

## Artículo Original / Original Article

### Cafeteria diet in breastfeeding dams promotes anxiolytic effects, accumulation of adipose tissue, and impacts offspring development

### La dieta de la cafetería en las madres que amamantan promueve los efectos ansiolíticos y la acumulación de tejido adiposo, y afecta el desarrollo de la descendencia

#### ABSTRACT

The aim of this study was to evaluate the nutritional and behavioral effects of a cafeteria diet in dams during the breastfeeding period and in their offspring from weaning until early adulthood (70 days old). Pregnant Wistar rats were fed a chow diet until delivery. Postnatally (D0), litters were culled to 8 pups and lactating dams received control (CTRL  $n=6$ ) or cafeteria (CAF  $n=6$ ) diets and water ad libitum. At the end of the breastfeeding period, male offspring were placed in individual boxes receiving the same treatment from their respective dams (CTRL or CAF) until adulthood (70 days). All nutritional and behavioral evaluations were performed with the dams ( $n=12$ ) during the breastfeeding phase and with the male offspring ( $n=24$ ) after weaning to adulthood. CAF dams demonstrated a lower caloric and protein intake; higher intake of fats; loss of weight; greater accumulation of adipose tissue; and an anxiolytic effect. CAF male offspring showed lower caloric intake; higher intake of fats; and accumulation of adipose tissue. In addition, these animals continued to have decreased body weight, body length and tibia-femur length in relation to CTRL. In dams, a cafeteria diet promoted alterations in body composition and anxiety, and in offspring the diet resulted in adequate development.

**Keywords:** Cafeteria diet; Food behavior; Marble burying test; Nutritional evaluation; Plus-maze test.

#### RESUMEN

El objetivo de este estudio fue evaluar los efectos nutricionales y de comportamiento de la dieta de la cafetería en las madres durante el período de lactancia materna y en su descendencia desde el destete hasta la edad adulta temprana (70 días de edad). Ratas Wistar embarazadas fueron alimentadas con una dieta estándar hasta el parto. Postnatalmente (D0), las camadas se ajustaron en 8 crías y las madres lactantes recibieron las dietas control (CTRL  $n=6$ ) o cafetería (CAF  $n=6$ ) además de agua ad libitum. Al final del período de lactancia materna, las proles machos fueron colocados en cajas individuales recibiendo el mismo tratamiento de sus respectivas madres (CTRL o CAF) hasta la edad adulta (70 días). Todas las evaluaciones nutricionales

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y comportamentales se realizaron con las madres ( $n=12$ ) durante la fase de lactancia y con la prole masculina ( $n=24$ ) después del destete hasta la edad adulta. Las madres CAF demostraron una menor ingesta calórica y proteica; mayor ingestión de grasas; pérdida de peso; mayor acumulación de tejido adiposo; y un efecto ansiolítico. La prole masculina CAF presentó menor consumo calórico; mayor ingestión de grasas; y la acumulación de tejido adiposo. Además, estos animales presentaron menor peso corporal, longitud corporal, y longitud de la tibia-fémur, en relación a CTRL. En las madres, la dieta de cafetería promovió cambios en la composición corporal y ansiedad, y en la prole la dieta comprometió el desarrollo adecuado.  
**Palabras clave:** Comportamiento alimentario; Dieta de la

*cafeteria; Evaluación nutricional; Laberinto en cruz elevada; Prueba de enterramiento de mármol.*

## INTRODUCTION

The breastfeeding period is one of the most nutritionally demanding phases for the mother during offspring development, thus, a balanced diet during the postpartum period is critical to promoting maternal health. However, recent research suggests that good eating habits developed during gestation (balanced prenatal diets) and altered in breastfeeding may have an impact on health in short and long term<sup>1,2,3</sup>.

Animal studies indicate that dams treated with hypercaloric diets during breastfeeding experience changes in milk composition<sup>4</sup>, accumulation of adipose tissue<sup>5</sup> and behavioral disorders<sup>6</sup>. Besides complications to the mother's health, there are also direct associations between maternal nutrition and the full development of the offspring at the cellular, tissue and behavioral levels. In humans, an unbalanced diet during breastfeeding can predispose mothers and offspring to chronic non-communicable diseases such as type 2 diabetes, high blood pressure, cardiovascular disease and cancer<sup>3</sup>.

