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Maternal rat prenatal and neonatal treatment with pequi pulp reduces anxiety and lipid peroxidation in brain tissue of rat offspring at adolescence

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ABSTRACT

The Pequi fruit (*Caryocar Brasiliense cambess*), typical of the Brazilian *cerrado* or savannah, is a source of essential fatty acids, carotenoids, and phenolic compounds. The aim of this study was to analyze the effects of consuming this fruit on anxiety behavior and lipid peroxidation in the brains of rats whose mothers were treated (by gavage) during pregnancy and lactation with Pequi fruit (pulp or nuts) at 2000 mg/kg of body weight. Anxiety parameters were assessed using the open field (OF), elevated plus maze (EPM), and light/dark box (LDB) tests. The brain was removed to measure malondialdehyde (MDA) levels. Data were analyzed using One-way Anova ($p < 0.05$). In the OF, the animals in the pulp group presented more time spent in the central area (20.37 ± 0.73 vs Control: 12.51 ± 0.39; Nuts: 8.28 ± 0.40) and increased locomotion (159.7 ± 6.10) compared to the other groups (Control: 127.3 ± 5.54; Nuts: 139.08 ± 6.57). In the EPM, the pulp group entered into the open arms (8.57 ± 0.36) and stayed more time in the central area (19.44 ± 1.17) compared to the Nuts group (7.14 ± 0.34; 13.00 ± 1.57). In the LDB the pulp group entered more (8.00 ± 0.42 vs Control: 7.16 ± 0.16 and Nuts: 7.42 ± 0.75) and stayed longer in the clear light side (92.18 ± 6.42) than all the other groups (Control: 71.44 ± 3.53; Nuts: 80.57 ± 6.50), respectively. Pulp group presented lower MDA in the brain (55.34 ± 3.04) compared to Control (72.06 ± 4.66) and Nuts (66.57 ± 2.45). We conclude that Pequi pulp consumption during pregnancy and lactation reduces lipid peroxidation in brain tissue and induces anxiolytic-like behavior in rat offspring. These effects were not observed in the Pequi nuts group.

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1. Introduction

Adequate nutrition is essential for normal development of the body, and if lacking, development is impaired [1]. Brain formation is characterized by stages that include neurogenesis, neural migration, selective apoptosis, synaptogenesis, and myelination. These stages are sequential, providing shape and functionality to brain tissue [2]. The formation and maturation of the central nervous system starts in the intrauterine phase and continues throughout the first years of life [3]. Nutrients from the mother are extremely important for the formation of the *conceptus* (the embryo and its appendages or adjunct parts), and are passed to the fetus through the placenta [4]. After birth, they are passed on through breast milk [5]. Fatty acids are the main constituents of the central nervous system [6], and are responsible for increases in membrane fluidity and synaptic plasticity that contribute to brain functions [7,8].

Studies have shown that fatty acids act as nutritional antioxidants [9,10] as do vitamins C [11] and E [12], carotenoids [13], and phenolic compounds [14], and help reduce lipid peroxidation in brain tissue. Lipid peroxidation is a consequence of oxidative stress, and directly related to behavioral disorders such as anxiety [15,16]. The brain is particularly vulnerable to oxidative damage, due to its high oxygen consumption, high content of polyunsaturated fatty acids, and mitochondrial activity. Compared to other organs and tissues, the brain presents low antioxidant capacity [17]. In brain cell membranes, stress alters both composition and function, and it reduces dendrite growth in the hippocampus, amygdala, and prefrontal cortex, all of which are involved in anxiety and depressive behaviors [18].

Pequi (Caryocar brasiliense Cambess) is a fruit from the Brazilian *cerrado.* Consumption of its pulp, nuts and oil is common in many places. The pequi consists of a greenish brown exocarp, an external mesocarp formed by a white pulp, and an internal mesocarp (an edible portion of the fruit), which is light-yellow to dark-orange in color. The pequi's thorny endocarp protects the edible almond, which is covered by a thin brown integument [19]. Pequi fruit have lipid content higher than 40%, in addition to proteins, carbohydrates, fiber, ash, and vitamin C. Its yellow-orange pulp is indicative of the presence of carotenoids such as β-carotene and β-cryptoxanthin, which are carotenoids with pro-vitamin A activity [20–22]. These nutrients suggest that pequi may protect neuronal lipids from oxidation.

Works in the literature have investigated the characteristics of pequi pulp oil [23,24], pequi almond oil [25], and its extract [26]. However, research investigating the effects of pequi pulp and almonds *in vivo* are scarce, especially when this fruit is offered in early life.

Based on the above, we aimed in this study to analyze the effects of consuming pequi pulp and almonds on the parameters of anxiety and cerebral lipid peroxidation in the offspring of rats treated during pregnancy and lactation.

2. Materials and methods

2.1. Characterization and research locale

This experimental research involved biological assays, and was conducted at the Laboratory of Experimental Nutrition (LANEX), the Bromatology Laboratory (LABROM) – CES/UFCG, and at the Pharmacology Laboratory - III/UFRN.

