Macauba (*Acrocomia aculeata*) pulp oil has the potential to enhance the intestinal barrier morphology, goblet cell proliferation and gut microbiota composition in mice fed a high-fat diet

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Abstract

Macauba (*Acrocomia aculeata*) is a palm tree native from Brazil, whose pulp is rich in oil that has a high content of oleic acid and carotenoids. Macauba pulp oil can bring health benefits due to its bioactive compounds; however, its effects on gut health are unknown. Thus, the objective of this study was to evaluate the effect of macauba pulp oil on the intestinal health in mice fed a high-fat (HF) diet. Male C57BL1/6 mice were randomly divided into three groups (10 animals/group): control diet, HF diet and HF diet with 4 % of macauba pulp oil (HFM). Concentration of short-chain fatty acids (SCFA), faecal pH and histomorphometric analysis of the colon were performed. Content of colon samples was used on microbiome analysis using 16S rRNA amplicon sequencing. Animals from the HFM group had higher butyric acid content and goblet cells number, greater circular and longitudinal muscle layer and higher α-diversity compared with the HF group. Moreover, consumption of MPO reduced *Desulfobacterota* phylum, *Ruminococcaceae*, *Oscillospiraceae*, *Prevotellaceae*, *Bifidobacteriaceae* family, *Faecalibacterium*, *Prevotella*, *Ruminococcus* and *Enterorhabdus* genus. Therefore, macauba pulp oil was able to modulate the gut microbiota and enhance intestinal barrier morphology, showing preventive effects on gut dysbiosis in mice fed a HF diet.

Keywords: **HPLC: Butyrate: Oleic acid: Carotenoids: Gut microbiota: Goblet cell**

The role of gut microbiota in the development of diseases has received increased attention from researchers worldwide[1]. These diseases share a common mechanism because the activation of the immune system leads to greater inflammation, and components originating from gut microbiota, such as lipopolysaccharide, peptidoglycan, flagellin and bacterial DNA, can cause immune system activation[2]. People with obesity generally have a lower abundance of beneficial gut microbiota, and studies have found that people with obesity have different gut microbiota compositions compared with lean people[3,4].

Diet has a marked influence on the intestinal microbiota, generating different enterotypes with a predictable composition according to the type of diet consumed[5]. Compared with diets enriched with SFA, diets with a high percentage of unsaturated fatty acids have been associated with a lower stimulatory effect on weight gain and hepatic lipid accumulation, related to the diet-induced changes observed in gut microbiota[6]. Furthermore, bioactive compounds such as carotenoids are capable of modulating the intestinal microbiota, providing positive beneficial effects on health[7,8].

To date, however, little is known about the potential impact of specific plant-derived dietary oils on the composition of gut microbiota and host metabolic health. Macauba (*Acrocomia aculeata*) is a palm tree found naturally in almost all Brazilian territory and it is considered a promising alternative source of vegetable oil[9]. The oil extracted from the pulp is predominantly composed of MUFA, oleic acid being its main constituent. Furthermore, it also presents compounds that play an important role in the health benefits, such as carotenoids[10].

A recent study carried out by our research group demonstrated that macauba pulp oil prevented adipogenesis, inflammation and oxidative stress in mice fed a high-fat (HF) diet[11]; however, there is no information in the literature on the intestinal health benefits of macauba pulp oil. In the present study, we hypothesised that macauba pulp oil would modulate the microbiota composition and improve the intestinal morphology,

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alleviating the disorder caused by HF diet consumption in mice. This experimental model is widely used in research and may be the first indication of effects in humans. Thus, considering the impact of metabolic diseases on public health and the association between these diseases and change microbiota, and the influence of diet on this parameter, this work focused on the effect of macauba pulp oil in gut microbiota modulation of mice fed a HF diet.

Material and methods

Materials

Macauba fruits utilised in this study were harvested in Araponga – Minas Gerais (Brazil) in March 2019, in mature stage, and then, they were peeled and pulped. Macauba pulp oil was extracted using a manual hydraulic press (Laboratory Press, Fred S. Carver Inc) at room temperature, centrifuged at 5000 rpm for 20 min and stored in a freezer at −80°C until use.

Carotenoids and fatty acid composition of macauba pulp oil

Carotenoid analysis was carried out by HPLC with detection of 450 nm, using the chromatographic conditions: HPLC system (Shimadzu, SCL 10AT VP), chromatographic column Phenomenex Gemini RP-18 (250 mm × 4·6 mm, 5 mm), fitted with a guard column RP-18 Phenomenex ODS column (4 mm × 3 mm). The mobile phase consisted of methanol: ethylacetate:acetonitrile (70:20:10, v/v/v) with a flow of 2·0 ml/min and a run time of 15 min[12,13]. In the fatty acid analysis, the oil was converted to fatty acid methyl esters (FAME) to obtain the fatty acid profile[14]. Samples were injected in a gas chromatograph equipped with a Flame Ionisation Detector (Shimadzu, GC-2010) and a capillary column of 100 m × 0·25 mm (SP-2560, Sigma-Aldrich). The analysis was performed by direct injection of 1 μl of the sample. Helium gas was used as the dragging gas and maintained at a constant flow rate of 363 kPa. The FAME were separated using a linear heating ramp from 100°C to 270°C, at a heating rate of 20°C/min, and high linear velocity for better peak resolution. Peak identification was confirmed by comparison with the standard FAME mix (Supelco 37 FAME mix, Sigma-Aldrich).

Ethical approval

The study was approved by the Ethics Committee of the Federal University of Viçosa, Brazil (Protocol 09/2019; date of approval: 28th May 2019). All experimental procedures with animals were performed in accordance with the ethical principles for animal experimentation and Animal Research guidelines: the ARRIVE Guidelines[15].

