 15% w/w, respectively) for four weeks improves systemic and white adipose tissue inflammation and liver function in obese mice [11]. Like most phytochemicals with antioxidant properties, jaboticaba phenolics may protect against the development and progression of NAFLD by reducing the accumulation of triglycerides in hepatocytes [10,12]. It should be noted that most of these effects were observed after supplementation with jaboticaba peel [8,9,11,12], while only a few studies have investigated the combined health properties of the jaboticaba peel and seeds, or of the seeds only, which are fractions with different phenolic compositions, which, therefore, may affect the biological effects of the plant [11]. It is also important to mention that the bioactivity of dietary phenolic compounds depends on their bioavailability, which, in turn, is mainly influenced by the gut microbiota [13]. Thus, there are few studies that associate the consumption of jaboticaba with the liver-gut axis, the metabolism of phenolic compounds, and beneficial effects on NAFLD [8,9,11].

Therefore, this study aimed to evaluate the effect of jaboticaba peel and seed powder (JPSP) consumption on metabolic parameters, liver remodeling, and gut microbiome composition in high-fat diet (HFD)-fed mice.

2. Material and methods

2.1. Jaboticaba peel and seed powder and experimental diets

Jaboticaba fruit (*Myrciaria jaboticaba*, cv. Sabará) were purchased from Rio de Janeiro's agricultural trading center. The fruit were selected, washed, and sanitized (sodium hypochlorite 100 ppm) for 15 min. Then, they were depulped (NPC Equipamentos, Itabuna, Brazil) to obtain the peel and seeds, which were freeze-dried (Thermo Fischer Scientific®, Waltham, MA, USA) for 72 h. The dried peel and seeds were milled (MF 10 Basic; IKA® Werke, Staufen, Germany) and sieved to obtain a powder with a particle size of 25 mesh to obtain the JPSP, which had been previously characterized for its macronutrients and phenolic compounds [14].

The experimental diets included a low-fat control (AIN-93M) maintenance diet and a high-fat obesogenic diet including 0%, 5%, 10%, and 15% of JPSP prepared by Prag Soluções Biosciences Com. and Serv. Ltda (Jaú, São Paulo, Brazil). The diets followed the recommendations set by the American Institute of Nutrition for rodents [15]. The fiber was adjusted accordingly with cellulose based on the fiber content in the JPSP (Table 1).

2.2. Analysis of phenolic compounds in experimental diets by HPLC-DAD

The extraction of soluble and insoluble phenolic compounds from diets with JPSP was performed in triplicate, as previously described [16]. All extracts were filtered through a 0.45-μm cellulose ester membrane (Millipore®, São Paulo, Brazil) before HPLC analysis. The liquid chromatography system (Shimadzu®, Japan) included two LC-20 CE parallel pumps, an SIL-20AHT automatic injector, a CBM-20A system controller, a DGU-20A5 degasser, and an SPD-M20A diode-array detector (DAD). Reverse-phase C18 columns and gradient elution were used for the chromatographic separation of anthocyanins and non-anthocyanin phenolic compounds [16]. Anthocyanins were monitored at 530 nm and non-anthocyanin phenolic compounds were monitored from 190 nm to 370 nm. The phenolic compounds were identified by comparing the retention time and absorption spectrum of commercial standards. Quantification was performed using external standardization. Data were acquired using Lab Solutions software (Shimadzu Corporation®, version 5.82 SP1, 2008–2015). The results are expressed as mg of phenolic compounds per kg of diet (Table 2).

2.3. Animals

Eighty male 3-month-old C57BL/6 J mice were kept in polypropylene boxes under standard laboratory conditions (temperature: 23 ± 2 °C; humidity 60% ± 10%; 12 h light/dark cycle) with unrestricted access to food and drinking water. The mice were randomly divided into two groups, one fed with the AIN93-M standard maintenance diet (control, n = 16) and the other fed with an HF diet with 50% of the total energy from lipids (HF, n = 64) to induce obesity, both for nine weeks. After this period, the animals from the HF group were randomly subdivided into four groups (n = 16 each), one that continued to receive the HF diet and the other three groups that received the HF diet supplemented with JPSP in a concentration-dependent dose (5%, HF-J5; 10%, HF-J10; 15%, HF-J15) for four weeks. All animal experimental procedures were approved by the Committee of Ethics of Animal Experimentation of Rio de Janeiro State University (UERJ; number 51/2016).

Table 1
Ingredients and composition of experimental diets.

	Control	HF	HF-J5	HF-J10	HF-J15
Ingredients (g/kg)					
Corn starch	465.7	232.7	245.0	257.2	269.5
Maltodextrin	155.0	115.0	115.0	115.0	115.0
Sucrose	100.0	100.0	100.0	100.0	100.0
Casein	140.0	175.0	175.0	175.0	175.0
Soybean oil	40.0	40.0	40.0	40.0	40.0
Lard	0.0	238.0	238.0	238.0	238.0
Cellulose	50.0	50.0	37.8	25.5	13.3
Minerals	35.0	35.0	35.0	35.0	35.0
Vitamins	10.0	10.0	10.0	10.0	10.0
Cystine	1.8	1.8	1.8	1.8	1.8
Choline	2.5	2.5	2.5	2.5	2.5
JPSP	0.0	0.0	50.0	100.0	150.0
Composition (g/kg) and energy value (kJ/g)					
Protein	140.0	175.0	175.0	175.0	175.0
Carbohydrate	770.7	497.7	497.7	497.7	497.7
Lipid	40.0	278.0	278.0	278.0	278.0
Energy value	16.7	21.7	21.7	21.7	21.7

JPSP: jaboticaba peel and seed powder; kJ: kilojoules. HF: high-fat diet; HF-J5: high-fat diet with 5% of JPSP; HF-J10: high-fat diet with 10% of JPSP; HF-J15 high-fat diet with 15% of JPSP.

Table 2
Content of phenolic compounds (mg/kg of diet)[a] in experimental diets added with jabuticaba peel and seed powder (JPSP).

	HF-J5	HF-J10	HF-J15
Ellagic acid	874.3	1666.7	2799.4
Gallic acid	808.5	1785.7	2276.0
3,4-Dihydroxybenzoic acid	27.4	53.2	65.7
Quercetin-3-*O*-rutinoside	29.8	36.8	51.5
Myricetin-3-*O*-rhamnoside	8.3	11.3	25.7
Quercetin	1.6	4.6	6.0
Cyanidin-3-*O*-glucoside	5.4	24.8	49.9
Delphinidin-3-*O*-glucoside	ND	2.7	4.8
Total phenolics	**1755.3**	**3585.9**	**5279.1**

[a] Results are presented as mean of triplicates with coefficient of variation lower than 10%. HF-J5: high-fat diet with 5% of JPSP; HF-J10: high-fat diet with 10% of JPSP; HF-J15 high-fat diet with 15% of JPSP; ND: not detected.

2.4. Weight gain, feeding behavior, and glucose sensitivity

Body mass and dietary intake were assessed using an analytical balance (Shimadzu® UX 4200H) throughout the experiments. Energy consumption (kJ/day) was calculated using the average feed intake (g) and energy values (kJ). The intake of phenolic compounds (mg/day) was calculated using feed intake and total phenolic compound content. The oral glucose tolerance test (OGTT) was performed after 6 h of food deprivation. Glucose (1.0 g/kg) was administered by orogastric gavage to induce glucose overload. Blood was collected by milking the tip of the tail at 0 min, 15 min, 30 min, 60 min, and 120 min after glucose administration. Blood glucose was measured using a glucometer (Accu-Chek, Roche, Germany). The area under the curve (AUC) was calculated for the OGTT from 0 min to 120 min [17].

2.5. Ellagic acid urinary metabolites by HPLC-DAD-MS and LC-MS/MS

For urine collection, the mice were placed in metal cages and allowed to grasp the cage bars. A gentle pressure was applied to the caudal area of the mouse as the free hand held a vial beneath the mouse to allow the voided urine to be directly collected into the sterilized Petri dish, which was transferred to the vial [18], lyophilized, and stored at −80 °C until analysis. Urine samples were reconstituted with Milli-Q water, vortexed, centrifuged at 14,300 × *g* for 10 min at 10 °C (Sigma 1–16 K; Sigma Laborzentrifugen, Osterode, Germany), and filtered through a 0.22-μm PVDF filter (Millipore). Urolithins (ellagic acid gut microbiota metabolites) were analyzed as previously described [19]. The liquid chromatography system was an Agilent 1200 HPLC system equipped with a diode array detector and a single quadrupole (6120 Quadrupole; Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation of metabolites was achieved using a Poroshell 120 EC-C18 column (2.7 μm, 100 mm × 3 mm; Agilent Technologies) operating at 25 °C with gradient elution. The optimal ESI-MS parameters using nitrogen as the nebulizer gas were as follows: capillary voltage, 3500 V; drying gas flow, 10 L/min; nebulizer pressure, 45 psi; drying temperature, 300 °C. ESI was operated in the negative ionization mode and MS in selective ion monitoring (SIM) mode. Urolithins were monitored by DAD at 305 nm and their identification was carried out by their spectral properties, molecular masses, and chromatographic comparisons with authentic standards [19].