The experimental protocol followed the ethical recommendations of the National Institute of Health Bethesda (Bethesda, USA), regarding animal care, taking into account the well-being of the laboratory animals, such that any suffering or stress would be minimized as much as possible. This study protocol was submitted to the Ethics Committee on the Use of Animals (CEUA) of the Rural Health and Technology Center – CSTR/UFCG, under protocol number CEUA/CSTR Nº04/2020.

2.2. Materials

The Pequi fruit was purchased in the city of Chapada do Araripe in the state of Ceará - Brazil (−7.12732, −39.59378). It was manually selected, cleaned, and sanitized with a sodium hypochlorite water solution at a concentration of 2.0–2.5%, it was then manually peeled with a stainless-steel knife, and submitted to a simple heat treatment at 70 °C (pre-baking). Being cooled in running water, it was then manually pulped (stainless steel knife), and its pulp and almond (seed) were dried in a forced air circulation oven at a temperature of 55 °C, respectively for 24h and 48h adapted by Ref. [27]. Afterwards, the pulp was crushed in a conventional blender, vacuum packed, and stored in a freezer at −18 °C for later use. The kernels were broken in half to manually remove the almond, which was also crushed in a conventional blender, vacuum packed, and stored in a freezer at −18 °C for later use.

2.3. Total antioxidants analysis of pequi almond and pulp

2.3.1. Extraction by maceration

The extracts were obtained from the sample previously ground, weighed (1g) and transferred to a falcon tube wrapped in aluminum foil with a capacity of 50 mL. To the sample was added 80% methanol solvent at a 1:10 ratio (m/v), and being then vortexed for 1 min, and kept at rest for 24 h at an approximate room temperature of 22 ± 2 °C. Afterwards, the extracts were filtered on qualitative filter paper and stored in amber vials in a freezer (−18 °C) until the moment of analysis [28–31].

2.3.2. Determination of total phenolic compounds

To determine the total phenolic compounds content, the Folin-Ciocalteu method described by Ref. [32] was used. The previously diluted extracts were transferred to test tubes and 2.0 mL of an aqueous solution of 0.2 N Folin-Ciocalteau reagent was added, shaken,

and left to stand in the absence of light for 6 min, then 1.6 mL of sodium carbonate (Na_2CO_3) 7.5% (m/v) was added. These were then shaken and incubated for 5 min in a water bath at 50 °C. After further water cooling, reading in a spectrophotometer at 760 nm, (calibrated with a reference solution of gallic acid) was performed. The results were expressed in milligram gallic acid equivalents - mg GAE/100 g of almond flour, or mg GAE/100g of pulp.

2.3.3. Determination of total flavonoids

The total flavonoid content was determined according to the method proposed by Ref. [33]. An aliquot of 0.5 mL of the previously diluted extracts were placed in test tubes and 2 mL of distilled water was added, followed by 0.15 mL of sodium nitrite ($NaNO_2$), and after 5 min, 0.15 mL of aluminum chloride ($AICI_3$). These were left to rest for 6 min in the dark, and after the addition of 2 mL of 1 M sodium hydroxide solution (NaOH) and 1.2 mL of distilled water, the solution was shaken and the absorbance read in a spectrophotometer at 510 nm, being calibrated with a catechin reference solution. The content of total flavonoids was expressed in mg catechin equivalents - mg CE/100g of almond flour or mg CE/100g of pulp.

2.3.4. Determination of total carotenoids

For determination of total carotenoids, the methodology employed by Ref. [34] was used. 1 g of the sample was weighed in test tubes wrapped with aluminum foil, and 10 mL of acetone was added to each tube and shaken for 30 s in a tube shaker (model AP56, Phoenix Luferco, Araraquara, São Paulo, Brazil). Subsequently, this mixture was filtered using qualitative filter paper (40 Whatman®, 125 mm). The sample was then read in a spectrophotometer (BEL Photonics) at the wavelengths of 470 nm, 645 nm, and 662 nm, against an acetone blank. The total carotenoids content was expressed in mg C/100g of almond flour or mg C/100g of pulp.

2.3.5. $ABTS^{\bullet+}$ radical capture method

To determine antioxidant activity, the ABTS radical method (2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) was performed according to the methodology described by Ref. [35] with some modifications. The $ABTS^{\bullet+}$ radical was formed by the reaction of ABTS solution at 7 mM with a 140 mM potassium persulfate solution, incubated at 25 °C, then left in the dark for 12–16 h, and afterwards diluted in distilled water until obtaining an absorbance value of 0.700 ± 0.020 at 734 nm. From each extract, six different dilutions were prepared in triplicate. In a dark environment, a 15 μL aliquot of the previously diluted extract was transferred to test tubes containing 1.5 μL of the $ABTS^{\bullet+}$ radical. The reading was carried out after 6 min of reaction, in a spectrophotometer at 734 nm. A control solution was prepared according to the procedure described above, without adding the sample. As a reference, Trolox was used and the results expressed in μmol equivalent to trolox/g of the sample (μmol TEAC g^{-1}).