Animals and diets

The experimental design was done according to our previous study[11]. C57BL1/6 mice, male, lineages Inbred and with 8 weeks old were used in this study. Mice were obtained from the Center for Reproductive and Biology (Federal University of Juiz de Fora). Animals were kept in a temperature-controlled room

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

Ingredients	CD	HF	HFM
Albumin*	17·97	17·97	17·97
Dextrinised starch	15·5	15·5	15·5
Sucrose	10	10	10
Cellulose	5	5	5
AIN-93M mineral mix	3·5	3·5	3·5
AIN-93M vitamin mix	1	1	1
L-cystine	0·18	0·18	0·18
Choline bitartrate	0·25	0·25	0·25
Maize starch	42·60	11·40	11·40
Soyabean oil	4	4	–
Lard	–	31·2	31·2
Macauba pulp oil	–	–	4
Energetic density (kcal/g)	3·85	5·41	5·41

CD, control diet – AIN93M; HF, high-fat diet; HFM, high-fat diet with macauba pulp oil.
* Purity of 78 %.

(22 (SD 2)°C), with automatically controlled light-dark cycles of 12 h, and in individual stainless steel cages, with water and respective experimental diets supplied *ad libitum*.

The animals (*n* 30) were randomly divided by body weight into three groups (10 animals/group): control diet – AIN93M (CD); HF and high-fat diet with macauba pulp oil (HFM). The number of animals per group was calculated based on the sample calculation equation and was considered α-level = 5 %, α-error type I = 1·96 and data of fat mass mean from Schoemaker *et al.*[16]. Experimental diets were based on AIN-93M and HF diet[17]. The HF diets were prepared in the following energetic proportions: 59 % from fats, 28 % from carbohydrates and 13 % from protein. In diets with macauba pulp oil, this was added in the proportion of 4 %, replacing the soyabean oil used in the AIN-93M diet (Table 1). The diets were kept under freezing temperature (−20°C) and were placed daily for the animals. Food intake and body weight were recorded weekly. After the intervention period (8 weeks), animals were anaesthetised with isoflurane (Isoforine, Cristália), according to the body weight of the animal. Colon and its content were collected and stored in a freezer at −80°C until further analysis. For histological analysis, part of the colon was fixed in 10 % formaldehyde.

Faecal pH

Newly excreted faeces were weighed (100 mg), diluted in MilliQ water (1:10, and homogenised by vortexing for 15 s. The pH readings were then performed using a pH metre (Kasvi®)[18].

SCFA measurement

The SCFA analysis was performed in the caecum content following the methodology proposed by Siegfried *et al* with modifications[19]. Throughout the analysis, the samples remained under low temperature. Briefly, 100 mg of caecum faeces was homogenised in MilliQ water following a Vortex shaking protocol with calcium hydroxide and cupric sulphate to extract the SCFA. The quantification of SCFA was performed by HPLC. The SCFA were determined in a Dionex Ultimate 3000 Dual detector HPLC apparatus (Dionex Corporation) equipped with a refractive index detector Shodex RI-101 maintained at

40°C. The SCFA were separated on a Bio-Rad HPX-87H column (300 × 4·6 mm) (Phenomenex Inc.) maintained at 45°C. Analyses were performed isocratically under the following conditions: mobile phase sulphuric acid 5 mmol⁻¹, flow rate 0·7 ml/min, column temperature 45°C and injection volume 20 μl. Stock solutions of the standards were prepared using the acetic, propionic and butyric acid. All SCFA were prepared with a final concentration of 10 mmol/l.

Histomorphometric analysis of the colon

The colon samples were fixed in 10 % formaldehyde, dehydrated, cleared and embedded in paraffin. Sections were cut at 3 μm thick, mounted on glass slides and stained with haematoxylin and eosin. Analyses were performed under a photomicroscope (Leica DM750®). The histological sections images were captured in a 10× objective. Crypt width and depth, goblet cells number (20 villi/sample), thickness of the circular and longitudinal muscle layer were evaluated[20]. Scale of 50 μm was used, and twenty random fields per animal were selected. The images were processed using the ImagePro-Plus software version 4.5 (Media Cybernetics).

Gut microbiota analysis by 16S rRNA gene sequencing

The genomic DNA was extracted from approximately 100 mg of caecal content (*n* 10 animals/group) following a mechanical disruption by beat-beating and phenol/chloroform extraction protocol[21]. The concentration and quality of DNA were determined spectrophotometrically by measuring the A260/280. Amplicons of the 16S rRNA V3-V4 region were generated using forward primer 341F (5'-CCTAYGGGRBGCASCAG-3') and reverse primers 806R (5'-GGACTACNNGGGTATCTAAT-3') and a barcoded primer set adapter for the Illumina NovaSeq platform (Illumina)[22]. Samples were loaded onto an Illumina flow cell for paired-end sequencing reactions using the Illumina NovaSeq PE250 platform in the Novogene Corporation at the University of California at Davis campus. Amplicons were sequenced on a 2 × 250 bp NovaSeq run using customised sequencing primers and procedures[22]. The sequences obtained for all samples were submitted to Sequence Read Archive database on the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/sra) under the accession number PRJNA906643.

16S rRNA sequence processing

Data processing and analysis were performed using the software Mothur (version v.1.44.3). The R1 and R2 paired-end reads were joined, and sequences smaller than 380 or greater than 440 bp were removed. Sequences were discarded if they had homopolymers with at least eight nucleotides or contained ambiguous bp. Chimera sequences were detected and filtered with a reference-based approach using UCHIME version 4.2[23]. After cleaning the sequences, they were aligned with the 16S rRNA gene using the SILVA database v.138[24]. Taxonomic classification was performed using SILVA database v.138, and the operational taxonomic units were grouped with a 97 % sequence similarity cut-off. The coverage of all samples was assessed by Good's coverage estimator (bacteria > 97 %). To correct for sampling bias due to unequal amplicon library sizes, the samples were normalised for the lowest number of sequences produced from any sample.