Dams treated with cafeteria diets (a hypercaloric model diet) during breastfeeding, demonstrate higher levels of triglycerides and lower levels of proteins in breast milk (in relation to dams with a standard diet), factors that lead offspring to increased adult adipose tissue, deficits in body development<sup>5</sup>, anxiolytic effects<sup>7</sup> and changes in eating behavior<sup>8,9</sup>.

Periods involving breastfeeding and post-breastfeeding are also seen as being particularly sensitive to promote the programming feeding behavior. In this context, Bayol et al.<sup>10</sup> identified that the offspring of rats treated during the breastfeeding phase with a cafeteria diet had a higher preference for foods high in sugars and fats in adult life besides higher food and energy intake<sup>5,8</sup>. Nonetheless, most of the studies deal with the ingestion of hypercaloric diets only during breastfeeding, and there is a lack of data that deals with the consumption of this type of diet starting during the suckling period and continuing into the adulthood of the offspring. Therefore, we hypothesize that if animals are fed a hypercaloric diet from lactation to adulthood, there may be a feeding programming for the development of hyperphagia.

The objective of the present study was to evaluate the nutritional and behavioral effects of a hypercaloric diet (cafeteria diet) in dams during the breastfeeding phase and in their offspring up to adulthood.

## MATERIALS AND METHODS

### Ethical considerations

The project was developed in the Laboratório de Nutrição Experimental (LabNutrex) of Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM) in Brazil. Management and euthanasia was carried out according to the ethical

principles of animal use<sup>11</sup>. The Ethics Committee on the Use of Animals of UFVJM approved this study (protocol 043/15).

### Animals and diets

All Wistar rats (*Rattus norvegicus*) used in this study were from LabNutrex/UFVJM. They were housed in conditions of natural moisture; with a temperature of 22±2 °C (controlled by air conditioning); and a 12-hour cycle of light and darkness, with the light cycle beginning at 6:00 a.m.

Initially, Wistar female rats (80 days old) housed in pairs or trios were placed for mating with males (2-3 females per male). These animals received a standard diet (Nuvilab<sup>®</sup>) during the mating and gestation phases. After delivery (mean weight of all dams: 259.09 ± 9.26 g), litters of up to 8 pups (6 male and 2 female) were culled. This was considered "day 0" (D0) and the beginning of the experiment, at which time the dams were divided to receive one of the following diets during the breastfeeding period (D0-D21):

- Control (CTRL) - received lab chow (Nuvilab<sup>®</sup>) and water ad libitum (n= 6);
- Cafeteria (CAF) - received a cafeteria diet and water ad libitum (n= 6).

Lab chow Nuvilab<sup>®</sup> is a commercial chow intended for the feeding of small rodents, obtained from Quimtia S/A, Paraná, Brazil with a final energy density of 342.96 kcal per 100 g. The cafeteria diet consisted of 3 parts lab chow (Nuvilab<sup>®</sup>), 2 parts chocolate (Bel<sup>®</sup>), 2 parts ground, roasted peanuts (Pachá<sup>®</sup>) and 1 part cornstarch biscuit (Aymoré<sup>®</sup>) with a final energy density of 474.00 kcal per 100 g.

The chocolate, ground, roasted peanuts and biscuits were bought from the local market. The cafeteria diet was prepared weekly. All components were powdered, mixed and placed in stainless steel containers inside the cages of the animals.

Weaning occurred on D21, at which time male offspring were placed in their individual cages, receiving the same diet initially offered to their respective dams (CTRL dams – CTRL male offspring; CAF dams – CAF male offspring). All nutritional and behavioral evaluations were performed with the dams (n= 12) during the breastfeeding period (D0-D21), and with the male offspring (n= 24) after weaning until adulthood (D21-D70).

### Nutritional evaluations

Throughout the experiment all animals were weighed weekly. During the breastfeeding period the dams and litters were weighed, and the weight gain of both were determined by calculating the difference between the final weight (D21) and initial weight (D0). After weaning, the male offspring were weighed individually, and weight gain was determined by the difference of weight between D70 and D21.

The food (lab chow and cafeteria diet) was weighed daily to obtain the food and caloric intakes. The feed efficiency was obtained from the ratio of weight gain from the total food intake. All of these evaluations were made in dams

during the breastfeeding period (D0-D21), and on male offspring after weaning (D21-D70) in the morning period (08:00 to 12:00 am).