2.3.6. Iron reducing capacity – FRAP

Antioxidant activity was also determined through reduction of iron (FRAP), the methodology described by Ref. [36] adapted by Ref. [37]. FRAP reagent was prepared at the time of analysis, by mixing 11 mL of acetate buffer (0.3 M, pH: 3.6), 1.1 mL of TPTZ (tripyridyltriazine) solution, (10 mM in 40 mM HCl), and 1.1 mL of aqueous ferric chloride solution (20 mM)). An aliquot of 200 μL of the previously diluted extract was added to 1800 μL of the FRAP reagent and incubated at 37 °C in a water bath for 30 min. A blank was performed for each sample, without addition of extract. Absorbances were measured after the incubation period at 593 nm. As a reference, Trolox was used and the results expressed in μmol equivalents of trolox/g of sample (μmol TEAC g^{-1}).

2.3.7. Identification of pequi nuts and pulp fatty acids

The extraction of lipids was performed by Ref. [38] method and the identification and quantification of fatty acids, according to the method described by Ref. [39] in gas chromatography. Fatty acids were quantified by normalizing the areas of the methyl esters and the results were expressed in percentage of area (%).

2.4. Biological tests

2.4.1. Animals and experimental diets

Primiparous females (n = 21) of the Wistar rats (90 days of life/250 ± 50g) were mated (one male per female) to obtain the pups. After confirmation of pregnancy, they were housed in individual polypropylene maternity cages under standard laboratory conditions (temperature 22 ± 1 °C, humidity 55 ± 5%, 12/12 h light/dark cycle with 6 h of artificial light 12:00 to 18:00 h). Females were randomly divided into three groups: Control – receiving distilled water; Pequi Pulp – treated with 2000 mg of pequi pulp/kg of body weight, and Pequi Nuts – treated with 2000 mg of pequi almond/kg of body weight, being administered from the 7th day of gestation until the 21st day of lactation by gavage. The total weight gain and feed intake during pregnancy and lactation were measured. Standard chow (Presence®) and water were provided *ad libitum*. The litters were standardized at ten male offspring to each experimental group.

The litters were standardized with six pups, being composed preferably of males. When there were not enough males, litters were completed with females. After weaning, the pups were kept in collective cages receiving standard feed (Presence®) and water *ad libitum*. At 35 days of life, behavioral anxiety tests were performed. Only male offspring were used for behavioral analysis to avoid hormonal influence on the results. At the end of the experiments, the animals were euthanized (60 days of life).

The experimental design of the study is presented in Fig. 1.

2.4.2. Assessment of behavioral parameters

2.4.2.1. Assessing anxiety using the open field test (OF). The open field, as described by Ref. [40] consists of a square wooden box with black walls and floor space measuring 60x60 × 60cm; subdivided into 9 units delimited by white lines. Each animal is placed in the center of the open field one animal at a time, where they remain for 10 min for free exploration. The ambulation parameters (number of segment crossings by the animal with all four legs), rearing behavior count, and the time spent in the central quadrant of the device were evaluated. Before starting the tests, the device was sanitized with 70% alcohol and paper towels sanitized with 10% alcohol at each animal change to eliminate the animal odor so as not to interfere with the behavior of the next animal. The handling of the animals was always carried out by the same researcher, and all sessions were recorded with a camera positioned on the ceiling above the open field.

2.4.2.2. Anxiety assessment using the elevated plus maze (EPM). The EPM is made of wood in the shape of a cross, raised from the ground, and formed by two (walled) closed arms, and two (perpendicularly directed) open arms. The frequency of entries and the time spent by the animal in each type of arm is analyzed, in addition to any time spent in the central area. The animal is placed in the center of the apparatus, facing one of the closed arms, where free exploration is allowed for 5 (five) minutes [41]. All sessions were thus recorded with a camera positioned on the ceiling above the EPM.

2.4.2.3. Verification of anxiety parameters using the light dark box test (LDB). The light-dark transition test box is made of wood, lined with waterproof material, with total dimensions of: 27 cm (H) × 45 cm (L) × 27 cm (W) and consisting of two compartments, a larger lighted compartment (27 × 27 × 27 cm) with the floor divided into 9 squares (9 cm × 9 cm), and a smaller dark compartment (27 × 18 × 27 cm). In the divider between the two compartments there is a central opening measuring 7 cm × 7 cm. The box also has a black painted covering over the dark compartment, and a lamp over the lighted compartment. Each animal spent 5 min in the box, and the device was cleaned before and after each test with 70% alcohol and 10% alcohol at each change of animals. The following parameters were evaluated: number of entries into the lighted compartment; length of stay in the lighted compartment; and the total time spent in the dark compartment. All sessions were recorded with a camera positioned on the ceiling above the box, and a second camera was placed in the transparent (lighted) compartment to record the animal's movements.

2.4.3. Euthanasia and obtaining brain tissue

Euthanasia was performed by exsanguination (cardiac puncture). After confirmation, the brain was removed and immediately placed on an ice surface and divided into strips for further malonaldehyde (MDA) content analysis. Tissues were kept at −80 °C until the time of analysis.

