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AÇAÍ (Euterpe oleracea Martius) SUPPLEMENTATION IN A HIGH-FAT MATERNAL DIET MODULATES SHORT-CHAIN FATTY ACIDS CONCENTRATION IN DAMS AND OFFSPRING

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ABSTRACT

Introduction: maternal diet high in fat can metabolically program their offspring, increasing the risk of metabolic changes associated with obesity in adult life. The effect of dietary components on the microbiota has been studied as an adjuvant therapeutic strategy. Açaí, a fruit from the Amazon, is described in the literature as having these bioactive compounds. Objective: to evaluate the effect of supplementation with açaí pulp in a maternal HF diet on the concentration of short-chain fatty acids (SCFA) in colonic content and liver concentration of tumor necrosis factor (TNF) in post-weaning rats and pups. Methods: firstly, in vitro antioxidant characterization of the açaí pulp used was carried out. For the experimental model, 32 rats were divided into 4 groups: Control, HF, A (Control supplemented with 2% açaí pulp) and HFA (HF supplemented with 2% açaí pulp) before mating, during pregnancy and lactation. Açaí pulp has exceptional antioxidant activity. Results and discussion: we identified that rats in the HAF group had lower concentrations of total SCFA, acetic and propionic acid than those in the HF group. Açaí in the HF diet prevented the increase in hepatic TNF in rats. Açaí in the maternal HF diet increased the concentration of butyric acid and reduced propionic acid in their offspring. Conclusion: the results suggest that açaí, rich in fiber and polyphenols, can alter the SCFA profile in rats and puppies, contributing to its recognition as an adjuvant therapeutic strategy for metabolic complications associated with obesity.

Key words: Açaí. Bioactive compounds. High-fat maternal diet. Offspring. Short-chain fatty acids.

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RESUMO

A suplementação de açaí (Euterpe oleracea Martius) em uma dieta materna rica em gordura modula a concentração de ácidos graxos de cadeia curta em mães e filhotes

Introdução: a dieta materna rica em gordura pode programar metabolicamente seus descendentes, aumentando o risco de alterações metabólicas associadas à obesidade na vida adulta. O efeito de componentes dietéticos sob a microbiota tem sido estudado como uma estratégia terapêutica adjuvante. O açaí, fruto da Amazônia, é descrito na literatura por apresentar esses compostos bioativos. Objetivo: avaliar o efeito da suplementação com polpa de açaí em dieta materna HF sobre a concentração de ácidos graxos de cadeia curta (AGCC) no conteúdo colônico e concentração hepática do fator de necrose tumoral (TNF) em ratas e filhotes pós-desmame. Métodos: primeiramente, foi realizada a caracterização antioxidante in vitro da polpa de açaí utilizada. Para o modelo experimental, 32 ratas foram divididas em 4 grupos: Controle, HF, A (Controle suplementada com polpa de açaí 2%) e HFA (HF suplementada com polpa de açaí 2%) antes do acasalamento, durante a gestação e lactação. A polpa de açaí apresenta excepcional atividade antioxidante. Resultados e discussão: identificamos que ratas do grupo HAF apresentaram menor concentração de AGCC total, ácido acético e propiônico do que as do grupo HF. O açaí na dieta HF preveniu o aumento hepático de TNF nas ratas. O açaí na dieta materna HF aumentou a concentração de ácido butírico e reduziu propiônico em seus filhotes. Conclusão: os resultados sugerem que o açaí, rico em fibras e polifenóis, pode alterar o perfil de AGCC, em ratas e filhotes, contribuindo para o seu reconhecimento como estratégia terapêutica adjuvante para as complicações metabólicas associadas à obesidade.

Palavras-chave: Ácidos graxos de cadeia curta. Compostos bioativos. Descendentes. Dieta materna rica em gordura. Polpa de açaí.

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INTRODUCTION

The growing global prevalence of obesity and overweight, associated with a series of metabolic complications, represents an important public health challenge in all age groups of the population (Koliaki, Dalamaga e Liatis, 2023).

The developmental origins of health and disease are based on the idea that different stimuli during critical periods of development (such as the neonatal period and fetal growth) cause physiological responses in the fetus that may predispose to the onset of metabolic diseases in adulthood (Bianco-Miotto e colaboradores, 2017).