As other metabolites with absorption spectra similar to ellagic acid were detected, they were monitored by DAD at 360 nm and quantified using the ellagic acid calibration curve. To confirm the identification of these metabolites, HPLC-ESI-MS/MS was employed. The liquid chromatography system was an Agilent HPLC 1200 series equipped with a diode array detector and an ion-trap mass spectrometer detector in series (Agilent Technologies, Waldbronn, Germany), operated in negative ion mode.

2.6. Euthanasia and biochemical parameters

At the end of 13 weeks, the animals were anesthetized with sodium pentobarbital (60 mg/kg body mass, intraperitoneal). Blood samples were collected by cardiac puncture, stored in a heparinized tube, and centrifuged (800×*g* at 4 °C) to separate the plasma, which was stored at −80 °C. The liver and intestines were dissected carefully. The liver was sectioned into two parts, one fixed in 10% formalin and the other frozen in liquid nitrogen and stored at −80 °C. Fasting blood glucose (mg/dL) and plasma insulin concentration (pg/mL) were measured using the insulin 125I Ria Kit (MP Biomedicals®, LLC, Orangeburg, NY). Insulin resistance was calculated using the homeostatic model assessment of insulin resistance (HOMA-IR) index. Plasma concentrations (mg/dL) of total cholesterol, triglycerides, and low-density lipoproteins (LDL-c), and plasma activities (U/L) of aspartate transaminase (AST) and alanine aminotransferase (ALT) were determined using colorimetric assays (Bioclin®, Belo Horizonte, Brazil). Plasma concentrations (pg/mL) of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) were determined using ELISA kits (#BMS603-2 and #BMS607-3, respectively; Thermo Fisher Scientific Inc., Waltham, MA, USA). The plasma concentration of LPS (EU/mL) was determined using the lipopolysaccharides ELISA kit (#ABIN6574100; CUSABIO technology LLC, Houston, TX, USA).

2.7. Liver analysis stereology

After being fixed in formalin, hepatic tissue was placed in Paraplast Plus (Sigma-Aldrich, St Louis, MO, USA) [20]. Subsequently, 5-μm sections were placed on slides and stained with hematoxylin-eosin or picrosirius. The slides were analyzed and images were captured randomly (JPG format, 36-bit color, 1360 × 1024 pixels) under a light microscope (Olympus BX51 with DP71 digital camera; Olympus Optical, Tokyo, Japan). The immunofluorescence silanized slides were incubated in citrate buffer (pH 6.0, 60 °C, 20 min), blocked with glycine buffer (2% glycine in PBS/BSA), and incubated overnight at 4 °C with α-actin primary antibody (Santa Cruz Biotechnology) 1:50 in 1% PBS/BSA. The slides incubated for 1 h at 24 °C with a specific secondary antibody conjugated to the fluorochrome IgG-Alexa 546 (anti-mouse) (Invitrogen, CA, USA). The slides were washed with PBS, mounted with Slow Fad Antifade (Invitrogen, Molecular Probes, Carlsbad, CA, USA), and photographed using a confocal laser scanning microscope (LEICA®, TCS, SPE; LEICA Microsystems, USA).

2.8. Hepatic triacylglycerols and cholesterol

The hepatic lipid content was assessed using a previously reported method [21]. Briefly, 50 mg of the liver was homogenized in isopropyl alcohol (Vetec®, Duque de Caxias, Brazil) and centrifuged (5900×*g*) for 10 min at 4 °C. The triacylglycerol (TAG) and cholesterol contents of the supernatant were quantified using colorimetric kits (Bioclin®).

2.9. Gene expression

To evaluate the genes involved in hepatic lipid metabolism, total RNA from hepatic tissue was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies). TaqMan gene expression assays for *Mus musculus* (Applied Biosystems) were used to detect AMPK, SREBP-1, HGMCoA, and ABCG8 mRNA expression (Mm01296700_m1, Mm00550338_m1, Mm01282499_m1, and Mm00445980_m1, respectively). PCR amplification was performed using ABI Prism 7.500 fast (Applied Biosystems, Waltham, MA, USA) under standard cycling conditions. The expression of each target gene was normalized to the relative expression of the 18S-ribosomal RNA as an internal efficiency control. The mRNA fold change was calculated using the 2(-Delta Delta C(T)) method [22].

2.10. DNA extraction and gut microbial analysis based on the 16 S rRNA gene

DNA extraction from the cecum content was performed using the QIAamp PowerFecal DNA kit (QIAGEN, Valencia, CA). The Illumina MiSeq Platform (2 × 300 cycles; Illumina Inc., San Diego, CA) was used to perform paired-end sequencing, as previously described [23]. Briefly, amplicon libraries were built according to the Illumina protocol that amplifies the V3-V4 region of the 16S rRNA gene (forward primer: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGC-WGCAG-3′; reverse primer: 5′-GTCTCGTGGGCTCGGAGATGTGTATAA GAGACAGGACTACHVGGGTATCTAATCC-3′). The Quantitative Insig ht into Microbial Ecology (QIIME 1.9.1) toolkit was used for the taxonomic profiling of the phylogenetic diversity (PD) whole tree. Alpha diversity indices (Chao1, Simpson, and Shannon) were calculated.

Total bacterial levels in the cecal fecal contents were evaluated using real-time PCR, as previously described [23]. Total bacterial primers (forward: 5′-CGGYCCAGACTCCTACGGG-3′; reverse: 5′-TTACCGCGGCT GCTGGCAC-3′) were used to quantify the bacterial load of the cecum and were normalized to the weight of the fecal content from which the bacterial DNA was isolated [24].

2.11. Statistical analysis

The results are expressed as the mean ± standard deviation. The *t*-test was used to determine the differences in body mass between the control and HFD groups during the first nine weeks of the experiment. To compare the five experimental groups, one-way ANOVA followed by Tukey's post-hoc test was used. Statistical significance was set at $p < 0.05$. All analyses were performed using GraphPad Prism version 6 for Windows (San Diego, CA, USA). Statistical significance for alpha diversity was determined in QIIME using the standard two-sample *t*-test with Monte Carlo permutations (999) to calculate the nonparametric *P*-value. Beta diversity was calculated using weighted (quantitative) and unweighted (qualitative) UniFrac distance metrics, and principal coordinate analysis (PCoA) was plotted using the phyloseq package in R (cite). The heatmap of abundance was visualized in R with the function 'pheatmap' and significant genera differences were determined by permutational ANOVAs corrected with the 'TukeyHSD' function for multiple comparisons [24].

3. Results

3.1. JPSP is rich in ellagic and gallic acids

Eight phenolic compounds were identified and quantified in the experimental diets containing JPSP (Table 2). Among these, the ellagic and gallic acids were the most abundant, corresponding, on average, to 50% and 46%, respectively, of total phenolics. Among the minor compounds, one phenolic acid and five flavonoids were observed in JPSP. Cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside, which are anthocyanins characteristic of jaboticaba, represented only 0.7% of the total phenolics. As expected, the total phenolic compound content in the diets was proportional to the added percentage of JPSP (Table 2).