On D22, dams were anesthetized using xylazine (20 mg/kg) and ketamine (40 mg/kg) and euthanized by exsanguination. Body length was determined by the distance from the snout to the anus of the animal. The tibia and femur were removed, weighed and the length measured with a digital pachymeter. Thereafter, these bones were muffled for a period of 4 hours at 550 °C for the incineration of organic matter and the total mineral content obtained<sup>12</sup>.

The organs (heart, kidneys, liver, spleen, adrenal glands) and abdominal adipose tissue (visceral and retroperitoneal) were removed. Relative organ weights were calculated using the following equation: (weight of organ or adipose tissue/final body weight) × 100. The Lee index was calculated by using the equation: ( $\sqrt[3]{\text{body weight}}$ )/body length. Males were euthanized on D70 and the same procedures and collections were carried out, with the addition of the epididymal fat to the abdominal fat.

### **Behavioral tests**

Dams were tested after weaning (D21) in the elevated plus maze and marble burying test. Male offspring were tested in same tests on D69. All tests were performed in a double-blind procedure, in an isolated room (light of 150 lux) in the LabNutrex (between 08:00 and 12:00 am).

Among males, groups were subdivided to perform the tests. Half of the animals in each group (n= 6) received intraperitoneal saline treatment (CTRL-S and CAF-S), while the others received a dose of diazepam 2.0 mg.kg<sup>-1</sup> (CTRL-D and CAF-D) under the same conditions 30 minutes prior to testing, both at volumes of 1.0 ml.kg<sup>-1</sup>.

The elevated plus maze consists of an apparatus made of wood with two closed arms (50 × 10 × 40 cm) perpendicular to two open arms (50 × 10 cm) and a central area (10 × 10 cm). The four arms are also raised to a height of 50 cm above the floor of the room. The procedure followed was described by Pellow et al.<sup>13</sup>, where the animals were taken to the behavior room 10 minutes prior to the test for acclimatization. Each animal was placed in the maze with its head facing one of the closed arms and its movements filmed for 300 seconds (Handcam Sony®). At the end of each test, the maze was cleaned with 70° alcohol to remove any olfactory signals.

The elevated plus maze test is an animal model based on the characteristic fear rodents have for open spaces and heights. When rodents are exposed to the apparatus, there is a paradigm between the tendency to explore the new space and its aversion to open and/or high environments. In general, they tend to avoid the open arms where they feel more exposed, preferring to stay within the closed arms<sup>13</sup>. The number of entries, defined by when the animal enters with all 4 paws, and time spent in each arm were evaluated by a trained observer. The ratios of entries and time spent were calculated.

After completing the elevated plus maze test, the same animals were subjected to the marble burying test. This test is based on the observation that rodents will bury aversive stimuli, such as inanimate objects, placed inside in their cages on their bedding. A greater number of buried objects can be understood as a greater degree of anxiety in the animal<sup>14</sup>. The protocol adopted by Almeida et al.<sup>14</sup> was followed, with modifications: twenty-five marbles were evenly distributed in a polyethylene box with dimensions 46 cm × 31 cm × 16 cm. The floor covered with 5 cm of sawdust and the rat exposed for 30 minutes. A ball was only considered buried if at least 2/3 of it was below the sawdust.

### **Statistical analysis**

All results are shown as mean ± standard deviation. Statistica® 10.0 was used to analyze the data and GraphPad Prism® 7.0 for preparing the figures. One-way analysis of variance (ANOVA) was used to evaluate the differences between diets, and repeated measures when the variables diet and time were analyzed. The Newman-Keuls test was used when necessary (p < 0.05).

## **RESULTS**

### **Breastfeeding period (D0-D21)**

The evaluations of the dams' mean weekly body weight showed a diet effect (p < 0.05), with the CAF group weighing less than CTRL, and a time effect (p < 0.001), where D7 and D14 presented higher body weights than D0 and D21. A diet-time effect was also identified for the body weight of dams (p < 0.05). The evaluation of D21 CAF dams showed lower body weights than the CTRL group (Figure 1A).