In both humans and animal models, offspring of mothers exposed to over-nutrition during gestation have elevated risks of obesity and liver dysfunction (Williams e colaboradores, 2014).

Dietary imbalances, such as excessive lipids intake, serve as a determinant for developing non-alcoholic fatty liver disease (NAFLD). NAFLD, recently redesignated as steatotic liver disease associated with metabolic dysfunction (Gowda e colaboradores, 2023), is a spectrum of liver diseases that includes simple steatosis, nonalcoholic steatohepatitis, fibrosis, and cirrhosis. Epidemiologic data show that the prevalence of this disease in children is approximately 8% in the general pediatric population and 34% among obese children (Lazarus e colaboradores, 2022; Yu e Schwimmer, 2021).

For a long time, the "two-hit" hypothesis was used to understand NAFLD, proposing that it primarily resulted from oxidative and inflammatory imbalances. However, the gut-liver relationship has gained significant attention as a crucial factor in the progression of this disease, given their bidirectional relationship via the portal vein (Ohtani, Kamiya e Kawada, 2023).

Although evidence points to the relationship between intestinal microbiota and the development of NAFLD, the underlying mechanisms are still not well understood (Plaza-Díaz e colaboradores, 2023).

One of the hypothesis could be related to short-chain fatty acids (SCFA), such as acetate, propionate, and butyrate (Xie e DeMarzio, 2019).

Patients with NAFLD present an imbalance in the concentration and/or metabolism of SCFAs. This imbalance promotes intestinal barrier dysfunction, resulting in metabolic endotoxemia and the formation of products and toxins derived from the intestine (lipopolysaccharide).

These toxins activate hepatic toll-like receptors, with subsequent production of pro-inflammatory mediators, such as tumor necrosis factor (TNF), and inflammation (Wang e colaboradores, 2021; Wang e colaboradores, 2020).

Lifestyle and diet changes are crucial for improving metabolic complications resulting from obesity (Cotrim e colaboradores, 2023).

In recent years, literature has demonstrated the properties of bioactive compounds present in foods, such as fibers and phenolic compounds, in affecting the activity and metabolism of the microbiota (Kasprzak-Drozd e colaboradores, 2023). This may serve as an adjunctive alternative in the treatment of metabolic diseases that affect the liver.

Açaí (Euterpe oleracea Martius) is a typical fruit from the Amazon region, described in the literature as containing various bioactive compounds.

Recently, we demonstrated that supplementation with açaí pulp in a HF maternal diet before mating, and during gestation, and lactation prevented the NAFLD in dams and the onset of early metabolic changes leading to NAFLD in their offspring.

The results suggested that açaí pulp supplementation played a protective role by acting on lipid metabolism and exhibiting antioxidant effects in dams and their offspring (Barbosa e colaboradores, 2021; Barbosa e colaboradores, 2020).

However, the impact of açaí pulp supplementation in a HF maternal diet on the modulation of microbe-derived metabolites has not been elucidated.

Our interest in this topic arose because açai's polyphenols are not fully degraded during the in vitro digestion process, and some of these components reach the colon.

Together with dietary fibers, they modulate the SCFA production in feces and the concentration of bacterial groups, leading to a decrease in the number of the Bacteroides-Prevotella spp. and Clostridium-histolyticum species (Alqurashi e colaboradores, 2021).

Therefore, this study aimed to investigate SCFA concentration in the colonic content and hepatic TNF concentration in dams and 21-day-old offspring in the NAFLD model induced by a HF maternal diet.

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MATERIALS AND METHODS

Ethical aspects

The procedures utilized in this study were conducted in accordance with the norms of the National Council for Control and Animal Experimentation approved by the Ethics Committee in Animal Research of the Federal University of Ouro Preto (protocol number 2015/15).

Açaí pulp

Pasteurized frozen açaí pulp without colorants or preservatives was obtained in a single lot from Icefruit Comércio de Alimentos Ltda (Tatui, São Paulo, Brazil), ensuring its homogeneity throughout the experiment. According to the manufacturer, the açaí pulp is pasteurized, vacuum packed, and kept at -20ºC and does not have the addition of any ingredient other than water. Nutritional analysis of the pulp showed moisture content of 90%, 3.9-g lipids, 2.3-g total carbohydrate, 0.9-g protein, 2.3-g insoluble fber and 0.4-g soluble fber per 100 g of pulp. The polyphenol content of açaí pulp used in this study is 549.5 mg GAE/100 g (mg of gallic acid equivalent (GAE) in100 g of açaí pulp) (Barbosa e colaboradores, 2020).