2.4.4. Lipid peroxidation marker - malonaldehyde (MDA)

Malonaldehyde (MDA) concentrations were determined in the animal brain tissue as described by Ref. [42]. The samples were thawed, minced, and homogenized (Ultra Stirrer Homogenizer, Model 80) with Tris HCl buffer (pH = 7.4) at a ratio of 1:5 (m/v). The homogenate obtained was centrifuged at 2500 G for 10 min at 4 °C, and a chromogenic reactant (1-methyl-2-phenylindole and acetonitrile) and hydrochloric acid (HCl - 37%) were added to the supernatant. The MDA content was calculated through interpolation of a standard curve using 1,1,3,3 - tetraethoxypropane. Absorbance readings were performed in a Polaris® Microplate Reader (Celer Biotecnologia SA) at 586 nm, and data expressed in nmol/g of tissue. All reagents were purchased from Sigma-Aldrich® (St Louis, MO, USA).

2.5. Statistical analysis

The results were expressed as mean ± standard error of the mean (SEM) and analyzed by One Way ANOVA, followed by the Tukey test for comparison between groups. A significance level for rejecting the null hypothesis of $p \leq 0.05$ was considered.

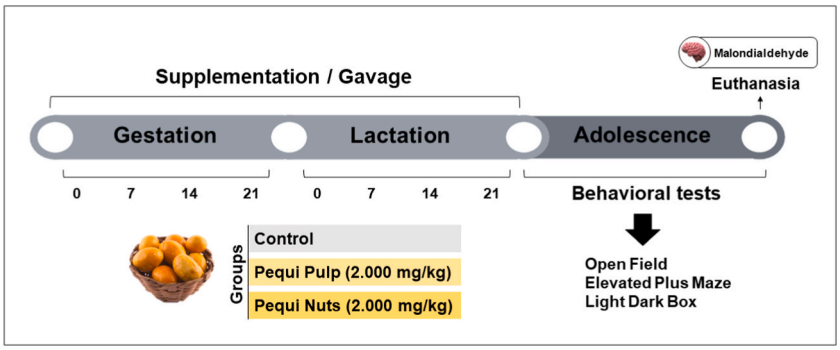

Fig. 1. Experimental design.

3. Results

3.1. Total body weight gain and feed intake

During pregnancy, the rats belonging to the pulp group (58.8 ± 4.2) presented lower weight gains compared to the nuts group (72.4 ± 13.7) and controls (86.5 ± 19.8) [F (2.25) = 10.66] ($p < 0.05$). In the lactation period, the pulp group (18 ± 6.9) also presented the lowest weight gain compared to the nuts group (24 ± 5.03) and controls (31 ± 1.15) [F (2.19) = 16.00] ($p < 0.05$).

As for feed intake during pregnancy, the pulp group (328.5 ± 37) was significantly lower than both the control (397.4 ± 74) and nuts (378.25 ± 61) groups [F (2.23) = 3.289] ($p < 0.05$). And during the lactation period, the animals in the pulp group (411.5 ± 29.6) also presented lower consumption than the control (500.16 ± 29.5) and nuts (463.6 ± 40.8) groups [F (2.15) = 10.45] ($p < 0.05$).

3.2. Phenolic compounds, flavonoids, antioxidant activity and profile fatty acid - Pequi pulp and almonds

As compared to the Pequi nuts, the pulp presented higher total phenolics, flavonoids, and carotenoids, and greater antioxidant activity (Table 1).

3.3. Assessment of anxiety parameters

3.3.1. Open field

In the Open field ambulation tests, the pulp group presented greater exploratory activity than both the control and nuts groups (control: 127.3 ± 5.54; Nuts: 139.08 ± 6.57; Pulp: 159.7 ± 6.10) [F (2.29) = 6.613] ($p < 0.05$) (Fig. 2 A).

As for head rearing, the control group presented greater activity than the other groups, however, the pulp group performed more rearing than the nuts group (control: 60.71 ± 2.38; Nuts: 35.7 ± 1.01; Pulp: 48.2 ± 2.37) [F (2.24) = 37.19] ($p < 0.05$) (Fig. 2 B).

As to permanence in the center of the open field, the pulp group presented a higher time percentage as compared to both of the other analyzed groups. The nuts group spent less time in the center of the open field as compared to the control (control: 12.51 ± 0.39; Nuts: 8.28 ± 0.40; Pulp: 20.37 ± 0.73) [F (2.29) = 26.20] ($p < 0.05$) (Fig. 2C).

3.3.2. Elevated plus maze (EPM)

The animals in the almond group entered more often into the open arms when compared to the control group, and the animals in the pulp group entered more often into the open arms than both other groups control (control: 5.16 ± 0.79; Nuts: 7.14 ± 0.34; Pulp: 8.57 ± 0.36) [F (2.17) = 10.85] ($p < 0.05$) (Fig. 3A). There was no difference in the permanence time % between the groups in the open arms (control: 5.16 ± 0.79; Nuts: 7.14 ± 0.34; Pulp: 8.57 ± 0.36) [F (2.29) = 26.20] (Fig. 3B).