Gut microbiota diversity and composition analysis

The normalised data table was used for calculating α- and β-diversity and the relative abundance of operational taxonomic units. All the analyses were performed considering the taxonomic classification at genus level[24]. The α-diversity of each sample was analysed using the Chao1, Shannon and Simpson index for microbial community composition. β-diversity metrics were calculated using the Jaccard dissimilarity index. Principal coordinate analysis plots were performed on calculated distance matrices in the bacterial communities. The statistical significance of β-diversity across sample groups was assessed with the non-parametric permutational multivariate ANOVA (Monte Carlo permutations) test using the Past software (version 4.05). Linear discriminant analysis effect size was performed to identify the functional microbial pathways that were differentially expressed in the different experimental groups using the Galaxy website[25,26].

Statistical analysis

The results are expressed as the mean values and standard deviations. The results were analysed by ANOVA followed by post hoc Tukey test. The significance of differences was defined at the $P < 0.05$ level. SPSS software version 26.0 was used to carry out the statistical analysis. For the microbiome results, the Chao 1, Shannon and Simpson index α-diversity metric was utilised to determine the bacterial richness in the samples, and the differences among the groups were analysed by ANOVA. For assessing β-diversity, the Jaccard distances were calculated by the pairwise PERANOVA test. For correlation between caecal microbiota and intestinal parameters, Spearman's correlation was used. SAS version 9.3 (SAS Institute) was used for the microbiota analyses statistics.

Results

Carotenoids and fatty acid composition of macauba pulp oil

The macauba pulp oil used in this study contained high content of MUFA (55 %); with 49·32 % of oleic acid (Table 2), this lipid profile is different from soyabean oil, which has a lower content of oleic MUFA (24 %) and high linoleic acid (54·6 %)[27]. Additionally, macauba pulp oil showed 207·52 μg/g of total carotenoids (Table 2).

Effect of macauba pulp oil in body weight gain and food consumption

At the end of 8 weeks, the food consumption was significantly higher ($P < 0.05$) in the CD group compared with the HF and HFM groups (CD = 4·07 (SD 0·16); HF = 2·53 (SD 0·41); HFD = 2·63 (SD 0·42) g/d), but that did not represent a difference in the weight gain among groups, there was no difference

Table 2. Carotenoids and fatty acid composition of macauba pulp oil

	Macauba pulp oil
Total carotenoids (µg/g)	207·52
β-carotene	163·63
α-carotene	21·03
Lutein	8·75
Lycopene	14·11
Total SFA (%)	24·07
Palmitic	22·84
Stearic	1·23
Total MUFA (%)	55·30
Palmitoleic	5·93
Oleic	49·32
Total PUFA (%)	20·68
Linoleic	19·63
Linolenic	1·05

$(P > 0.05)$ in the experimental groups $(CD = 4.01$ (SD 1·74); $HF = 4.14$ (SD 2·23); $HFM = 3.66$ (SD 2·23) g).

Macauba pulp oil promotes increased SCFA production, enhances intestinal barrier morphology and increased goblet cells

The group that consumed macauba pulp oil did not differ in relation to faecal pH $(P > 0.05$; Table 3). After HFM intervention, the butyric acid was higher to than in the HF group $(P < 0.05$; Table 3). However, the HFM group did not differ in comparison with other groups for acetic acid and propionic acid contents $(P > 0.05$; Table 3).

The animals that consumed macauba pulp oil showed an increase in the goblet cells number compared with control CD and HF groups $(P < 0.05$; Fig. 1(a)). Furthermore, HFM showed greater circular and longitudinal muscle layer than HF group

Table 3. SCFA and faecal pH of mice after consuming the experimental diets for 8 weeks (Mean values and standard deviations)

	CD		HF		HFM	
	Mean	SD	Mean	SD	Mean	SD
Acetic acid (mmol/l)	2·12	0·37[a]	2·36	0·82[a]	2·17	0·47[a]
Propionic acid (mmol/l)	1·13	0·95[a]	2·19	1·05[a]	2·26	0·70[a]
Butyric acid (mmol/l)	1·27	0·46[a]	0·18	0·04[b]	0·44	0·04[a]
Faecal pH	8·3	0·08[a]	8·0	0·20[a]	8·2	0·42[a]