Preparation extract of açaí pulp

The extraction method used in this study is a modification of the procedure outlined (AOAC, 1998; Larrauri e colaboradores, 1997; Instituto Adolfo Lutz, 1985) Frozen açaí pulp (50 g) was weighed and subjected to two extractions at room temperature with continuous stirring for 1 hour. Initially, the pulp was extracted with 20 ml of methanol-water mixture (50:50 v/v), followed by a second extraction with 20 ml of acetone-water mixture (70:30 v/v). Intermittent centrifugation was employed at 4000 g for 15 minutes between each extraction. The supernatants obtained from both extractions were combined in volumetric flasks, and the volumes were adjusted to 50 ml with distilled water. These extractions were performed in triplicate, and the total phenolic compound content and all in vitro antioxidant characterization analyses were performed on these extracts.

ANALYSIS OF IN VITRO ANTIOXIDANT CAPACITY

Determination of antioxidant activity by the method radical ABTS•+ (22,20 -Azinobis-3-ethylbenzotiazoline-6-sulfonic acid)

The ABTS•+ assay was performed following a method developed (Miller e colaboradores, 1993) with modifications. ABTS•+ radical cations were produced by reacting 7 mM ABTS stock solution with 145 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The ABTS•+ solution was then diluted with ethanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm. Upon addition of 30 µl of sample or trolox standard to 3 ml of diluted ABTS•+ solution, absorbances were recorded at 6 min after mixing. Calibration was performed using ethanolic solutions of known trolox concentrations, and the results were expressed as µM trolox/g açaí pulp.

Determination of antioxidant activity by the method frap (power ferric reducing)

The antioxidant capacity of the sample was estimated by FRAP assay, following the procedure described in the literature Benzie and Strain, (1996) with modifications. Briefly, 2.7 ml of freshly prepared FRAP reagent (2,4,6-tris(2-pyridyl)-s-triazine, $FeCl_3$, and acetate buffer) at 37ºC was mixed with 90 µl of sample and 270 µl of distilled water. Absorbance at 595 nm was measured at 30 minutes, using a blank containing only the FRAP reagent as a reference. Aqueous solutions of known Fe (II) concentrations in the range of 100–1500 µM (Fe_2SO_4) were used for calibration.

Determination of antioxidant activity by the method radical DPPH• (2,2-difenil-1-picril hidrazil)

The antioxidant capacity was determined by the modified DPPH• method Brand-Williams, Cuvelier, e Berset, (1995). A methanol solution containing 0.06 mM DPPH• was prepared. After adjusting the blank with methanol, an aliquot of 100 µl of the sample was added to 3.9 ml of this solution. The decrease in absorbance at 515 nm was measured at 1-minute intervals for the first 10 minutes, followed by measurements at 5-minute intervals

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until stabilization (for açaí:120 minutes) (Rufino e colaboradores, 2010).

The antioxidant capacity was expressed as the concentration of antioxidant required to reduce the original amount of free radicals by 50% (EC50) and values expressed as g açaí pulp/g DPPH. Additionally, we calculated the antioxidant activity index (AAI) using the formula:

AAI = final concentration of DPPH (µg ml-1) ⁄ IC50 (µg ml-1).

Where values of AAI < 0.5 indicate low antioxidant activity, between 0.5 and 1, moderate activity, between 1 and 2, strong activity, and > 2, very strong antioxidant activity (Scherer e Godoy, 2009).

Animals and diets

Thirty-two female Fischer rats (90 days of age) were obtained from the Laboratory of Experimental Nutrition at the School of Nutrition of the Federal University of Ouro Preto (Minas Gerais, Brazil). The animals were divided into four groups and fed different diets: control diet (C), high-fat diet (HF, 60% of total calories as fat, 53% saturated fat, 6% soybean oil and 1% cholesterol), control diet supplemented with açaí pulp (A, control supplemented with 2% of açaí pulp) or high-fat diet supplemented with açaí pulp (HFA, high-fat diet supplemented with 2% of açaí pulp). The control diet was based on the AIN-93G diet (Barbosa e colaboradores, 2020) and high-fat diet as described in previous studies (Zhang e colaboradores, 2022; Guerra e colaboradores, 2015). The supplementation with açaí pulp was based on the study (Oliveira e colaboradores, 2010). The ingredients used in the preparation of the experimental diets are described in table 1.