As for the length of stay of the animals at the center of the EPM, the animals in the pulp group remained for longer time periods, than the animals of the other groups. The nuts group did not differ from the control group arms (control: 12.50 ± 1.42; Nuts: 12.64 ± 1.26; Pulp: 11.22 ± 1.56 [F (2.24) = 5.799] ($p < 0.05$) (Fig. 3C).

Table 1
Composition for pequi nuts and pequi pulp.

Antioxidant values	Pequi nuts	Pequi pulp
Total phenolics (mg AGE/100g)	75.69 ± 1.471[a]	120.48 ± 2.942[b]
Total flavonoids (mg CE/100g)	4.50 ± 0.297[a]	15.23 ± 0.735[b]
Total carotenoids (mg/100g)	0.044 mg/100g[a]	0.122mg/100g[b]
FRAP (μmol TEAC/g)	0.49 ± 0.00[a]	0.75 ± 0.00[b]
ABTS (μmol TEAC/g)	3.06 ± 0.000[a]	5.21 ± 0.000[b]
Fatty acids (%)		
Saturated (SAF)		
Palmitic acid C16:0	23.87	31.48
Myristic acid C14:0	0.06	–
Stearic acid C18:0	2.33	1.58
Total SAF	26.26	33.06
Monounsaturated (MUFA)		
Oleic acid C18:1 ω9	69.73	57.37
Total MUFA	69.73	57.37
Polyunsturated (PUFA)		
Linoleic acid C18:2 ω6	2.39	9.28
Eicosenoic acid C20:2 ω6	–	0.36
Total PUFA	2.39	9.64

Values expressed as Standard Deviation (±SD) ANOVA. Different letters between columns signify differences between groups ($p < 0.05$). Total flavonoids and phenolics expressed in mg/100g of pequi; ABTS and FRAP expressed in μmol trolox TEAC/g pequi.

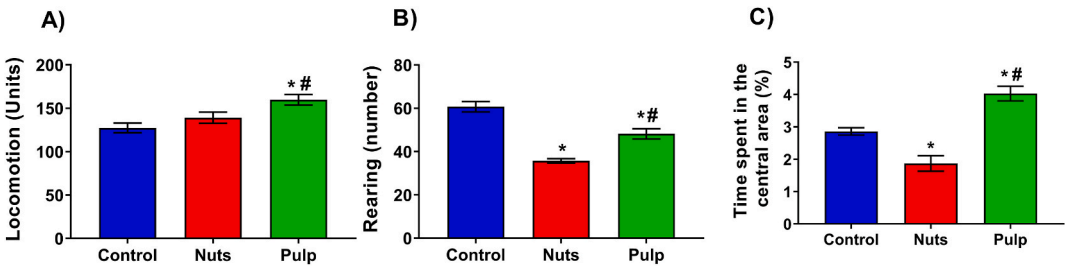

Fig. 2. Evaluation of anxiety parameters performed in the open field test for animals treated with Pequi almonds or pulp during the initial phase of life. Locomotion (Units) (A), Rearing (number) (B), Time spent in the central area (%) (C) were verified. Data were expressed as mean ± SD, n = 10 per group (ANOVA One-way); ($p < 0.05$), *versus control group, #versus almond group.

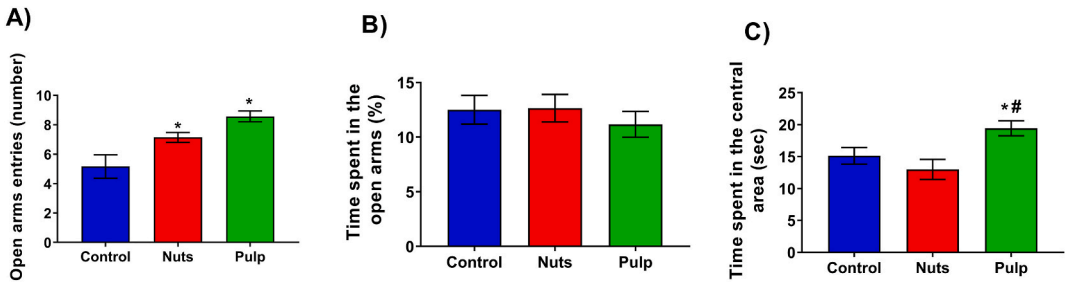

Fig. 3. Performance of the offspring of rats supplemented with Pequi almonds and pulp during pregnancy and lactation in the elevated plus maze: Open arms entries (number) (A), Time spent in the open arms (%) (B), Time spent in the central area (sec) (C), were measured. Data were expressed as mean ± SD, n = 10 per group (One-way ANOVA); ($p \leq 0.05$), *versus control group, #versus almond group.

3.3.3. The light dark box test

Using a light and dark box, the number of entries in the lighted compartment, the time spent in the lighted compartment, and the time spent in the dark compartment were analyzed.