CD, control diet – AIN93M; HF, high-fat diet; HFM, high-fat diet with macauba pulp oil.
Different letters indicate a statistical difference by Tukey test $(P < 0.05)$.
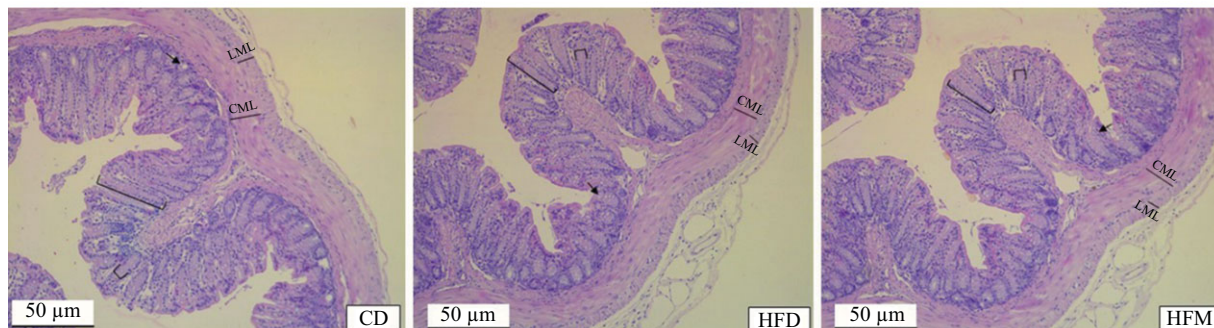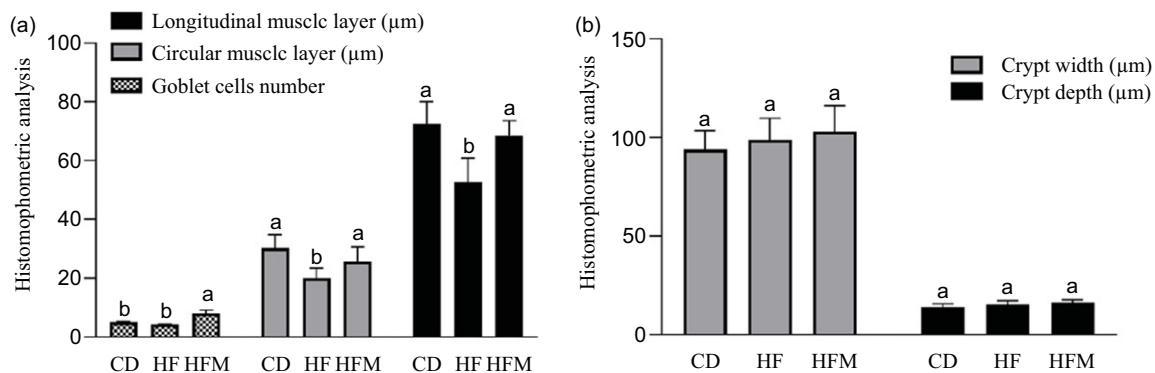

Fig. 1. Effects of macauba pulp oil consumption in colonic histomorphometric characteristics in mice fed a high-fat diet. Data are expressed as the mean values and standard deviations (*n* 6 animals/group). Different letters indicate a statistical difference by Tukey test $(P < 0.05)$. CD, control diet – AIN93M; HF, high-fat diet; HFM, high-fat diet with macauba pulp oil; CML, circular muscle layer; LML, longitudinal muscle layer. Black arrows represent goblet cells in the crypt. Black brackets represent the crypt depth and width.
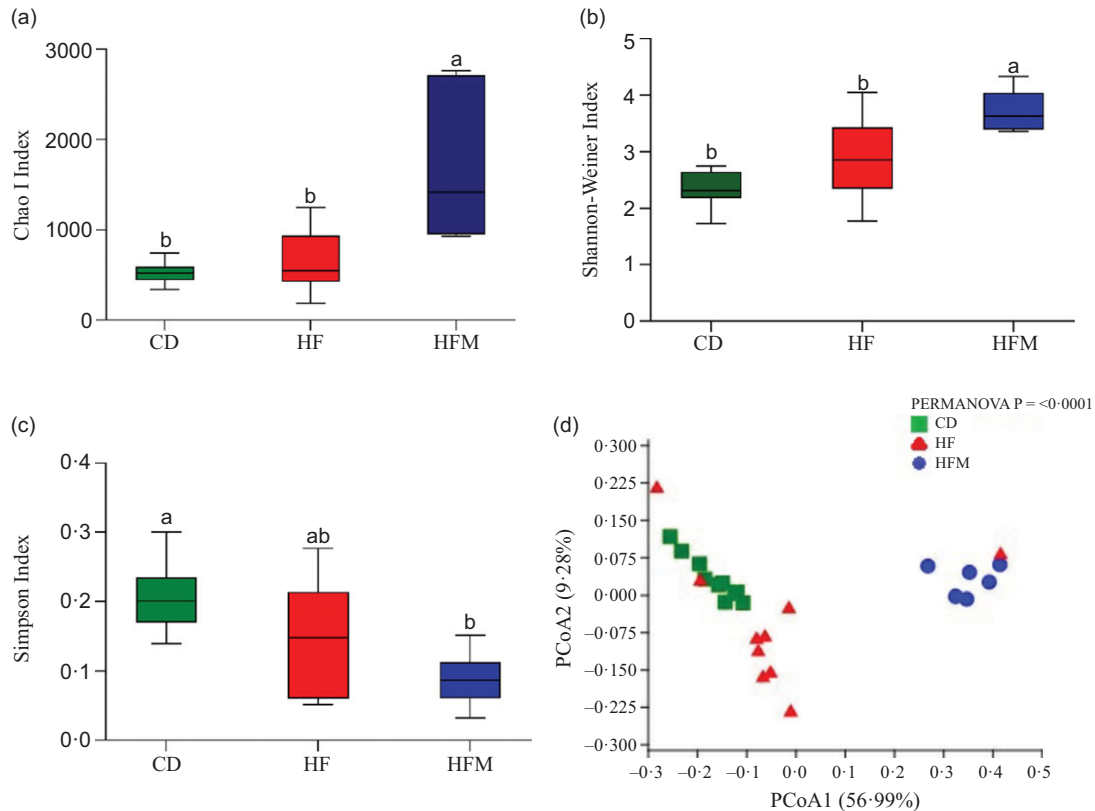
Fig. 2. Microbial diversity of the caecal microbiome after the consumption of macauba pulp oil for 8 weeks. Measure of α-diversity using the (a) Chao 1, (b) Shannon and (c) Simpson index. (d) Principal coordinate analysis (PCoA) based on Jaccard similarity distance of caecal microbial communities. Each dot represents one animal, and the colours represent the experimental groups. CD, control diet – AIN93M; HF, high-fat diet; HFM, high-fat diet with macauba pulp oil. Different letters indicate a statistical difference by Tukey test ($P < 0.05$). PERMANOVA, permutational multivariate analysis of variance.