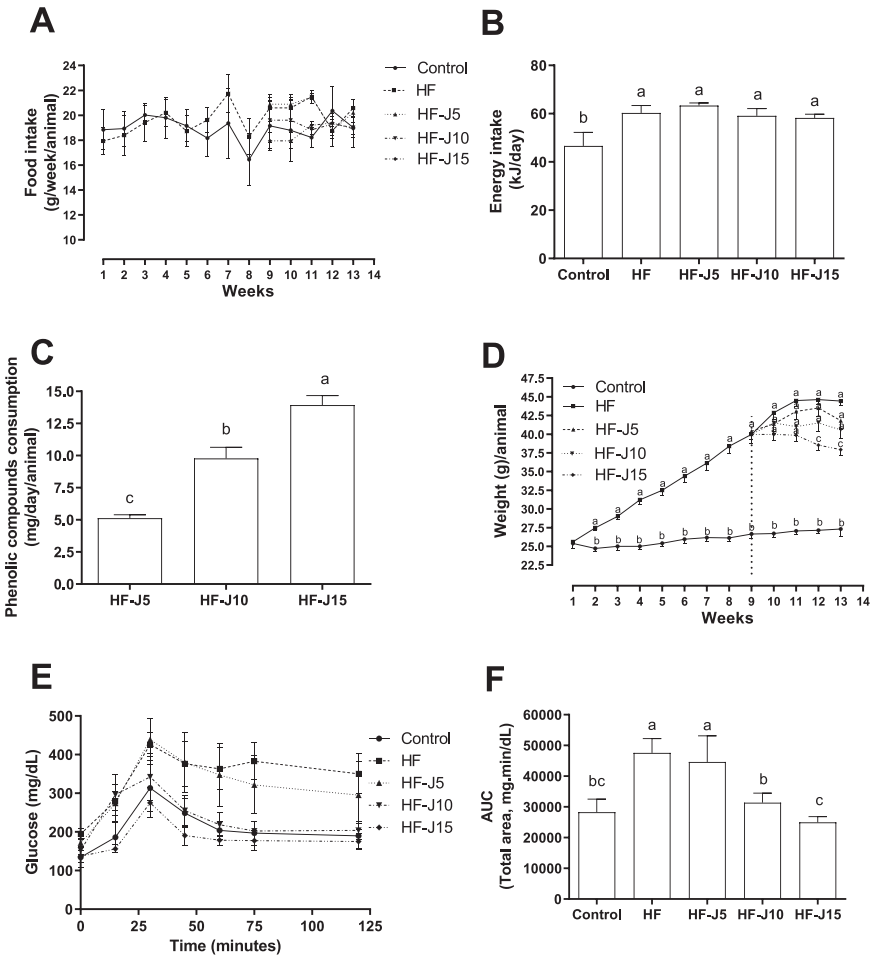

Fig. 1. Feed intake of diets with or without jaboticaba powder (JPSP) and weight gain. HF: high fat; J5: high-fat diet with 5% JPSP; J10: high-fat diet with 10% JPSP; J15 high-fat diet with 15% JPSP. A, Grams; no statistical difference between the experimental groups. B, Energy (kilojoules); significance indicated by different letters ($p < 0.05$ in comparison to the other experimental groups). C, weight gain (g)/animal; significance indicated by different letters, in which: (b) $p < 0.0001$ in relation to the HF group; (c) $p < 0.05$ relative to the HF group. Until the ninth week of the experiment *t*-test was used to compare the control and HF groups. To compare all groups, one-way ANOVA with Tukey's post-hoc test were performed (n = 12). The results are expressed as mean ± SD.

3.2. JPSP increased phenolic consumption and did not change the feeding behavior of the mice, apart from increasing ellagic acid urinary metabolites

There were no food intake differences between the experimental groups (Fig. 1A). The HF groups that were either supplemented or not with JPSP had higher energy intake when compared to the low-fat diet control group ($p < 0.05$) (Fig. 1B).

The daily consumption of phenolic compounds ranged from 5.1 mg (HF-J5) to 13.9 mg (HF-J15) ($p < 0.0001$) (Fig. 1 **C**), consistent with the percentage of JPSP in the diets. Ellagic acid urinary metabolites were evaluated to investigate whether the JPSP phenolic compounds were metabolized by the mice. Following the consumption of JPSP, five ellagic acid metabolites (Table 3) were identified: ellagic acid dimethyl ether diglucuronide, two isomers of ellagic acid methyl ether glucuronide, and two isomers of ellagic acid dimethyl ether glucuronide. Urolithins and nasutins, which are common ellagic acid urinary metabolites in humans and animals [25], respectively, were not detected. The urinary excretion of total ellagic acid metabolites by the HF-J10 and HF-J15 groups was approximately three times higher than that of the HF-J5 group ($p < 0.05$) (Table 3).

3.3. JPSP impaired weight gain and improved glucose sensitivity

At the beginning of the experiment, mice from all groups had similar body weights. From the second week until the end of the experiment (week 13), the HF group gained weight compared to the control group ($p < 0.0001$) (Fig. 1 **D**). Although the consumption of JPSP showed a concentration-dependent tendency to attenuate weight gain, only the HF-J15 group showed a significant reduction (-5.8 ± 1.2 g) in this parameter in comparison with the HF group in the last two weeks of the experiment ($p < 0.05$) (Fig. 1D).

The HF group showed an oral glucose intolerance when compared to the control group (Fig. 1E); that is, glucose levels did not return to basal levels 120 min after glucose administration. This result is in accordance with the area under the curve of the glycemic curve for this group (Fig. 1F), which was significantly higher than that of the control group (45%; $p < 0.001$). The HF-J5 diet did not improve glucose intolerance induced by the HF diet (Fig. 1E), with both groups showing similar area under the curve (AUC) values (Fig. 1F). Notably, the HF-J10 and HF-J15 groups were able to maintain glucose levels similar to those of the control group during the experiment (Fig. 1E). The AUC values of the glycemic curve for the HF-J10 and HF-J15 groups were lower than those of the HF group (-43% and -47%, respectively; $p < 0.001$), and similar to that of the control group (Fig. 1F).

3.4. JPSP consumption improved glucose homeostasis, lipid metabolism, and inflammatory markers in obese mice

The HF group showed an imbalance in glucose homeostasis, characterized by higher plasma concentrations of glucose and insulin, and a higher HOMA-IR index than the control group ($p < 0.001$) (Table 4). JPSP consumption progressively decreased glucose levels and HOMA-IR index values compared to those in the HF group. The HF-J15 group had a similar glucose level to that of the control group, while the HOMA-IR index was higher (Table 4). Lower insulin levels were observed in the HF-J5, HF-J10, and HF-J15 groups than in the HF group. It is worth mentioning that the control group had plasma insulin values similar to those of the HF-J5 and HF-J10 groups (Table 4).

All experimental groups showed similar TAG levels (Table 4). The HF group showed a higher total cholesterol level ($p < 0.0001$) than the control group (Table 4). Among the groups fed with JPSP, only HF-J15 showed a lower total cholesterol level than the HF group ($p < 0.05$). The plasma levels of LDL-c were lower in the HF-J10 and HF-J15 groups than in the HF group ($p < 0.001$).

The HF group showed higher IL-6 and TNF-α plasma levels than the control group. JPSP consumption progressively decreased ($p < 0.01$) IL-6 and TNF-α levels in comparison to the HF group, with the HF-J10 and HF-J15 groups showing similar levels of these inflammatory markers in comparison to those of the control group (Table 4).

3.5. JPSP consumption ameliorated liver steatosis and fibrosis induced by a high-fat diet

In comparison with the control group, mice fed the HF diet showed increased liver mass (Fig. 2A) and volume (Fig. 2B). JPSP consumption attenuated these increases by up to 0.8 g and 0.9 cm^3, respectively. Regarding hepatic TAG (Fig. 2C) and cholesterol (Fig. 2D), the HF group showed increases of 113.8 mg/g and 14.3 mg/g, respectively, in comparison with the control group. JPSP consumption attenuated TAG increase by up to 85 mg/g (Fig. 2C). With regard to total cholesterol, JPSP supplemented at 10% and 15% reversed the increase caused by the HF diet (Fig. 2D).

The activity of AST in the HF-J5, H-J10, and HF-J15 groups decreased by up to 21 U/L in comparison to the HF group and showed no change compared to the control group (Table 4). The activity of ALT in the HF group showed an increase of 35 U/L in comparison to the control group, with JPSP consumption reversing this change, leading to values similar to those of the control group (Table 4).

Histologically, the liver tissue of the HF group showed numerous macro and micro lipid vesicles in the hepatocyte cytoplasm when compared to the control group (Fig. 3A and B), a hallmark of hepatic steatosis. In general, JPSP consumption reversed steatosis: in the HF-J5 group, a considerable reduction in lipid droplets was observed, while in the HF-J10 and HF-J15 groups, steatosis was almost completely reversed (Fig. 3C–E). These structural data corroborate the stereological analyses of hepatic steatosis (Fig. 3P) and binucleation (Fig. 3Q). Compared to the control group, the HF group exhibited a 60% increase in steatosis. JPSP consumption progressively reduced steatosis, with the HF-J15 group showing values similar to those of the control group (Fig. 3P). Hepatic nuclear binucleation indicated the recovery of hepatocytes in the JPSP-treated groups. The HF group showed an 89.7% reduction in binucleation compared to the control group, which was reversed by JPSP consumption (Fig. 3Q).

Sirius red staining revealed that the HF group had a large deposition of collagen fibers in the hepatic stroma and regions of inflammatory infiltrate when compared to the control group (Fig. 3F and G). JPSP

Table 3
Ellagic acid urinary metabolites (mmol) on the experimental groups fed with jaboticaba peel and seed powder (JPSP)[1].