The pulp group presented a greater number of entries (control: 7.16 ± 0.16; Nuts: 7.42 ± 0.75; Pulp: 8 ± 0.42) [F (2.21) = 0.7253] and permanence (control: 71.44 ± 3.53; Nuts: 80.57 ± 6.50; Pulp: 92.18 ± 6.42) [F (2.24) = 3.581] in the lighted compartment when compared to both the control and nuts ($p < 0.05$) (Fig. 4 A and 4 B). As for the time spent in the dark compartment, the pulp group spent less time in the dark area of the box (control: 228.55 ± 3.53; Nuts: 234.5 ± 9.30; Pulp: 207.81 ± 6.42) [F (2.29) = 3.771] ($p < 0.05$) (Fig. 4C).

3.4. Brain lipid peroxidation marker – malonaldehyde

The animals in the Pequi pulp group presented a lower lipid peroxidation index as compared to the control and nuts groups, thus demonstrating a protective effect against oxidative stress in the animals' brain tissue (control: 72.06 ± 4.66; Nuts: 66.57 ± 2.45; Pulp: 55.34 ± 3.04) [F (2.18) = 6.542] ($p < 0.05$) (Fig. 5).

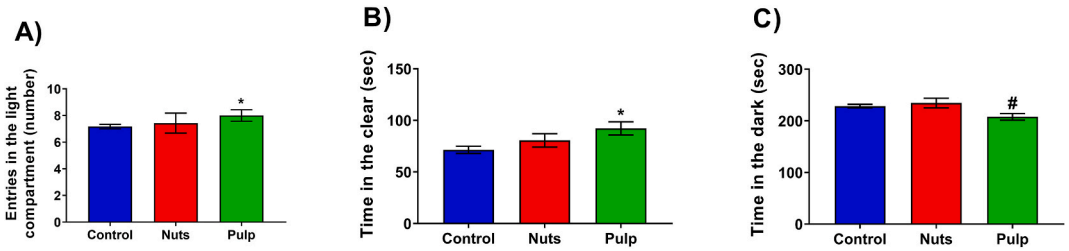

Fig. 4. Light and dark box performance of rat offspring receiving Pequi almond and pulp during pregnancy and lactation; where (A) Entries in the light compartment (number) (B), Time in the clear (sec) (C), Time in the dark (sec). Data were expressed as mean ± SD, n = 10 per group (One-way ANOVA); ($p \leq 0.05$) *versus control group, #versus almond group.

4. Discussion

Both pequi pulp and nuts are sources of phenolic compounds, flavonoids, carotenoids, and fatty acids. However, in the pulp, the amounts of these functional compounds are higher. These biological compounds bring benefits to the organism by eliminating free radicals, modulating signal transduction and gene expression, and restoring neuronal communication [43–45]. In the present research, we investigated how consumption of pequi pulp and nuts, when offered to female rats during the initial phases of life, can affect the brain functions of their offspring. In the offspring of mothers who received the pulp, there were reductions in lipid peroxidation in the brain together with anxiolytic-like behavior. Anxiety behavior was assessed using the open field, elevated plus maze, and light/dark transition box. From a nutritional point of view, pequi pulp and nuts present high lipid content, comparable to that of avocados [46]. When compared to the nuts, the pulp presents more essential fatty acids, and higher levels of phenolic compounds, antioxidants, and carotenoids [47]. Because of its distinctive flavor and it being a source of lipids and antioxidant vitamins (A and E), the pulp and nuts (edible oil portions of fruit) are used as food and in the preparation of sauces and spices in regional dishes, and substituting for other sources of lipid [48].

Composition of pequi pulp fatty acids was analyzed and the predominance of two fatty acids, oleic and palmitic, was found. Oleic acid is an unsaturated fatty acid. Along with other unsaturated fatty acids, it makes up more than 50% of the lipids present in pequi, together with palmitic acid. Palmitic acid is a saturated fatty acid and the most abundant in this class, with more than 20% of the saturation present in these lipids. Oleic acid is an antioxidant that decreases oxidative stress and, consequently, promotes protection against DNA damage, besides having a healing effect [48].

In the open field test, we observed increased ambulation in the offspring of dams that consumed pequi pulp, yet less rearing compared to the controls; there were a higher number of rearings compared to the nuts group. Thus, the pequi pulp induced anxiolytic-like behavior in the rat offspring. The work of Ref. [49] reported on the effects of maternal consumption of unsaturated lipid diet sources such as pequi pulp on alteration of brain function in the offspring. A clinical study with women, found that consumption of monounsaturated and polyunsaturated fatty acids was inversely related to anxiety scores [50].

Analyzing locomotor activity and the exploration rate of the animals in the center of an open field, characterizing exploratory activity, the animals whose mothers received pequi pulp presented greater locomotion and greater affinity for the center of the apparatus than the other groups. Pequi pulp is rich in phenolic compounds, as other studies have demonstrated, phenolic compounds can induce anxiolytic effects [51,52]. Therapeutic effects against neurodegenerative disease have also been reported [53]. Maternal consumption of an extract of açai seeds reverted anxiety-like behaviors induced by periodic maternal separation from offspring. The tested offspring spent more time in the central area of the open field [54]. Açai seeds are a source of phenolic compounds, as is the pequi fruit.