Table 1 - Composition of experimental diets.

Nutrientes (g)	Diets			
	C	A	HF	HFA
Casein	200	200	260	260
Corn starch	530,7	510,7	170,7	150,7
Soybean oil	70	70	40	40
Choline	2,5	2,5	2,5	2,5
Minerals mix[1]	10	10	10	10
Vitamins mix[2]	35	35	35	35
Cellulose	50	50	50	50
Cholesterol	0	0	10	10
Açaí pulp	0	20	0	20

C, standard diet; CA, standard diet plus açai pulp 2%; H, hypercholesterolemic diet; HA, hypercholesterolemic plus açai pulp 2%.

[1]Salt mixture (g/kg of mixture):): NaCl – 139,3 / KI- 0,79 / MgSO4.7H2O- 57,3 / CaCO3- 381,4 / MnSO4.H2O – 4,01 / FeSO4.7H2O – 0,548 / CuSO4. 5H2O – 0,477 / CoCl2.6H2O – 0,023 / KH2PO4 – 389,0 [30].

[2] Vitamin mixture (IU or g/kg of mixture): retinol acetate, 2 000 000 IU; cholecalciferol, 200 000 IU; p-aminobenzoic acid, 10.00; inositol, 10.00; niacin, 4.00; calcium pantothenate, 4.00; riboflavin, 0.80; thiamin HCl, 0.50; pyridoxine HCl, 0.50; folic acid, 0.20; biotin, 0.04; vitamin B12, 0.003; sucrose, quantity sufficient to 1 kg; choline, 200.0; a-tocopherol, 10 000 IU [30].

All animals were housed in a standard environment at 23 ± 2°C, with 55% humidity and a 12-hour light/dark cycle, with food and water provided ad libitum. Initially, animals were their respective experimental diets for two weeks. After this period, mating was conducted with a male rat paired with two females for one week. Following the mating period, the females were separated and individually housed in cages to allow the natural progression of gestation, while they continued to receive their allocated diet during gestation and lactation. At birth, male puppies were kept, six per mother, to ensure homogeneous growth of the litters throughout the lactation period (21 days).

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At weaning, both dams and male offspring (n= 8 per group) were anesthetized under isoflurane, after 12 hours of fasting and sacrificed using total blood collection from the brachial plexus. Male pups were selected to reflect the higher incidence of NAFLD in the male population, with eight randomly chosen from each group for all the analyses (Fellows e colaboradores, 2018).

Following euthanasia, the livers from both dams and offspring were collected, washed with a cold saline solution, and weighed. The small hepatic lobe was submerged in liquid nitrogen and immediately stored at -80°C for further analyses. The colonic content was obtained by dissecting the colon from the ileocecal valve and stored at -80°C for analysis of SCFA concentration.

Analysis of fecal SCFA concentration

The extraction and determination of the SCFA concentration in the colonic content was performed using the High-Performance Liquid Chromatography (HPLC) technique according to the method proposed (Torii e colaboradores, 2010; AOAC, 1980) with some modifications.

Briefly, 20 µL aliquots of the extracted and filtered fecal material were analyzed on a DAD Shimadzu® detector HPLC equipment and Aminex HPX-87H column, 300 x 7.8 mm (Bio-Rad®, California, USA). The analysis was performed at a wavelength of 210 nm for 70 minutes, under a flow rate of 0.6 mL/min of 5 mM H_2SO_4, with the column oven set at 55°C. To mobile phase was prepared using 5 mM sulfuric acid (Sigma, San Luis, USA).

Calibration curves were established using SCFA standards (Sigma, San Luis, USA), at the following concentrations: acetic acid (1.2 to 83.0 µmol), propionic acid (1.05 to 67.0 µmol), and butyric acid (0.8 to 56.0 µmol).

Determination of the tnf concentration in the liver

The liver samples were homogenized in RIPA buffer containing 1% protease inhibitor (Thermo Fisher Scientific, USA). The liver tissue was ground using an electric homogenizer and then centrifuged. The total protein concentration in the supernatant was evaluated using the method proposed (Lowry e Colaboradores, 1951; Folin e Ciocalteu, 1927).