	Retention time (min)	MS $[M–H]^-$ (m/z)	MS^2 $[M–H]^-$ (m/z)	HF-J5	HF-J10	HF-J15
EA dimethyl ether diglucuronide	7.478	681	**505**, **329**, 314, 299	0.72 ± 0.02^c	4.90 ± 0.43^a	1.85 ± 0.27^b
EA methyl ether glucuronide	10.718	491	**315**, 300	0.41 ± 0.18^b	2.35 ± 0.38^a	2.51 ± 0.43^a
EA methyl ether glucuronide	11.201	491	**315**, 300	1.54 ± 0.15^c	2.57 ± 0.34^b	3.04 ± 0.03^a
EA dimethyl ether glucuronide	11.584	505	**329**, 314	1.15 ± 0.64^b	3.37 ± 0.23^a	3.47 ± 0.12^a
EA dimethyl ether glucuronide	11.896	505	**329**, 314	0.10 ± 0.07^b	0.78 ± 0.05^a	0.81 ± 0.15^a
Total metabolites				**3.83 ± 0.77^b**	**13.96 ± 0.67^a**	**11.69 ± 0.41^a**

[1] Results expressed as mean ± SD. No metabolites were detected in control and high-fat (HF) groups. Different superscript letters indicate statistical difference ($p < 0.05$) between the experimental groups (One-way ANOVA followed by Tukey's multiple comparison *post-hoc* test). The most abundant ions at MS^2 are given in bold font. EA: ellagic acid; HF-J5: high-fat diet with 5% of JPSP; HF-J10: high-fat diet with 10% of JPSP; HF-J15 high-fat diet with 15% of JPSP.

Table 4
Biochemical parameters of obese mice that received jabuticaba peel and seed powder (JPSP) in their diet.

	Control	HF	HF-J5	HF-J10	HF-J15
Glucose homeostasis					
Glycemia (mg/dL)	134.2 ± 20.9^{c}	194.7 ± 13.3^{a}	172.9 ± 11.3^{b}	152.2 ± 10.6^{c}	136.8 ± 15.3^{c}
Insulin (pg/mL)	454.2 ± 50.7^{c}	821.0 ± 110.1^{a}	584.3 ± 46.1^{bc}	540.1 ± 48.6^{bc}	602.2 ± 71.9^{b}
HOMA-IR	0.98 ± 0.2^{d}	2.58 ± 0.18^{a}	1.63 ± 0.11^{b}	1.32 ± 0.09^{c}	1.33 ± 0.15^{c}
Lipids					
Tryacylglycerols (mg/dL)	46.6 ± 9.6^{a}	44.7 ± 12.0^{a}	33.0 ± 14.4^{a}	43.1 ± 12.8^{a}	44.9 ± 14.4^{a}
Cholesterol (mg/dL)	70.1 ± 19.1^{c}	166.9 ± 17.9^{a}	174.5 ± 16.7^{a}	162.2 ± 26.3^{ab}	138.5 ± 13.9^{b}
LDL-c (mg/dL)	16.8 ± 4.8^{ab}	18.9 ± 3.9^{a}	16.4 ± 4.1^{ab}	10.8 ± 4.0^{b}	10.3 ± 2.6^{b}
Inflammatory citokines					
IL-6 (pg/mL)	16.07 ± 4.2^{c}	70.23 ± 8.3^{a}	30.59 ± 4.1^{b}	14.83 ± 2.5^{c}	14.84 ± 3.4^{c}
TNF-α (pg/mL)	3340 ± 1053^{b}	8441 ± 1573^{a}	6107 ± 1018^{a}	3727 ± 941.7^{b}	3643 ± 1187^{b}
Hepatic enzymes					
Aspartate transaminase (U/L)	29.2 ± 6.3^{ab}	39.6 ± 12.7^{a}	18.0 ± 5.3^{b}	23.3 ± 4.6^{b}	18.8 ± 5.2^{b}
Alanine transaminase (U/L)	14.9 ± 7.1^{b}	49.1 ± 16.6^{a}	26.6 ± 7.5^{b}	13.8 ± 5.5^{b}	13.1 ± 5.5^{b}
Endotoxin					
Lipopolysaccharide (EU/mL)	0.1 ± 0.2^{c}	3.1 ± 0.7^{a}	1.7 ± 0.5^{b}	0.4 ± 0.3^{c}	0.4 ± 0.3^{c}

Results expressed as mean ± SD. HF: high fat; HF-J5: high-fat diet with 5% of JPSP; HF-J10: high-fat diet with 10% of JPSP; HF-J15 high-fat diet with 15% of JPSP. Different letters denote statistical difference ($p < 0.05$) between the experimental groups (One-way ANOVA followed by Tukey multiple comparison post hoc test).

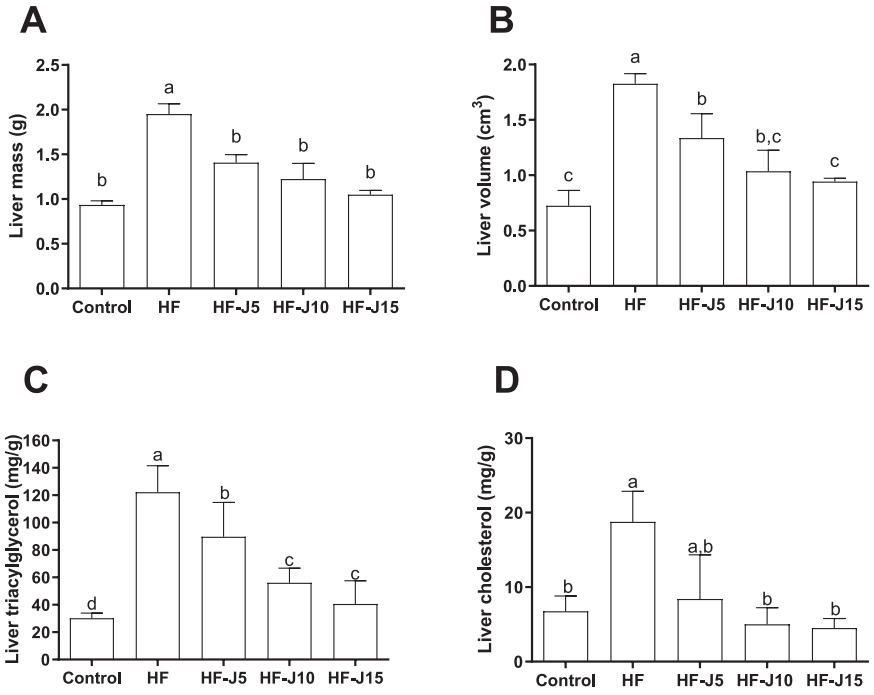

Fig. 2. Effect of jaboticaba powder (JPSP) consumption on liver steatosis. The results are expressed as mean ± SD. HF: high fat; HF-J5: high-fat diet with 5% JPSP; HF-J10: high-fat diet with 10% JPSP; HF-J15 high-fat diet with 15% JPSP. Different letters denote statistical difference ($p < 0.05$) between the experimental groups (one-way ANOVA followed by Tukey's multiple comparison post-hoc test).

consumption progressively improved liver fibrosis and tissue inflammation (Fig. 3H–J). These results were reinforced by the immunofluorescence analysis of activated hepatic stellate cells (HSCs). When active, these cells are easily marked with smooth muscle α-actin, resulting in the excessive deposition of collagen fibers and fibrosis. Only the HF group showed positive staining for activated HSCs, indicating that JPSP consumption reversed the activation of these cells (Fig. 3K–O).

3.6. JPSP consumption regulated hepatic lipid metabolism genes

The mRNA expression of SREBP-1, HMG-CoA, AMPK, and ABCG8, genes possibly involved in the hypolipidemic effect of JPSP, was evaluated in the liver (Fig. 4). The HF group showed higher expression levels of HMGCoA (Fig. 4A) and SREBP-1 (Fig. 4B) compared to the control group ($p < 0.001$). JPSP consumption progressively reversed these changes, leading to values similar to those of the control group for HMGCoA and SREBP-1, when 15% of JPSP was added to the feed (HF-J15) ($p < 0.001$). The expression of AMPK was 2-fold higher in HF-J10 and HF-J15 than in the HF group (Fig. 4C; $p < 0.001$). The ABCG8 levels were upregulated in a concentration-dependent manner up to 2.7-fold in HF-J15 compared to HF (Fig. 4D).