For comparison, adult rats treated with olive oil presented greater exploratory activity in an open field [55]. Olive oil is a good source of monounsaturated fatty acids, like the pequi. Both the pulp and the pequi nut are sources of monounsaturated fatty acids (MUFA); on the other hand, the proportion of polyunsaturated fatty acids (PUFA) of these two matrices are different (Pequi pulp showed 9.64% and Pequi nuts 2.39% of PUFA). This suggests that not only the MUFA levels, but the combination of MUFA and PUFA present in the pequi pulp, could have interfered in the improvement of the brain functioning of the offspring and in the induction of the anxiolytic-like behavior effect.

PUFA can be transferred through the placenta to the fetus, which has a limited capacity to synthesize these fatty acids [56]. So, the mother's organism is essential in the conversion of essential fatty acids into fatty acids that are important for the proper brain functioning of the offspring, such as docosahexaenoic acid (DHA) [57]. Researchers have already confirmed that maternal diet influences the composition of fatty acids in breast milk [58,59] with consequent deposition in the brain tissue of the offspring in rats.

Research evaluating the effects of composition of fatty acids in different areas on the behavior of young and old mice has observed that DHA was higher in young animals and arachidonic acid (AA) lower in animals that had better memory performance [60]. Animals treated with goat milk fat during pregnancy and lactation observed greater deposition of DHA and AA in the brain of their adolescent offspring resulting in better memory performance [59] and reduced anxiety [49]. In the present research, we did not measure composition of cerebral fatty acids in the offspring. As the pequi pulp presented a higher amount of PUFAs compared to the nut group, it may have caused a greater deposition of these fatty acids in the brain of the pulp group compared to the nuts group, thus justifying the anxiety-like behavior.

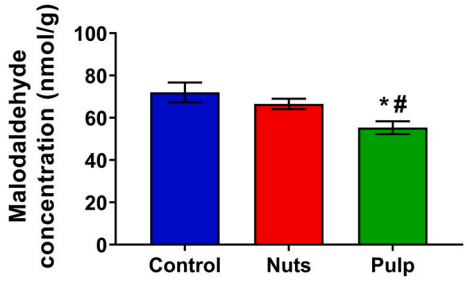

Fig. 5. Malonaldehyde concentration (nmol/g). Data expressed as mean ± SD, n = 10 per group (One-way ANOVA); ($p < 0.05$), *versus control group, #versus almond group.

In the present study, analysis of the data collected from the elevated plus-maze tests indicated that the offspring in the experimental groups entered the open arms of the apparatus more often, with no difference in permanence between them. However, regarding permanence in the central area of the apparatus, the animals in the pequi pulp group stayed for a longer time. Supporting this, Ref. [49] also observed that animals whose mothers had received a diet with conjugated linoleic acid (CLA) presented more entries and greater lengths of stay in the open arms of the EPM, and longer times in its central area, revealing the anxiolytic effect induced by maternal CLA consumption. Ref. [61], identified that the lipids present in goat milk provide an anxiolytic effect on the offspring of rats supplemented during pregnancy and lactation. Ref. [62] observed decreased anxiety behaviors in the offspring of rats treated with fish oil. These findings confirm that a maternal diet of essential fatty acids, such as those found in the pequi, consumed in the period of fetus neuronal formation and development can reduce the offspring's anxiety behavior in adulthood [63]. Fatty acids, such as those found in the pequi fruit, bind to a receptor that is associated with chloride channels in the brain, gamma-aminobutyric acid-A ($GABA_A$), which act to help control anxiolytic behavior [64,65].

A similar result to the present research was observed when animals were treated with hibiscus extracts (a source of flavonoids and phenolics) [66], and theaflavins (TF) [52]. These animals spent more time on the open arms of the EPM. Both pequi pulp and nuts are sources of flavonoids, phenolic compounds, and carotenoids. The pulp presents a greater amount of these bioactive compounds, which could explain the longer times spent by the animals in the center of the EPM. Flavonoids are found in many plants and can improve cognitive function and promote antioxidant activity [67]. Ref. [52] found that theaflavins can be metabolized by the intestinal microbiota into gallic acid, which is involved in promoting anxiolytic-like behavior. In the present research we did not measure organic acids as produced by intestinal microbiota in the pequi-treated animals, but we suggest that the results are based on the flavonoid levels found in the pequi fruit. However, further studies may be necessary to elucidate the mechanism.

In the light and dark box tests used in the present study, the animals in the pulp group presented a greater number of entries and permanence in the lighted compartment. In offspring whose mothers were treated with CLA, Ref. [49] observed a higher number of animal entries into the lighted area, but no greater permanence. The test using the light/dark box is based on the innate aversion that rodents have for lighted areas, and on their spontaneous exploratory behavior in response to mild stressors [68]. This seems to be mitigated by a diet containing essential fatty acids which protect brain tissue during its formation and prevent and/or reduce anxiety [69].