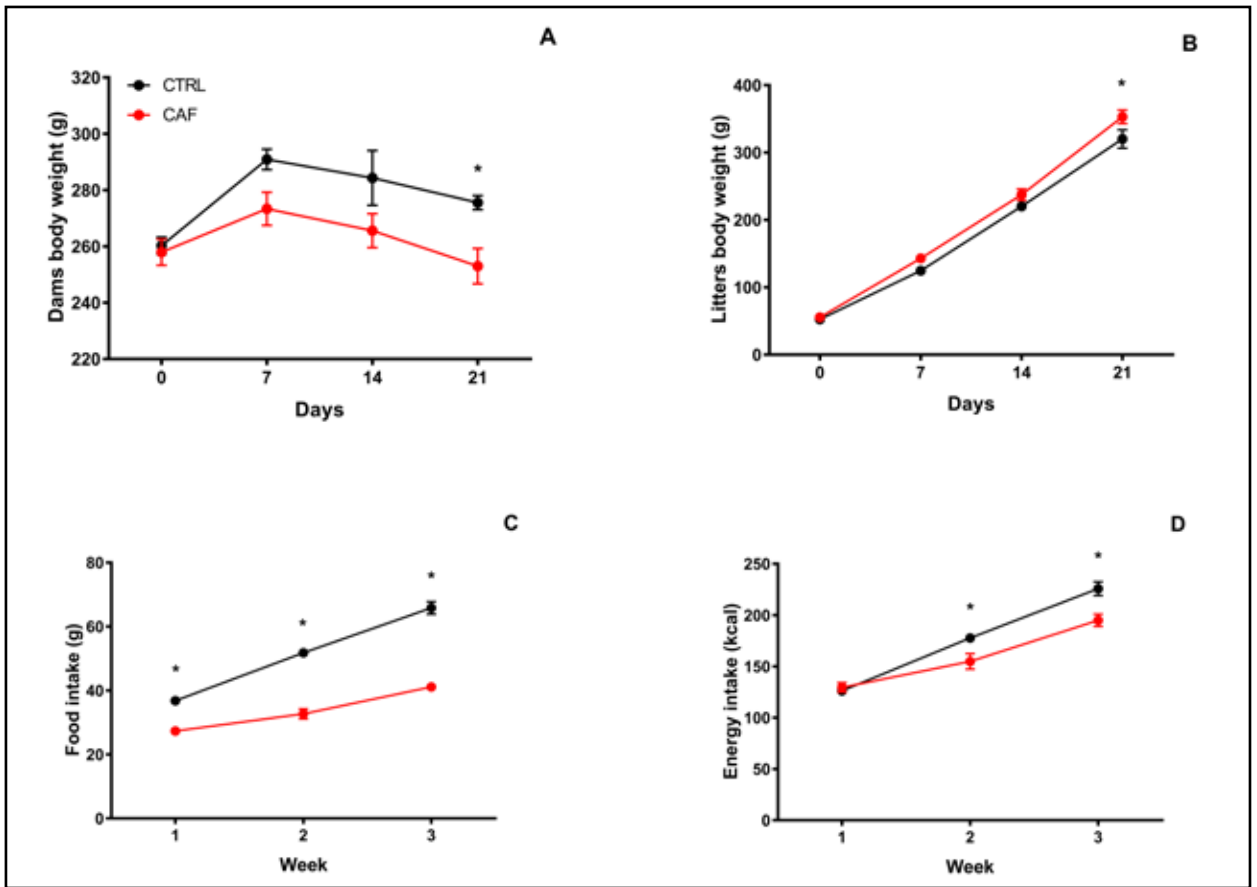
A time effect was observed with the litters' body weight (p < 0.001). Animals showed progressive weight gain during the three weeks of breastfeeding. A diet-time interaction was observed (p < 0.01), where CAF litters weighed more than CTRL on D21 (Figure 1B).

For the analysis of dams' food intake, a diet effect was observed (p < 0.001) with the CAF group being lower than CTRL, and a time effect (p < 0.001) where the animals consumed the least food in the first week, and progressively more in the second and third weeks. A diet-time effect was observed (p < 0.001), where the CAF group consumed less food than CTRL group during the three weeks (Figure 1C).

Energy intake of dams showed a diet effect (p < 0.05), where the CAF group intake of energy was lower than CTRL. A time effect also was observed (p < 0.001), whereby the animals increased the number of calories ingested over time. Finally, a diet-time effect (p < 0.001) confirmed that the CAF animals consumed less energy than CTRL in the second and third weeks (Figure 1D).

For the dams, the CAF group had significantly lower values than the CTRL group for total food intake (p < 0.001), weight gain (p < 0.001) and feed efficiency (p < 0.01). However, the CAF group presented a greater degree of weight gain in litters compared to CTRL (p < 0.05) (Table 1).