3.7. JPSP consumption improved gut barrier and changed microbiome composition

The HF group showed a decrease of up to 0.3- and 0.5-fold in the levels of mucin 2 (Fig. 5A; $p < 0.001$) and claudin 1 (Fig. 5B; $p < 0.001$), respectively, compared to the control group. JPSP consumption, independent of concentration, reversed the decrease in mucin 2 or even surpassed the level of claudin 1 in the control group. The levels of claudin 3 were similar in all groups (Fig. 5C). The HF group presented a higher LPS plasma concentration than the control group ($p < 0.001$)

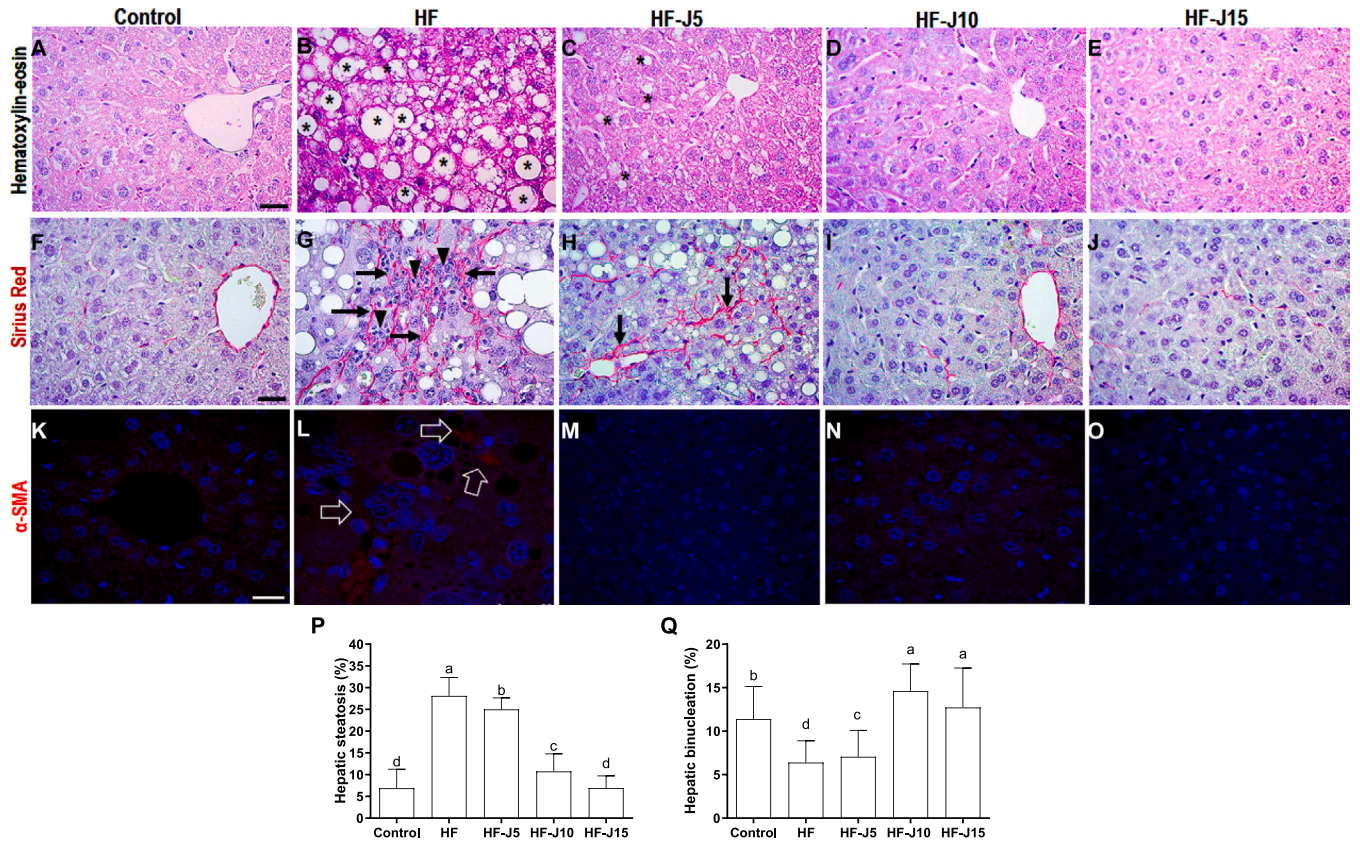

Fig. 3. Effect of jaboticaba powder (JPSP) consumption on liver remodeling. HF: high fat; J5: high fat diet with 5% JPSP; J10: high fat diet with 10% JPSP; J15: high-fat diet with 15% JPSP. Histological hepatic sections showing the accumulation of lipids (asterisk – fat droplet) (A–E) and collagen fibers on hepatic tissue (arrowheads – collagen fibers) (F–J). K, percentage of hepatic steatosis; statistical significance indicated by different letters, in which: (b) $p < 0.0001$ in relation to the control, HF, J10, and J15 groups; (c) $p < 0.0008$ in relation to the control group, $p < 0.0001$ in relation to the HF and J15 groups; (d) $p < 0.0001$ in relation to the HF group. L, percentage of hepatic binucleation; statistical significance indicated by different letters, in which: (b) $p < 0.0001$ in relation to the HF, J10, and J15 groups, $p < 0.001$ in relation to the J5 group; (c) $p < 0.002$ in relation to the HF group and $p < 0.0001$ in relation to the J10 and J15 groups. M, Immunofluorescence; α-actin was detected by immunofluorescence demonstrating lower activation of this protein in the J10 group (arrows – α-actin). Scales bar: A–J 40 μm.

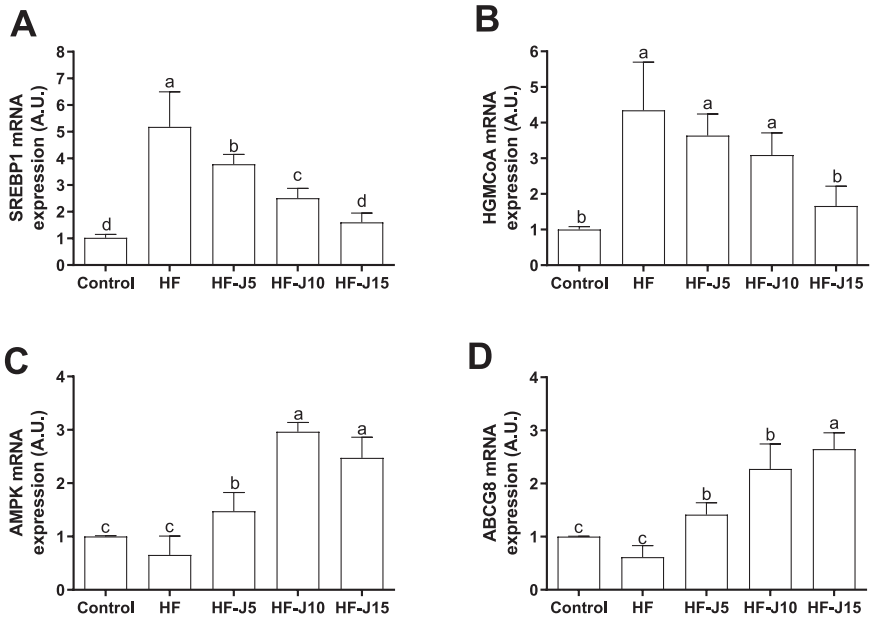

Fig. 4. Effect of jaboticaba peel and seed powder (JPSP) on hepatic lipid metabolism genes. HF: high fat; J5: high fat diet with 5% JPSP; J10: high fat diet with 10% JPSP; J15: high-fat diet with 15% JPSP. Different letters denote statistical difference ($p < 0.05$) between the experimental groups (one-way ANOVA followed by Tukey's multiple comparison post-hoc test).