Subsequently, the concentration of cytokine TNF was measured. For this assay, the Invitrogen ELISA Kit (Rat TNF-alpha ELISA, For Lysates, Kit - Catalogue Number ERA57RB 96 tests, California, USA) was used, and the analysis was conducted according to the manufacturer's recommendations. The values of TNF concentration in the liver were expressed in pg/mg of total protein.

Statistical analyses

Statistical analyses were performed using GraphPad Prism 6 for Windows (GraphPad Software, San Diego, CA). All data were tested for normality using the Kolmogorov–Smirnov test. Parametric data from the four groups were analyzed by ANOVA two-way followed by Bonferroni test to detect differences between the groups and expressed as mean ± standard deviation (SD). Results were considered statistically significant for p values <0.05.

RESULTS

Characterization of the in vitro antioxidant capacity of the açaí pulp

The results of the in vitro antioxidant activity of açaí pulp are outlined in Table 2. The antioxidant activity using the ABTS+• radical method for açaí pulp was determined to be 31.15 ± 0.05 µM of trolox/g of pulp. Additionally, the antioxidant activity of açaí pulp assessed by the FRAP method was calculated to be 9.52 ± 0.03 µM of ferrous sulfate/g of açaí pulp. Açaí pulp exhibited an AAI of 2.4, indicating strong antioxidant activity. Furthermore, the EC50 value for açaí pulp was determined to be 0.5 g of pulp per gram of DPPH.

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Table 2 - Characterization of the in vitro antioxidant capacity of the açaí pulp*.

	ABTS•+ (µM de trolox/g)	DPPH•	FRAP (µM Fe2SO4/g)
Açaí pulp	31.15 ± 0.05	AAI**= 2.4	9.52 ± 0.03

* Values expressed as mean ± standard deviation.
**AAI: Antioxidant Activity Index.
***EC50: Concentration of antioxidant required to reduce the original amount of DPPH• by 50%.

Table 3 - Short-chain fatty acids concentration in the colonic content of dams and their offspring.

	GROUPS				Two-way ANOVA		
	C (µmol/mg)	**A** (µmol/mg)	**HF** (µmol/mg)	**HFA** (µmol/mg)	**Diet effect**	**Açaí effect**	**Interaction**
Dams							
Total SCFA	57.28 ± 3.17	53.84 ± 3.78	55.04 ± 3.28	36.36 ± 2.18§	**0.0051**	**0.0021**	**0.0251**
Acetic	36.61 ± 1.61	34.48 ± 2.31	33.32 ± 1.98	22.90 ± 1.18§	**0.0006**	**0.0026**	**0.0341**
Propionic	15.60 ± 1.22	12.22 ± 0.99	15.50 ± 1.44	8.14 ± 0.16§	0.0552	**<0.0001**	0.0670
Butyric	4.90 ± 0.53	3.53 ± 0.38	5.75 ± 1.10	3.66 ± 0.33	0.4578	**0.0146**	0.5837
Offspring							
Total SCFA	58.65 ± 2.57	47.17 ± 3.47	59.77 ± 2.42	60.18 ± 3.12	**0.0255**	0.0734	0.0559
Acetic	35.77 ± 1.35	28.87 ± 2.30	35.41 ± 1.82	36.78 ± 2.11	0.0646	0.1679	**0.0442**
Propionic	14.78 ± 0.63	11.60 ± 0.56	18.23 ± 1.59	10.38 ± 0.63§	0.2474	**<0.0001**	**0.0208**
Butyric	5.69 ± 2.31	6.71 ± 1.31	7.58 ± 0.52	13.27 ± 1.20§	**0.0006**	**0.0043**	**0.0367**

§ versus HF
C: control diet; HF: high-fat diet; A: açaí diet; HFA: high-fat açaí diet; SCFA: short-chain fatty acid. For the statistical analysis, 8 animals were used per group, the value of $p < 0.05$ was considered statistically significant. two-way ANOVA test followed by Bonferroni test was used to detect statistical differences between experimental groups. The results are shown as the mean and SEM.

Effect of açaí pulp supplementation in a high-fat maternal diet on the scfa concentration

In Table 3**,** the results for total SCFA, acetic, propionic, and butyric acids in dams are presented. The proposed dietary modifications, including the HF diet and açaí pulp supplementation, demonstrated an isolated effect on the total SCFA concentration in colonic content (p=0.0051 and p=0.0021, respectively), as reflected by the sum of acetic, butyric, and propionic acids concentration. Additionally, an effect of the interaction on total SCFA concentration was observed (p=0.0251).