($P < 0.05$; Fig. 1(a)), which were similar to CD group. Crypt length and depth showed no statistical difference between groups ($P > 0.05$; Fig. 1(b)).

Macauba pulp oil alters the diversity of gut microbiota

The sequencing of the 16S rRNA gene from the faecal samples generated 4 381 792 raw sequences. After filtering and cleaning the sequences, 726 539 sequences of good quality were obtained. The Good's coverage obtained in the samples was > 99 %, indicating good coverage of the sequencing. The summary of sample sequencing data is presented in online Supplementary Table S1.

α-Diversity is measuring by Chao1, Shannon and Simpson index. The abundance and uniformity of species are indicated by Chao 1 index, and compared with that in the HF group, the Chao 1 index was significantly increased (Fig. 2(a)). In the HFM group, Shannon index was significantly increased (Fig. 2(b)), and the Simpson index was reduced (Fig. 2(c)). These results indicate that the α-diversity increased in the HFM group.

In the β-diversity analysis, the principal coordinate analysis revealed that there is a difference in the overall gut bacterial microbiota in animals fed the macauba pulp oil. Animals in the HFM group exhibited a distinct cluster compared with animals in the HF and NC groups, showing an evident distinction in

bacterial communities of HFM group. Moreover, we observed that animals from the HF and NC groups showed low dissimilarity in the bacterial community (Fig. 2(d)).

Macauba pulp oil shapes gut microbiota in different taxonomic levels

The taxonomic classification of samples showed 13 phyla, 20 classes, 50 orders, 89 families and 224 genera. At the phylum level, the relative abundance of *Desulfobacterota* in the HFM group was significantly reduced ($P < 0.05$, Fig. 3(a–b)); however, there was no significant difference in *Firmicutes*, *Bacteroidota* and *F/B* ratio ($P > 0.05$, Fig. 3(c)).

The relative abundance of *Bacilli* and *Desulfovibrionia* class in the HFM group was significantly reduced, while those of *Clostridia* and *Coriobacteriia* were significantly increased ($P < 0.05$, online Supplementary Fig. S1). The relative abundances of *Oscillospirales* and *Clostridiales* order were higher in the HFM group than in the HF group. After HFM intervention, the abundance of *Bifidobacteriales* and *Christensenellales* was similar to than in the CN group ($P < 0.05$, online Supplementary Fig. S1).

At the family level, the relative abundances of *Desulfovibrionaceae* were significantly reduced in the HFM group compared with those in the HF group, while those of *Ruminococcaceae*, *Oscillospiraceae*, *Bifidobacteriaceae*,
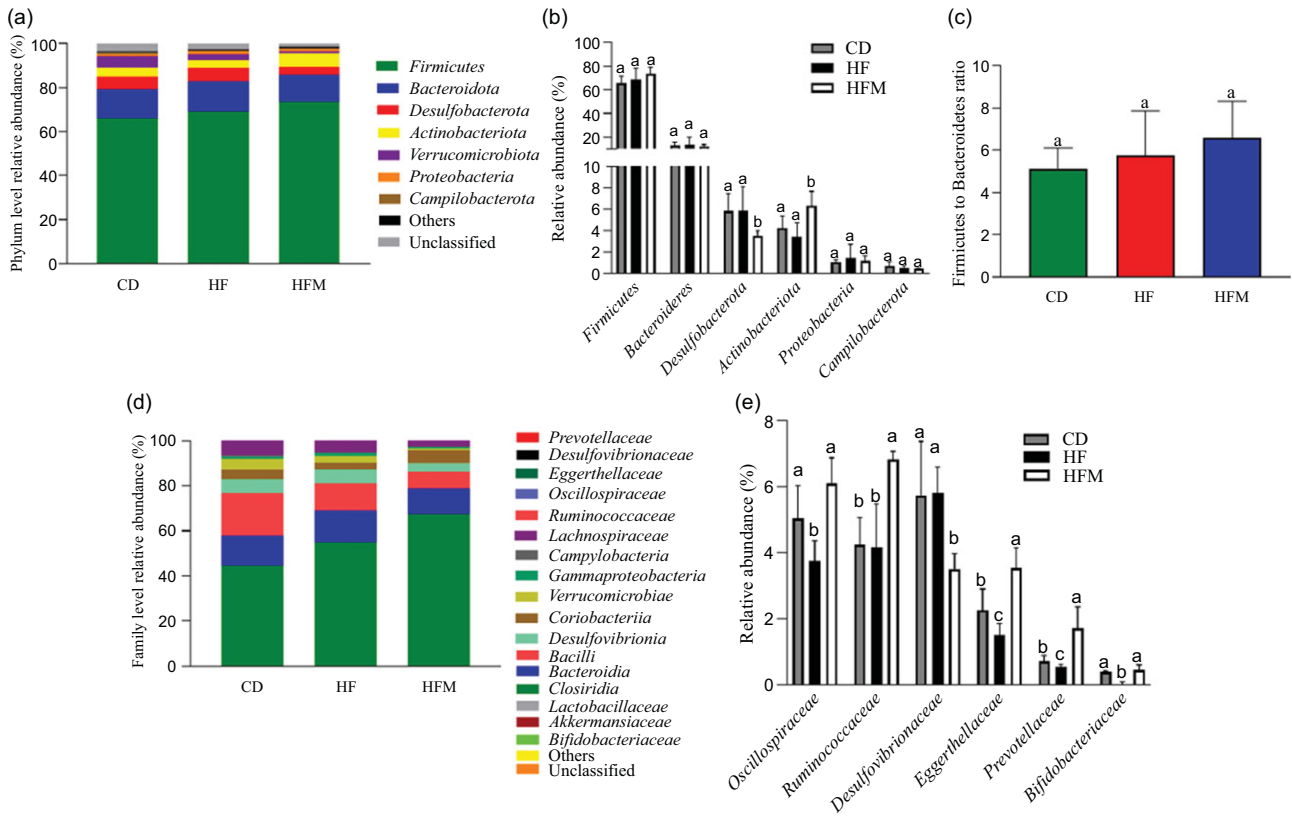
Fig. 3. Gut microbiota at phylum and family classification levels. (a) Distribution of mice gut microbiota at the level of phylum classification; (b) relative abundance of the gut microbiota at the level of phylum classification; (c) Firmicutes/Bacteroidetes ratio; (d) distribution of mice gut microbiota at the level of family classification; (e) relative abundance of the gut microbiota at the level of family classification. CD, control diet – AIN93M; HF, high-fat diet; HFM, high-fat diet with macauba pulp oil. Different letters indicate a statistical difference by Tukey test ($P < 0.05$).