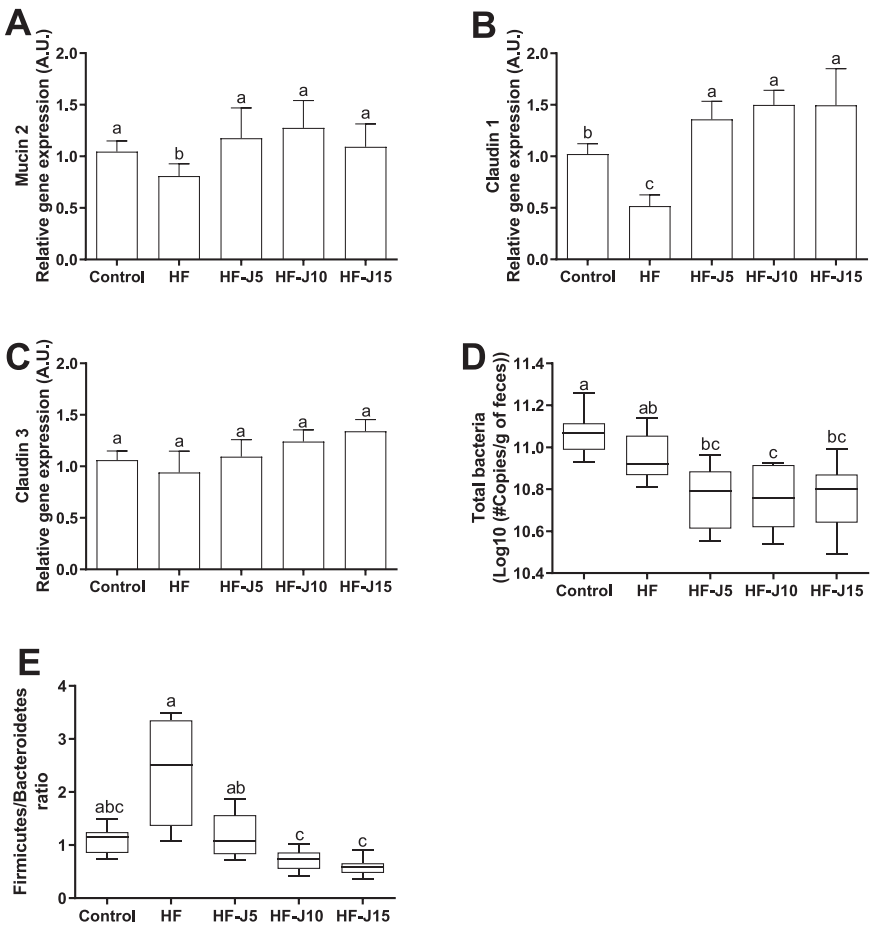

Fig. 5. (A) total bacteria and (B) Firmicutes/Bacteroidetes ratio. HF: high fat; HF-J5: high-fat diet with 5% JPSP; HF-J10: high-fat diet with 10% JPSP; HF-J15 high-fat diet with 15% JPSP. Different letters denote statistical difference ($p < 0.05$) between the experimental groups (one-way ANOVA followed by Tukey's multiple comparison post-hoc test). The results are expressed as mean ± SD.

(Table 4). JPSP consumption progressively reversed this change, leading to values similar to those of the control group when 10% (HF-J10) or 15% (HF-J15) of JPSP was added to the feed ($p < 0.001$). In comparison to the control group, the HF group had a similar number of total bacteria, as indicated by the 16S rRNA gene copy numbers, while mice that consumed JPSP had lower numbers (up to 0.4 log units) (Fig. 5D). The *Firmicutes* to *Bacteroidetes* ratio was similar between the HF and control groups (Fig. 5E). JPSP consumption at 10% (HF-J10) or 15% (HF-J15) decreased this ratio by up to 3-fold compared to the HF group.

To assess the effect of JPSP consumption on bacterial community composition, 16S rRNA gene amplicon sequencing was performed on the cecal contents. The PCoA plots for the weighted (Fig. 6A) and unweighted (Fig. 6B) UniFrac distance metrics indicated distinct clustering of microbial communities according to JPSP consumption. Alpha diversity indices (Chao 1, Simpson, Shannon, and the PD whole tree) showed a decrease in richness and abundance in the HF-J10 and HF-J15 groups, while the HF-J5 group showed a numerical increase, but this increase was not significant compared to the HF group (Fig. 6C; $p < 0.001$).

Six predominant phyla were observed: *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Tenericutes*, and *Verrucomicrobia* (Fig. 7). As can be noted in the heatmap, the jaboticaba diets drastically reduced many bacterial taxa, coinciding with reduced alpha diversity, while enriching a smaller subset of bacteria. Some of the changes in the microbial community that were associated with the HFD were inhibited by the jaboticaba treatments (Fig. 7). In particular, the increased relative abundance of *Desulfovibrionaceae, Clostridiaceae, Peptostreptococcaceae, Anaeroplasma*, and *Dorea* in HF mice, was not observed in HF-J5, HF-J10, and HF-J15 mice. The HF-J10 and HF-J15 groups also presented notable reductions in the members of several families found in both the control and HF groups, including *Bifidobacteriaceae*, S24–7, *Mogibacteriaceae*, *Christensenellaceae*, *Clostridiaceae*, *Dehalobacteriaceae*, *Peptococcaceae*, *Peptostreptococcaceae*, and *Ruminococeaceae*. HF-J5 resulted in more modest changes in the microbial community, which were often intermediate between HF and HF-J10 and HF-J15. HF-J5 also resulted in increased levels of some taxa relative to the HF and control groups, including *Dehalobacterium, Roseburia, Ruminococcus,* and *Oscillospira*, which decreased with the HF-J10 and HF-J15 diets. The *Lachnospiraceae* and *Enterobacteriaceae* families and the *Parabacteroides*, *Sutterella*, *Allobaculum*, and *Akkermansia* genera showed higher relative abundance in the HF-J10 and HF-J15 groups than in the HF group ($p < 0.05$) (Fig. 7).

4. Discussion

The results of this study demonstrate that JPSP supplementation in an increasing concentration manner can control adverse liver remodeling induced by an HF diet. These beneficial outcomes were associated with a reduced microbial load, as well as large shifts in microbial composition and reduced endotoxemia. JPSP also attenuated weight gain, improved dyslipidemia, insulin resistance, and the plasma concentrations of AST and ALT, which are markers of altered metabolic conditions in obesity, ameliorating NAFLD, and impairing its progression toward liver fibrosis.

The consumption of 15% JPSP reduced the weight gain of mice throughout the experiment. Undoubtedly, this result is associated with the treatment, as the energy intake did not differ among mice fed the HF

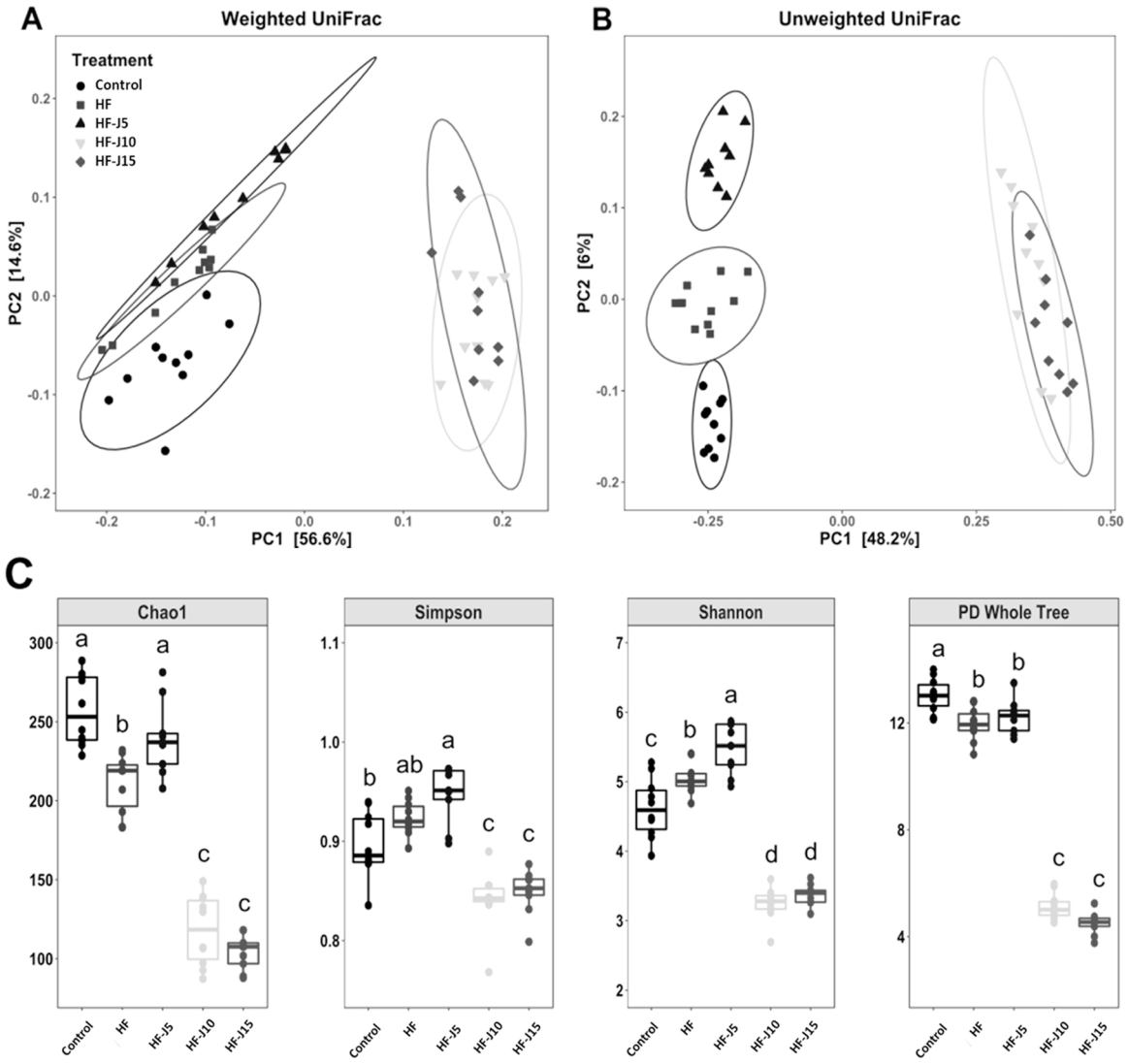

Fig. 6. Principle coordinate analysis (PCoA) plots and alpha diversity indices. The results are expressed as mean ± SD. HF: high fat; HF-J5: high-fat diet with 5% JPSP; HF-J10: high-fat diet with 10% JPSP; HF-J15 high-fat diet with 15% JPSP. Different letters denote statistical difference ($p < 0.05$) between the experimental groups (one-way ANOVA followed by Tukey's multiple comparison post-hoc test).