Studies have shown that high levels of anxiety are directly related to increased oxidative stress in the brain [15,16,70]. Oxidative stress is an imbalance between oxidants and antioxidants that causes cell damage. This imbalance can generate damage to lipids, proteins, carbohydrates, nucleic acids and other oxidizable substances [71]. Lipid peroxidation of brain tissue can induce tissue apoptosis with consequent changes in brain plasticity, neurodegeneration and brain damage [72]. This is a consequence of oxidative stress, since it promotes a chain reaction in polyunsaturated fatty acids (in cell membranes), affecting their integrity, fluidity, and permeability [73]. Brain membrane lipids are very rich in polyunsaturated fatty acids, which explains the susceptibility of brain tissue to lipid peroxidation [74]. Fatty acids and antioxidant compounds can promote a reduction in lipid peroxidation [75,76]. This protective activity can prevent brain damage and neurological disorders such as anxiety [77].

Malonaldehyde is a product of main chain reactions related to polyunsaturated fatty acid oxidation. Studies show that malonaldehyde presents cytotoxic and genotoxic activity, and is found in high concentrations in pathologies associated with oxidative stress [72,78].

In the present research, maternal consumption of pequi pulp was able to induce MDA reductions in the offspring's brain. The results from the pequi nuts group were similar to results from the control group in that anxiety-like behavior was not affected. A study inducing anxiety with periodic maternal separation in the offspring of rats treated with *Euterpe oleracea* Mart. (Açaí) seed extract, observed a reduction in MDA levels and catalase, and an increase in superoxide dismutase in the offspring's brain [79]. The extract served as an antioxidant defense in the central nervous systems of the offspring, similar to the pequi pulp studied in the present work.

Studies performed with different sources of polyunsaturated fatty acids have also reported protective effects against lipid peroxidation. Campos et al. [80], tested different concentrations of Omega 3 in mice, and observed a reduction in malonaldehyde (MDA) levels in brain tissue. Ref. [81] verified that a diet low in PUFAs, but rich in carbohydrates, induces a decrease in the levels of antioxidants in animal serum. As found in the present study, a work investigating the effects of a diet source of CLA offered during early life showed induced reductions in MDA in the offspring's brain tissue [49]. As was also found by Ref. [81], Ref. [82] reported that foods rich in sugar can induce anxiety-like behavior. On the other hand, the dietary sources of unsaturated fatty acids employed by Ref. [49] during animal early life, caused a reduction in lipid peroxidation with a consequent reduction in anxiety in the offspring. This occurs because stress alters both the composition and functioning of brain cell membranes, leading to a reduction in dendritic branching in the cingulate gyrus, hippocampus, amygdala regions, and prefrontal cortex, promoting anxiety and depression-like behaviors [18].

Thus, the effect of reducing MDA, with its consequent reduction in anxiety, is understandable in the group treated with pequi pulp. Pequi pulp is rich in unsaturated fatty acids, carotenoids, phenolic compounds, and flavonoids, which significantly increase its potential antioxidant activity [83]. A study observed that β-carotene efficiently prevents oxidative damage in human erythrocytes through reduction of both lipid peroxidation and hemoglobin oxidation [84]. Ref. [66] treated animals with hibiscus and found anxiolytic-like behavior. In tests of its *in vitro* antioxidant and free radical scavenging activities, hibiscus inhibited linoleic acid oxidation and scavenged the DPPH (1,1-difenil-2-picrilhidrazil) radical. The authors attributed these findings to the flavonoids and phenolics identified in the hibiscus. Reductions in oxidative stress and free radical injury can prevent various pathological conditions, including neurodegenerative disease.

Our study observed that the composition of the pequi pulp was different from that of the nut and that its biological effects on offspring were also different when consumed by the mother during pregnancy and lactation.

A limitation of the study was that it was not possible to determine other antioxidant brain enzymes in addition to MDA and the fatty

acid profile of the offspring's brain.

5. Conclusion

Maternal consumption of pequi pulp during the initial phase of life induces a reduction in anxiety behavior and in the cerebral lipid peroxidation in the offspring of rats during adolescence.

This same effect does not happen when the animals were treated with pequi nuts, probably because the volume of bioactive compounds and antioxidants in pequi nuts is much lower than in pequi pulp.

Future research can be carried out to investigate how maternal feeding with pequi can interfere with other behavioral parameters in the offspring, such as memory and depression.

Author contribution statement

Conceived and designed the experiments: Suedna Costa, Michelly Queiroz, Juliana Soares.
Performed the experiments: Suedna Costa, Jany Santos; Maria Lúcia Oliveira, Paloma Cristina.
Analyzed and interpreted the data: Suedna Costa, Juliana soares, Mayara Barbosa, Larissa Dutra.
Contributed reagents, materials, analysis tools or data: Vanessa Bordin, Gerlane Guerra, Daline Araújo, Juliano Rufino.
Wrote the paper: Suedna Costa, Juliana Soares, Marília Frazão, Larissa Dutra.

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Declaration of competing interest

The authors declare no known conflict of interest.

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