Prevotellaceae and *Eggerthellaceae* were significantly increased ($P < 0.05$, Fig. 3(d–e)).

At the genus level, the relative abundances of *Ruminococcus*, *Prevotella*, *Faecalibacterium* and *Enterorhabdus* were significantly increased in the HFM group, while those of *Akkermansia* were significantly reduced ($P < 0.05$, Fig. 4(a–b)). There was a significant correlation between *Ruminococcus*, *Enterorhabdus*, *Faecalibacterium* and circular muscle layer, and between *Enterorhabdus*, *Faecalibacterium* and goblet cell number, and between *Prevotella* and butyric acid ($P < 0.05$, Fig. 4(c)).

Effect of macauba pulp oil on the dominant caecal microbiota

The linear discriminant analysis effect size method was used to investigate the bacterial biomarkers and isolate gut microbiome differences between the treatment groups. It was identified by twenty-eight dominant operational taxonomic units with effect size >3, with higher number of dominant taxa in CD group (*n* 15) when compared with HF (*n* 5) and HFM (*n* 8). In the macauba pulp oil group, we observed that the most differentially enriched taxa were related to members of the *Clostridia* and *Coriobacteriia* class. In CN group, the dominant community was *Faecalibaculum*, *Dubosiella* and *Akkermansia*.

Sutterellaceae family was the dominant in the microbiota of the HF group, showing a larger effect size (Fig. 5).

Discussion

In this study, macauba pulp oil changed the gut microbiota profile, increased SCFA production and goblet cells and enhanced intestinal barrier morphology in mice fed a HF diet. The intestinal microbiota has been identified to be a potentially important player in the development, exacerbation and/or alleviation of diseases and has a beneficial role in the maintenance of physiological homoeostasis, and the diet is an important environmental factor in modulating the gut microbiota, which is closely associated with individual health[1,2,4].

In the current study, consumption of macauba pulp oil resulted in an increase in the butyrate level when compared with HF diet. This SCFA can reduce translocation of bacteria in the intestine epithelium and increase epithelial integrity and mucus secretion[28]. SCFA are metabolites of beneficial bacteria colonised in the colon and are closely related to the positive effect in metabolic diseases, inhibiting the proliferation of harmful bacteria, and protect intestinal environmental homoeostasis, and it is an indirect index reflecting whether the structure
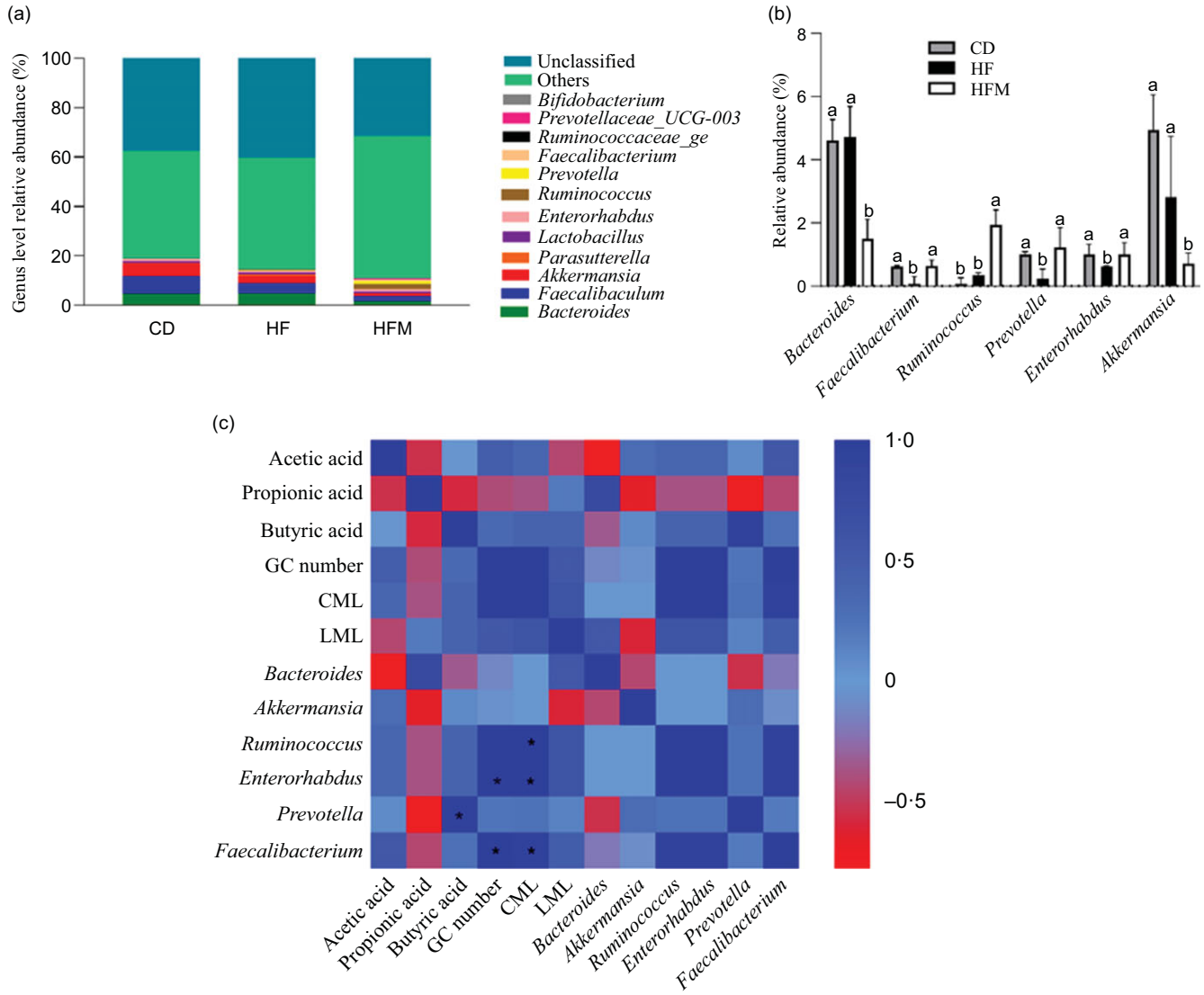
(a)