Following the post-test analysis, dams fed a HFA diet exhibited a 34% reduction in total SCFA concentration compared to those in the HF group. Regarding the concentration of acetic acid in dams, effects of the HF diet (p=0.0006), açaí pulp (p=0.0026), and the interaction (p=0.0341) were observed. Bonferroni's post-test showed a decrease of approximately 31% in HFA group compared to the HF group. The propionic acid concentration in dams was influenced by the açaí pulp (p<0.0001), showing a decrease of 47% in HFA group compared to the HF group following the post-test analysis.

However, concerning the butyric acid concentration in dams, an effect of açaí-pulp (p=0.0146) was observed, although no significant difference was found after the post-hoc analysis.

The results for SCFA in the offspring's feces are also presented in Table 3. Regarding the total SCFA concentration, an effect related to the HF maternal diet (p=0.0255) was observed, although no significant differences were found after the post-hoc analysis. Regarding acetic acid concentration, an effect of the interaction was observed (p=0.0442) with no significant differences at the post-hoc level. Propionic acid concentration showed an effect of the açaí pulp (p<0.0001) and the interaction

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(p=0.0208), showing a decrease of 43% in the offspring from the maternal HFA group when compared to the offspring from the maternal HF group.

Surprisingly, butyric acid concentration was influenced by the diet (p=0.0006), açaí pulp (p=0.0043), and the interaction (p=0.0367), showing an increase of 75% in butyric acid concentration if offspring from maternal HFA group when compared to offspring from maternal HF group.

Effect of açaí pulp supplementation in a high-fat maternal diet on the tnf hepatic concentration

In Figure 1, we present the evaluation of hepatic TNF concentration in dams and offspring. In dams, an effect of diet (p<0.01) and açaí pulp (p<0.001) on hepatic TNF concentration was observed, indicating a 65% increase in the HF group compared to the C group. However, açaí pulp supplementation in the HF maternal diet prevented this increase (Figure 1a).

Regarding the offspring, TNF concentration was influenced by the maternal HF diet (p=0.001). After the post-hoc test, an increase of 90% was observed in the offspring from the maternal HF group compared to offspring from the maternal C group, and 110% in the offspring from the maternal HFA group compared to offspring from the maternal A group (Figure 1b).

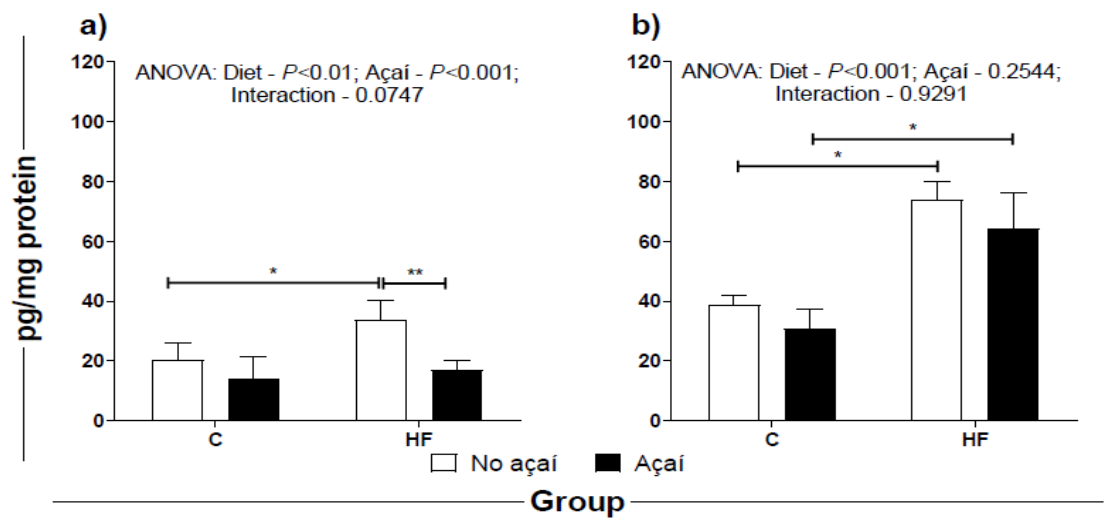

Figure 1 - Hepatic tumor necrosis factor concentration of dams and their offspring.
a: Hepatic TNF concentration of dams; b: Hepatic TNF concentration of offspring. For the statistical analysis, 8 animals were used per group, the value of p < 0.05 was considered statistically significant. two-way ANOVA test followed by Bonferroni test was used to detect statistical differences between experimental groups. The results are shown as the mean and SEM.