diet. This effect was accompanied by a significant improvement in metabolic parameters associated with obesity, such as inflammatory cytokines, dyslipidemia, and glucose intolerance. We have previously reported that, in the same experiment with obese mice, the JPSP treatment restored adipocyte function [11], which could possibly explain the altered metabolic status found in the present study.

Inflammation and insulin resistance are critical triggers in the development and progression of NAFLD by increasing lipolysis in the adipose tissue, which leads to higher free fatty acid influx into the liver and increased *de novo* lipogenesis (DNL). This increased demand exceeds the hepatic β-oxidation and TAG export capacity, favoring hepatocyte TAG accumulation and liver remodeling [26]. JPSP consumption decreased plasma hepatic enzymes, TAG, and cholesterol deposition, which may be associated not only with lower liver weight and volume, but also with the improvement in steatosis, as observed in the HF-J10 and HF-J15 groups. Moreover, JPSP consumption increased the percentage of binucleation, suggesting hepatic self-regeneration [27].

Increased hepatic cholesterol levels have been previously described as an indicator of inflammation, which is associated with NAFLD and may culminate in the activation of HSCs, promoting higher deposition of collagen fibers in the liver, a marker of NAFLD progression to non-alcoholic steatohepatitis (NASH), a more harmful obesity-associated hepatic comorbidity [28]. The presence of collagen fibers in liver histological sections of the HF group was attenuated by JPSP consumption (HF-J10 and HF-J15 groups), demonstrating its potential protective effects against NAFLD-NASH progression, through anti-inflammatory and anti-lipogenic effects caused by gut dysbiosis amelioration [29]. Therefore, the mechanism underlying the beneficial effects of JPSP on NAFLD can be partly attributed to the regulation of lipid homeostasis in the liver. In this study, JPSP consumption promoted the higher expression of AMPK in the HF-J10 and HF-J15 groups, which may favor the lower expression of SREBP1 in these groups owing to the inhibitory action of AMPK on the other DNL pathway proteins. This down-regulation is known to decrease hepatic TAG levels and improve steatosis [30]. We have previously reported that in an obese mouse model the consumption of polyphenol-rich acai berries positively regulates p-AMPK and lowers the expression of proteins involved in the DNL pathway [31]. Furthermore, an important aspect of hepatic lipid metabolism is cholesterol synthesis and excretion, as it influences plasma and tissue cholesterol concentrations [17]. The activity of the enzyme HMGCoA is considered a limiting factor in cholesterol synthesis [32] and its homeostasis is regulated mainly by DNL, intestinal absorption, and biliary and fecal excretion. Moreover, subfamily G transporters, such as ABCG8, remove excess body cholesterol [32]. The JPSP

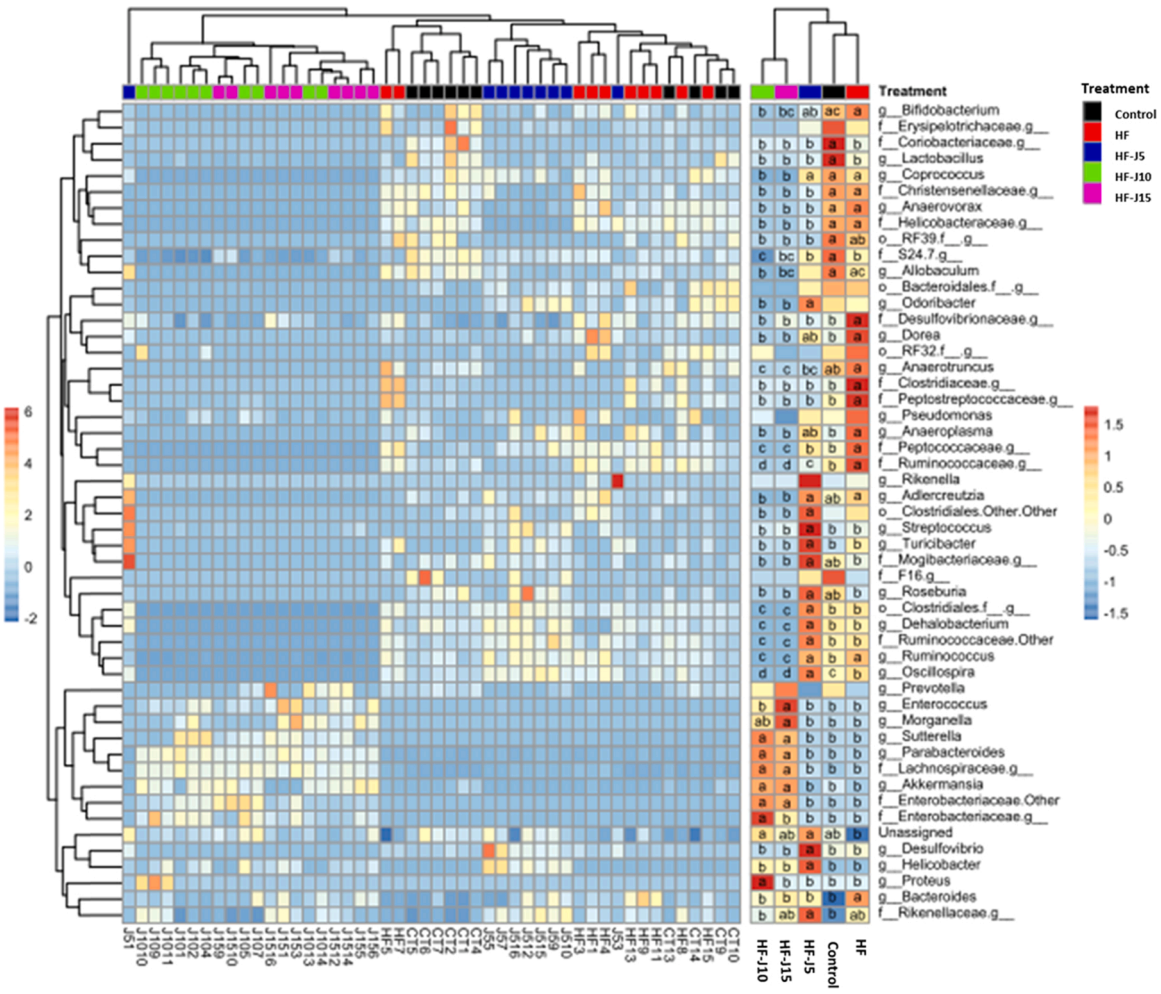

Fig. 7. Cecum microbial community heat map. The results are expressed as mean ± SD. HF: high fat; HF-J5: high-fat diet with 5% JPSP; HF-J10: high-fat diet with 10% JPSP; HF-J15 high-fat diet with 15% JPSP. Different letters denote statistical difference ($p < 0.05$) between the experimental groups (one-way ANOVA followed by Tukey's multiple comparison post-hoc test).

consumption decreased the HMGCoA gene expression, and the consumption of 15% JPSP in the diet promoted the higher expression of the ABCG8 cholesterol transporter, and in turn, lowered the concentration of liver cholesterol.