(b)

(c)

Fig. 4. Gut microbiota at genus class classification levels. (a) Distribution of mice gut microbiota at the level of genus classification; (b) relative abundance of the gut microbiota at the level of genus classification; (c) heatmap of Spearman's correlation between caecal microbiota and intestinal parameters. CD, control diet – AIN93M; HF, high-fat diet; HFM, high-fat diet with macauba pulp oil; GC, goblet cell; CML, circular muscle layer; LML, longitudinal muscle layer. Different letters indicate a statistical difference by Tukey test ($P < 0.05$).

of the intestinal microbiota is normal[29,30]. The high concentration of carotenoids in macauba pulp oil may have contributed to the increase in SCFA (butyric acid), since carotenoids are fat-soluble bioactive compounds, so they have low bioavailability in the blood[31] and can probably be fermented in the intestine by the microbiota. Gut microbes that synthesise more butyrate relative to acetate or propionate may decrease food intake or energy harvest, thereby decreasing hepatic glucose production, *de novo* lipid synthesis and adipogenesis and increasing lipolysis in white adipose tissue[32].

In addition, the consumption of macauba pulp oil improved intestinal barrier morphology, showing higher circular and longitudinal muscle layer and increased in the goblet cells number. The intestinal tract is essential to maintaining intestinal homoeostasis, through the intestinal barrier system that depends

on interactions among several barrier components, including mucus layer, epithelial layer and intercellular tight junctions[33]. Studies demonstrate that carotenoids can improve gut barrier integrity, regulating proliferation and differentiation in the intestinal epithelium, which is crucial to the maintenance of the gut barrier, and can regulate the levels of secretory IgA, which also influences the microbial composition in the gut[7]. Oleic acid can improve intestinal morphology by reducing bacteria that can reduce disulphide bonds in the mucus, which leads to the lysis of the MUC2 protein network (oligomeric mucus gel-forming), which are secreted by goblet cells, and with this improves intestinal morphology[34].

Macauba pulp oil consumption increased α-diversity, that is a marker most common indicator for assessing gut microbiota health. Low bacterial species diversity is closely associated with
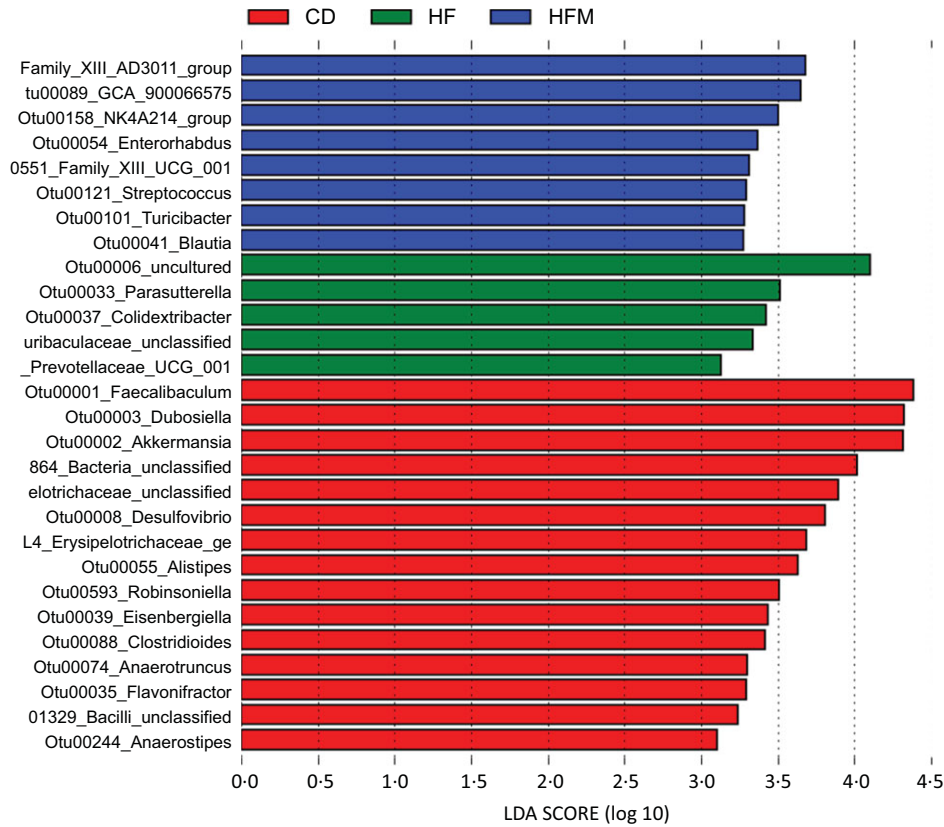
Fig. 5. Effect of macauba pulp oil in difference in dominant micro-organisms among groups. CD, control diet – AIN93M; HF, high-fat diet; HFM, high-fat diet with macauba pulp oil.