For dam body length (p < 0.05), CAF was lower than CTRL.



**Figure 1:** Body weight of dams (A) and litters (B); Food (C) and energy intake (D) of dams during the breastfeeding period. Legend: Control group (CTRL) (n = 6) and Cafeteria group (CAF) (n= 6). The symbol \* indicates a statistically significant difference between the groups by the Newman-Keuls test ( $p < 0.05$ ). In graph B, litter weight corresponds to the weight of the 8 pups (6 males and 2 females).

The length of tibia, femur and Lee index did not show any difference as well as for the relative organ weights of dams (heart, kidneys, liver, spleen and adrenal glands) (Table 1).

When the relative weight of abdominal adipose tissue was evaluated, a significant difference between groups was observed ( $p < 0.001$ ), where the CAF dams had a greater accumulation of abdominal fat (visceral and retroperitoneal) compared to CTRL (Table 1).

The evaluation of the ratio of entries in the closed arms (Figure 2A) and open arms (Figure 2B), as well as the ratio of time spent in each of these arms (Figure 2C and 2D) did not show statistical differences between the dams. In addition, no statistical difference was observed for the total number of entries and total time spent in each arm (data not shown).

The marble burying test performed by dams showed a statistically significant difference between the groups ( $p < 0.001$ ). The CAF dams buried fewer balls than CTRL (Figure 2E).

### Weaning to adulthood (D21-D70)

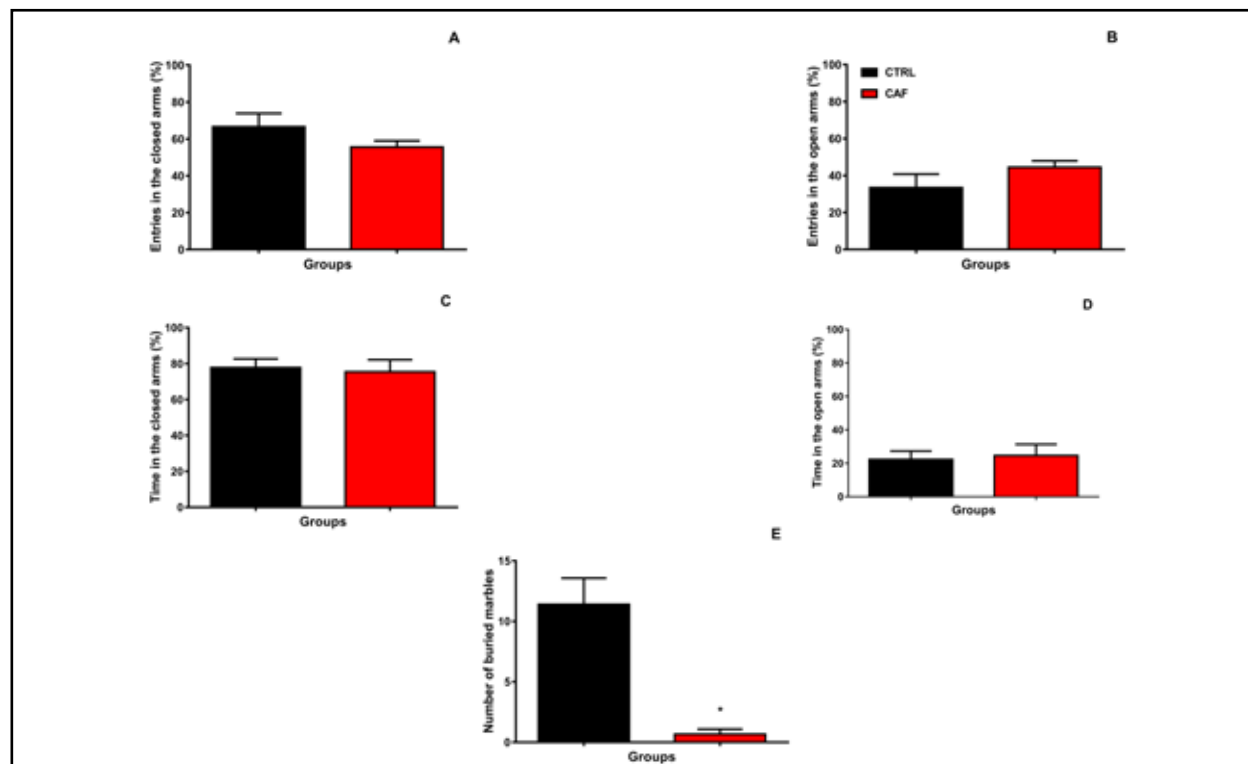
There was a diet effect in the mean weekly body weight values of male offspring after weaning ( $p < 0.001$ ). The CAF group presented lower weight than the CTRL group. For the time factor, a statistically significant difference ( $p < 0.001$ ) was identified, where the animals showed progressive weight gain from D21 to D70. An effect on diet-time interaction has been shown for male offspring body weight ( $p < 0.001$ ) as the CAF group presented lower weight than the CTRL group from D35 to D70 (Figure 3A).