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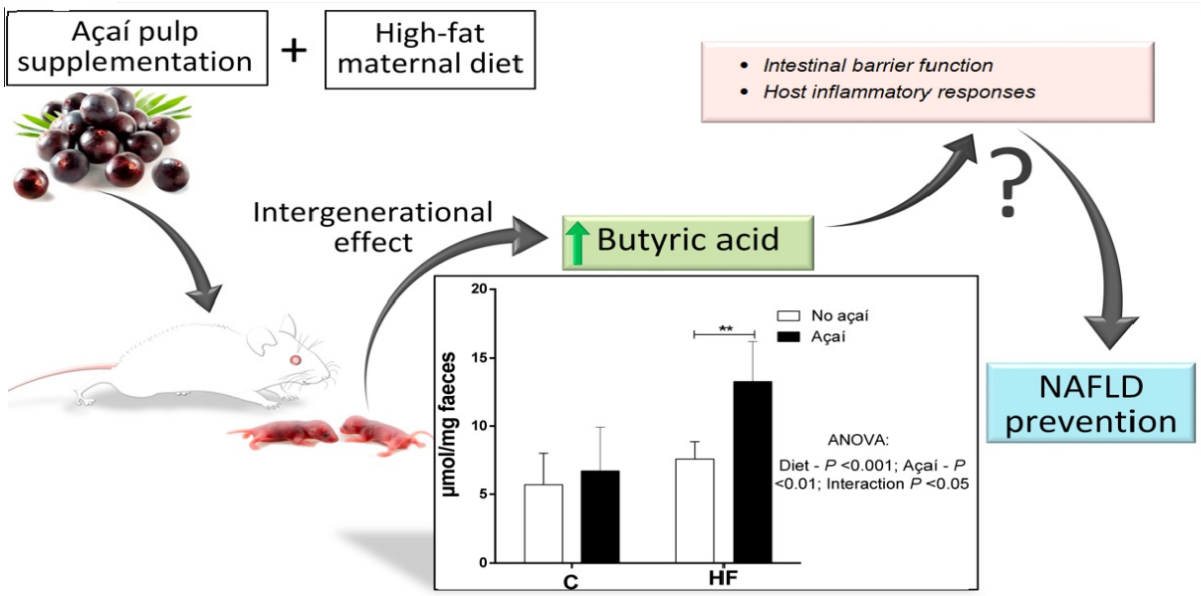

Figure 2 - Abstratc graph. Supplementation with açaí pulp (Euterpe oleracea Martius) promoted an increase in the concentration of butyric acid in the colonic content of the offspring of mothers fed an HF diet. We suggest that açaí pulp can be used as an adjuvant therapeutic strategy in the prevention of metabolic complications associated with obesity, such as NAFLD, as it can also modulate on the gut-liver axis.

DISCUSSION

The experimental model employed in our study focused on NAFLD induced by a HF maternal diet, and we previously showed that supplementing the HF maternal diet with açaí pulp attenuated NAFLD both in dams and their offspring (Barbosa e colaboradores, 2021; Barbosa e colaboradores, 2020).

In dams, HFA led to reductions in relative liver weight, fat, and cholesterol liver content, along with improvements in liver steatosis. Similarly, in offspring, HFA resulted in decreased relative liver weight and improved liver steatosis (Barbosa e colaboradores, 2020).

Our mechanistic studies suggest that açaí supplementation may attenuate NAFLD in dams and protect offspring from the detrimental effects of lipid excess from a maternal HF diet, primarily through the regulation of lipid metabolism and antioxidant effects (Barbosa e colaboradores, 2021; Barbosa e colaboradores, 2020).