the disease status, and in general, a high diversity provides the ecosystem with strong stability[35]. Previous study showed association between oleic acid consumption and increase in the intestinal microbiota diversity, similar to the current work with the consumption of macauba pulp oil that is high in oleic acid[36]. This relationship of diversity and health can be associated with the results obtained in a previous study, verifying that macauba pulp oil resulted in a reduction in inflammation, adipogenesis and oxidative stress in mice fed a HF diet[11].

At the phylum level, macauba pulp oil decreased potentially harmful bacteria phylum *Desulfobacterota*. *Desulfobacterota* phylum consists of various organisms capable of reducing sulphur compounds, followed by butyrate degradation via the butyrate β-oxidation pathway, which is not beneficial, as butyrate is a SCFA that is very important for intestinal health[37]. In the current study, the abundance of *Firmicutes* and *Bacteroidetes* and *Firmicutes:Bacteroidetes* ratio was not significative difference, but this ratio still cannot be considered parameter by gut health, because there are many unconfirmed issues about this possible biomarker[38]. It is important to know that more expressive changes are verified at lower taxonomic levels, such family, genus or species level, and not only phylum level.

When examining the macauba pulp oil effect at the family level, we demonstrated that macauba pulp oil increased the relative abundance of potentially beneficial bacteria. *Ruminococcaceae* may be involved in intestinal epithelium maintenance as it is inversely correlated with intestinal permeability and is closely related to the production of butyrate[39]. This association with maintenance of the intestinal epithelium is confirmed by the results of colon histology, which showed that macauba pulp oil increased muscle layer. We found that the consumption of macauba pulp oil reduced the abundance of lipopolysaccharide-producing and sulphate/sulphite-reducing bacteria, *Desulfovibrionaceae* family. Lipopolysaccharide is a major component of the outer cell membrane of gram-negative bacteria, and the increase in the concentration of lipopolysaccharide in the blood causes low-grade inflammation and, ultimately, obesity and related metabolic diseases, and this bacteria family produces hydrogen sulphide by sulphate/sulphite-reducing, that is, a genotoxic gas that causes barrier dysfunction and endotoxemia, impairing barrier function[32].

The present study showed several positive changes in the gut microbiota at the genus level, with the increase in *Faecalibacterium, Prevotella, Ruminococcus* and *Enterorhabdus* bacterial genus with potential healthy metabolic. The *Prevotella*, which belong to the phylum of *Bacteroides*, are anaerobic bacteria, which produce acetate and succinate through fermentation and were positively associated with glucose, obesity and the metabolic syndrome improvement[40,41]. *Ruminococcus* are

involved in SCFA production and, consequently, involved in intestinal barrier integrity by modulating the mucin expression and the glycosylation level in goblet cells[42]. This corroborates with the data from SCFA and histomorphometric analysis, which showed an increase in butyrate and goblet cells with the consumption of macauba pulp oil. *Enterorhabdus* and *Faecalibacterium* genus are indigenous butyric acid-producing bacteria in the gastrointestinal tract, and this SCFA has influence on the gut–brain axis, suppressive effects on excessive inflammatory response and enhancing effects in intestinal barrier function[43,44]. This is confirmed by the correlation analysis that showed an association between these bacterial genera and an increase in the longitudinal muscle layer, which results in an improvement in the intestinal barrier.

After 8 weeks of macauba pulp oil consumption, we observed a decrease in the relative abundance of *Akkermansia* genus, that is, a genus involved in maintaining the mucus thickness. Despite the reduction of this important bacterium, we observed an increase in the number of goblet cells in the group that consumed macauba pulp oil. Thus, the increase in goblet cells due the macauba pulp oil consumption might be due other factors besides the relative abundance of *Akkermansia* genus, because as demonstrated, there was no significant correlation between the relative abundance of *Akkermansia* and histological parameters. Further, these results can be explained due to the fact that *Akkermansia* are competitive exclusion bacteria, and in this case, we hypothesise that as the macauba pulp oil increased the relative abundance of *Ruminococcus*, *Prevotella*, *Enterorhabdus* and *Faecalibacterium* it could have promoted an environment of competition, thus resulting in the inhibition of *Akkermansia*[45].

There are metabolic and physiological differences between the experimental model used and humans, but considering the consumption of macauba pulp oil in the present study and converting it to Human Equivalent Dose (HED)[46,47], considering an adult, the current consumption would correspond to 20·32 g of macauba pulp oil/d, demonstrating that a small amount would already be able to generate changes benefits in the intestinal health, similar to what was observed in the present study with an animal model of mice.

Conclusion

The consumption of macauba pulp oil for 8 weeks changed the profile of gut microbiota, increasing the relative abundance of bacteria of the genus *Faecalibacterium*, *Prevotella*, *Ruminococcus*, *Enterorhabdus* and *Ruminococcaceae*, *Oscillospiraceae*, *Prevotellaceae*, *Bifidobacteriaceae* family. These changes may have enhanced intestinal barrier morphology by increased goblet cells number, greater muscle layer and butyric acid production in mice fed a HF diet. Therefore, macauba pulp oil has the potential to alleviate HFD-induced disorder of gut microbiota and can be a promising functional food, promoting intestinal benefits.

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Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114523002623

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