The beneficial health effects of jaboticaba have been associated with the high content of phenolic compounds in this fruit, especially anthocyanins and hydrolysable tannins, which are mainly concentrated in its peel and seed [14]. Considering that JPSP is rich in both hydrolyzable tannins (ellagitannins and gallotannins) and anthocyanins [33], the experimental diets supplemented with JPSP contained lower contents of anthocyanins and higher contents of ellagic and gallic acids than expected. While anthocyanins and ellagic and gallic acids accounted for 25% and 10% of the total phenolics, respectively, in JPSP, these compounds accounted for 0.7% and 96% of the total phenolics, respectively, in the experimental diets used in the present study. As anthocyanins are known to be thermolabile, the heat associated with the dehydration process used to prepare the diets probably caused their degradation. Likewise, this process probably caused the depolymerization of ellagitannins and gallotannins, yielding the corresponding acids. This result highlights the importance of analyzing polyphenols not only in JPSP, but also in experimental diets. Therefore, the phenolic profile of an experimental diet actually consumed by the animals may be completely different from that of the "active ingredient" added to their feed. This difference, which is usually overlooked in most studies, could lead to an incorrect association between biological activity and a class of phenolics. In the present study, for instance, the health effects of JPSP would have been erroneously associated with anthocyanins if the experimental diets had not been analyzed.

The beneficial effects of phenolic compounds are related not only to their intact forms found in food, but also to their metabolites, which are usually more bioavailable and bioactive than their precursors [34]. In the present study, the metabolism of ellagic acid, the most abundant phenolic compound in JPSP diets, was investigated. It is known that ellagic acid is poorly absorbed and extensively metabolized by gut microbiota, yielding urolithins and, less commonly, nasutins [34–36]. Although none of these classes of gut metabolites were detected in the urine of JPSP-fed mice, methyl ether glucuronides of ellagic acid were identified and quantified. These results are in agreement with studies that identified these metabolites in the urine and plasma of rats that received 4% (w/w) of *Myrciaria jaboticaba* berry peel (MJP) for 10 weeks [13] and in humans that received an acute dose of JPSP (20 g/day) [37]. These metabolites are formed upon the absorption of ellagic acid in the initial portion of the small intestine, metabolized by catechol-*O*-methyl transferase, and conjugated with glucuronic acid [38], indicating enterohepatic circulation.

The differences in ellagic acid metabolism reported by various studies (urolithins, nasutins, and/or ellagic acid conjugates) may be associated with the chemical structure of the ingested ellagitannins, most notably their degree of polymerization [36], as well as with gut microbiota composition [39]. Although the microorganisms responsible

for the complete metabolization of ellagitannins have not yet been fully identified, some intestinal bacterial species, such as *Gordonibacter urolithinfaciens, Gordonibacter pamelaeae*, and *Ellagibacter isourolithinifaciens* that all belong to the phylum *Actinobacteria*, have already been described in humans as urolithin producers [25,40]. In the present study, the low relative abundance of this phylum in the gut microbiota, which suggests no colonization of the intestine, may explain the absence of urolithins in the urine of JPSP-fed mice. Therefore, the beneficial effects of JPSP polyphenols are probably associated with the methyl ether glucuronides of ellagic acid.

The beneficial hepatic remodeling observed in the groups treated with 10% and 15% JPSP can be linked to an improvement in gut microbiota and liver function. Although the changes in the Firmicutes/Bacteroidetes ratio as a cause of obesity is highly contested [41], intestinal integrity and the translocation of LPS are clear contributors [42]. Both groups showed enhanced expression of gut mucin 2, a glycoprotein secreted by goblet cells that take part in the mucus barrier that protects the gut from damage [43]. Moreover, the HF-J10 and HF-J15 groups had high claudin 1 expression, implying preserved intestinal permeability [44]. This scenario was corroborated by markedly reduced LPS concentrations and hepatic steatosis.

The gut microbiota is a factor that has been associated with the pathophysiology of NAFLD in humans. NAFLD patients have a significant increase in intestinal permeability, favoring the translocation of LPS from the intestinal lumen to the circulation, which promotes metabolic endotoxemia [45]. Improvement in the integrity of the intestinal barrier has been associated with an increase in the relative abundance of *Akkermansia muciniphila* [46,47], which can be favored by the ingestion of phenolic compounds [47]. Thus, the higher abundance of *Akkermansia* sp. in the HF-J10 and HF-J15 groups may be associated with the lower plasma levels of LPS. This attenuates hepatic remodeling due to lower activation of Kupffer and stellate cells, which are closely associated with inflammation and liver fibrosis, respectively [45]. In summary, changes in the gut microbiome community caused by JPSP consumption may have contributed to several beneficial effects, such as decreases in insulin resistance, dyslipidemia, inflammatory cytokines, hepatic remodeling, and metabolic endotoxemia [48,49].

The reduction in alpha diversity following the consumption of JPSP (HF-J10 and HF-J15 groups), evidenced by distinct clusters of HF-J10 and HF-J15 in the UniFrac metric (weighted and unweighted), indicates a change in beta diversity. This could be explained by the antimicrobial activity of phenolic compounds, which directly affect the gut microbiota composition by inhibiting individual bacterial groups [50]. The major reduction in alpha diversity observed between HF-J5 and HF-J10 suggests that the antimicrobial threshold was reached between 5% and 10% supplementation. In this study, the *Firmicutes/Bacteroidetes* ratio was used as a parameter for both obesity status and changes in the gut microbiota [50,51]. An increase in this ratio has been associated with an increase in the incidence of obesity [23]. The lowest *Firmicutes/Bacteroidetes* ratio in the groups that consumed JPSP (HF-J10 and HF-J15) was caused by an increase in the relative abundance of *Bacteroidetes* (*Porphyromonadaceae* family) in detriment to *Firmicutes* (mainly of the *Clostridiales* order and of the *Ruminococcaceae* and *Erysipelotrichaceae* families), in accordance with other intervention studies with phenolic compounds [49,52]. The decrease in this ratio reinforces the implication that the consumption of phenolics may provoke less energy use from the diet, contributing to weight gain attenuation in HF-J15. It is also possible that the reduced energy harvest was associated with a reduced microbial population, irrespective of changes in composition.

In the groups treated with JPSP (HF-J10 and HF-J15), the higher abundance of microorganisms associated with the production of short-chain fatty acids (SCFAs), such as butyrate (*Lachnospiraceae* family) [53], acetate, and propionate (*Bacteroidetes* phylum) [54], suggests that JPSP has beneficial effects on colonocytes. In fact, an increase in the production of SCFAs in the gut following jaboticaba peel consumption has been reported [12]. One of the proposed mechanisms for the beneficial effects of SCFA production is their potential to increase AMPK activity, thus affecting the hepatic metabolism of lipids and glucose [55]. In addition, in the groups treated with JPSP (HF-J10 and HF-J15), the higher abundance of the phylum *Verrucomicrobia* and the genus *Akkermansia* may also be associated with beneficial health effects, as *A. muciniphila*, a gram-negative mucin-degrading bacterium belonging to this phylum and genus, has previously been implicated in prebiotic-induced beneficial effects on obesity, gut permeability, and insulin resistance [56,57]. In fact, it has been reported that the administration of a cranberry extract to obese mice increases the relative proportion of *Akkermansia*, which in turn plays a key role in the protection against liver steatosis and insulin resistance, as well as in the alleviation of metabolic endotoxemia and intestinal inflammation [48].

5. Conclusion

The consumption of JPSP, which is rich in phenolic compounds, promoted changes in the diversity of the intestinal microbiota of obese mice, in addition to favoring the reduction of metabolic endotoxemia, attenuation of hepatic remodeling, and improvement in glucose homeostasis, dyslipidemia, and inflammation. Thus, the changes observed in the intestinal microbial community and the local and systemic effects associated with the consumption of JPSP are consistent with the improvement in the metabolic parameters and NAFLD of obesity induced by a high-fat diet.

CRediT authorship contribution statement

Elaine Soares: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Aruanna C. Soares:** Conceptualization, Investigation, Writing – review & editing. **Patricia Leticia Trindade:** Conceptualization, Investigation, Writing – review & editing. **Elisa B. Monteiro:** Conceptualization, Investigation, Writing – review & editing. **Fabiane F. Martins:** Investigation, Writing – review & editing. **Andrew J. Forgie:** Data curation, Writing – review & editing. **Kim O.P. Inada:** Writing – review & editing, Visualization. **Graziele F. de Bem:** Writing – review & editing. **Angela Resende:** Resources, Writing – review & editing. **Daniel Perrone:** Resources, Writing – review & editing. **Vanessa Souza-Mello:** Investigation, Writing – review & editing. **Francisco Tomás-Barberán:** Resources, Writing – review & editing. **Benjamin P. Willing:** Resources, Writing – review & editing, Funding acquisition, Supervision. **Mariana Monteiro:** Resources, Writing – review & editing. **Julio B. Daleprane:** Conceptualization, Writing – review & editing, Funding acquisition, Project administration, Supervision.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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