The food intake of male offspring showed effects of diet ( $p < 0.001$ ) and time ( $p < 0.001$ ). The CAF animals consumed less food, with the consumption of all animals increasing from the first to the fourth week, stabilizing during the fifth and sixth weeks, and increasing again in the seventh week. There was also a correlation between diet and time factors ( $p < 0.001$ ), where the CAF group exhibited lower food intake from the second to the seventh week (Figure 3B).

**Table 1.** Nutritional assessment and relative organ weight of dams during the breastfeeding period.

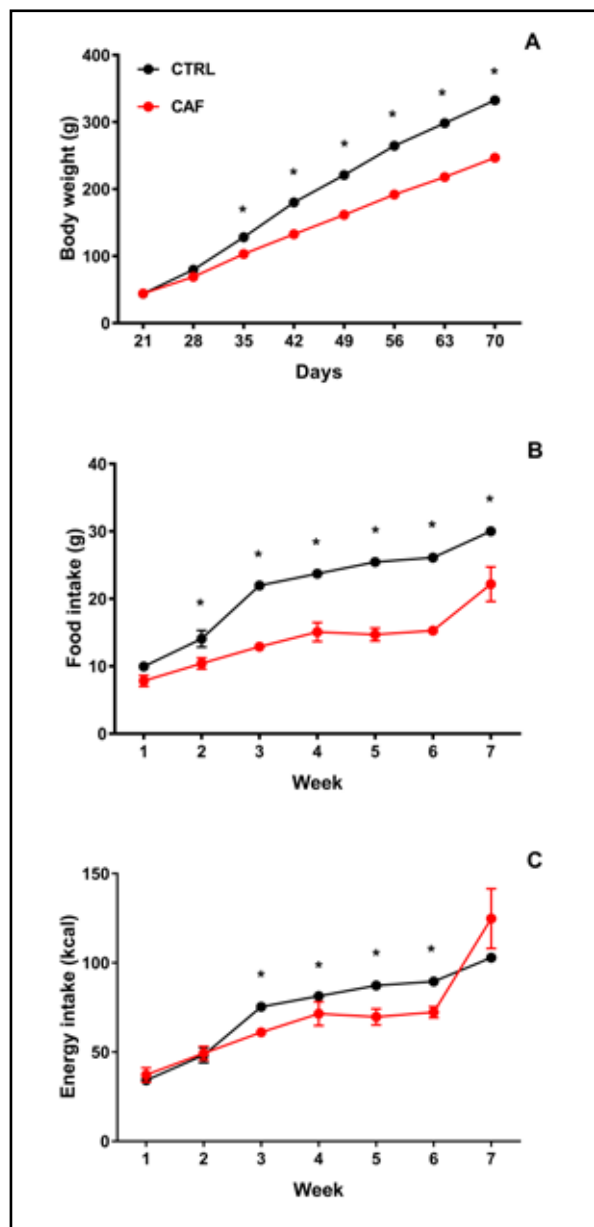
Variables	CTRL Nutritional assessment	CAF
Total food intake (g)	1030.23 ± 42.70	699.63 ± 51.31*
Total caloric intake (kcal)	3533.30 ± 146.44	3316.23 ± 243.22
Carbohydrate intake (kcal)	2013.91 ± 83.47	1317.54 ± 96.63*
Protein intake (kcal)	854.27 ± 35.41	451.96 ± 33.15*
Fat intake (kcal)	664.81 ± 27.55	1546.45 ± 113.42*
Weight gain of dams (g)	15.29 ± 5.61	-4.93 ± 7.72*
Weight gain of litters (g)	268.02 ± 20.93	297.58 ± 24.64*
Feed efficiency (g/g)	0.015 ± 0.00	- 0.006 ± 0.01*
Body length (cm)	22.98 ± 0.43	22.53 ± 0.16
Lee index	0.29 ± 0.01	0.28 ± 0.00
Tibia length (cm)	35.26 ± 0.69	35.58 ± 0.68
Femur length (cm)	31.20 ± 0.33	31.62 ± 0.50
	Relative organ weight	
Heart (%)	0.33 ± 0.06	0.29 ± 0.02
Kidney (%)	0.41 ± 0.04	0.34 ± 0.03
Liver (%)	4.91 ± 0.95	3.83 ± 0.19
Spleen (%)	0.25 ± 0.03	0.24 ± 0.01
Adrenal glands (%)	0.015 ± 0.001	0.017 ± 0.003
Abdominal adipose tissue (%)	1.38 ± 0.37	5.13 ± 0.98*