Faced with the hypothesis that alterations in gut microbiota may represent another manifestation of the “multiple hits” theory in NAFLD development, we aimed to evaluate the effect of açaí on SCFA concentration in the colonic content. This investigation stemmed from the well-established nutritional and antioxidant characterization of açaí pulp, which is rich in dietary fibers and polyphenols - bioactive compounds capable of modulating the intestinal microbiota (Zheng e colaboradores, 2024; Gallo e colaboradores, 2024).

Our study showed that açaí pulp modified the SCFA profile in both dams and offspring, and investigations into how açaí pulp supplementation in the HF maternal diet alters SCFA concentration are scarce. To our knowledge, this is the first study to examine the effect of maternal diet supplemented with açaí on the modulation of acetic, butyric, and propionic acids.

The reduction in acetic acid concentration in dams suggests a potential protective effect of açaí pulp against hepatic lipid accumulation, as acetic acid is essential for gluconeogenesis and lipogenesis in the liver (Yang e colaboradores, 2019, Ziętek, Celewicz e Szczuko, 2019).

Acetate, in particular, can potentially be used as a cholesterol or fatty acid precursor (Zhang e colaboradores, 2022), and this finding aligns with our previous research showing lower hepatic cholesterol levels in the HFA compared to the HF group (Wang e colaboradores, 2020).

Propionic acid is known to inhibit the activity of histone deacetylases (HDAC) (Silva e colaboradores, 2023; Li e Sun, 2019; Fellows e

colaboradores 2018). In experimental models of NAFLD induced by a HF diet, reduced hepatic expression and activity of the family of proteins classified as class III HDAC, the sirtuins (SIRT), have been observed, leading to changes in hepatic metabolism and promoting fat accumulation (Dornas e Schuppan, 2020; Zeng e Chen, 2022).

Our results showed that supplementation of the high-fat diet with açaí pulp led to a reduction in the concentration of propionic acid in dams and offspring, suggesting a lesser inhibition of SIRT. This observation may justify a higher gene expression of this protein in offspring from dams fed the HFA diet, as demonstrated in our previous study (Barbosa e colaboradores, 2021).

In this sense, we can propose a possible relationship between SCFA and SIRT1 as metabolic modulators that can attenuate NAFLD. However, molecular interaction experiments were not the focus of the present work, and further research is needed to confirm our hypothesis.

Intriguingly, açaí pulp increased butyric acid concentration in offspring from a HF maternal diet. Butyric acid plays a crucial role in the permeability of the intestinal mucosal barrier since it promotes the positive regulation of tight junction proteins integrity and mucins, which improves the gut barrier function and prevents the migration of toxic compounds, including pro-inflammatory molecules, to the liver (Dai e colaboradores, 2020; Chen e Vitetta, 2020).

Our findings suggest that maternal diet, whether rich in lipids or supplemented with fruit rich in bioactive compounds like açaí, affects the placental transfer and breast milk composition, influencing fetal development and genetic programming, justifying the results obtained in offspring (Kim e Yi, 2020).

We also evaluated the hepatic TNF concentration to understand how maternal diet and açaí pulp supplementation affect the inflammatory pathway related to the gut-liver axis in NAFLD development, as TNF is a mediator of hepatotoxicity. Our results show that açaí pulp exerts a protective effect against inflammation induced by the HF diet in dams, although this effect was not observed at the intergenerational level.

Our study paves the way for broadening the understanding of the beneficial effects that açaí may exert on the development of NAFLD, metabolic disease associated with obesity and thus supports dietary recommendations as a preventive measure for metabolic complications arising from unbalanced dietary patterns. However, it is important to acknowledge its limitations.

One such limitation is the absence of molecular experiments, such as the analysis of gastrointestinal microecology and the evaluation of other inflammation markers. allowing us to understand the role of these mediators in specific pathways related to the intestine-liver axis and the progression of NAFLD.

CONCLUSION

In conclusion, our findings suggest a beneficial effect of açaí pulp supplementation in the HF maternal diet, leading to changes in the SCFA profile in both dams and their offspring.

These results can be used to understand the functional effects of açaí pulp, highlighting its potential as an adjunctive therapeutic strategy for NAFLD prevention.

We believe that diverse array of nutrients and non-nutrients present in açaí pulp may act individually or synergistically to influence hepatic metabolism, potentially regulating various metabolic pathways beyond antioxidant and lipid metabolism.

This multifaceted action may help prevent metabolic complications associated with obesity, potentially reducing the need for pharmacological interventions, and improving the overall quality of life for affected individuals.

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