Legend: Control group (CTRL) (n= 6); and Cafeteria group (CAF) (n= 6). The symbol \* indicates a statistically significant difference between the groups by the Newman-Keuls test (p< 0.05).



**Figure 2:** Ratio of entries in the closed (A) and open arms (B); ratio of time spent in the closed (C) and open arms (D); number of buried marbles (E) by dams on day 21 of breastfeeding. Legend: Control (CTRL) (n= 6); Cafeteria group (CAF) (n= 6). The symbol \* indicates a statistically significant difference between the groups by the Newman-Keuls test (p< 0.05).

The energy intake demonstrated time ( $p < 0.001$ ) and diet-time effects ( $p < 0.001$ ) for male offspring. The animals showed an increase in energy intake from the first to the third week, stabilizing during the fourth to sixth weeks, and increasing again in the seventh week. In the interaction of factors (diet and time), CAF showed higher energy intake only in the seventh week (Figure 3C).



**Figure 3:** Body weight (A); Food (B) and energy intake (C) of male offspring after weaning. Legend: Control group (CTRL) ( $n = 12$ ) and Cafeteria group (CAF) ( $n = 12$ ). The symbol \* indicates a statistically significant difference between the groups by the Newman-Keuls test ( $p < 0.05$ ).

The CAF male offspring exhibited lower weight gain ( $p < 0.001$ ) and Lee index ( $p < 0.001$ ) than the CTRL. The total caloric intake did not show a significant difference, however, for the caloric intake of carbohydrates and proteins, CAF was lower than CTRL, whereas CAF was higher in fat. These evaluations are shown in table 2.

The evaluation of body length ( $p < 0.001$ ), tibia length ( $p < 0.001$ ) and femur length ( $p < 0.01$ ) demonstrated differences between the groups of male offspring. CAF had lower values compared to CTRL for all the aforementioned parameters. CAF also had a greater amount of total minerals ( $p < 0.01$ ) (Table 2).

Relative organ weights showed differences between the heart ( $p < 0.001$ ) and kidneys ( $p < 0.001$ ). Male offspring from the CAF group had higher heart, but lower kidney weights. The relative abdominal adipose tissue indicated a significant difference ( $p < 0.001$ ), and the CAF group had higher fat accumulation in the abdominal region than CTRL (Table 2).

No significant difference was observed for male offspring in the plus maze test for the number of entries and the time spent in the arms (data not shown). No difference was identified in the ratio of entries (Figure 4A and 4B) and ratio of time spent in the arms (Figure 4C and 4D).

The marble burying test also showed no differences between the male offspring groups (Figure 4E).

## DISCUSSION

The cafeteria diet demonstrated in both cases (dams during breastfeeding and male offspring until adulthood) the accumulation of adipose tissue in the abdominal region. For dams, it was effective in promoting an anxiolytic effect, while for offspring a delay in body development was observed.

### Breastfeeding period (D0-D21)

The breastfeeding period is directly related to a high caloric expenditure due to the synthesis of breast milk and the act of breastfeeding itself. This phenomenon, in turn, is linked to weight loss in infants and to the increase of caloric intake in order to compensate for this energy expenditure. These facts are corroborated in the works of Da Silva et al.<sup>15</sup> and De Los Ríos et al.<sup>16</sup>.

According to Słupecka et al.<sup>17</sup>, the breastfeeding phase places a high energetic demand on dams, which is accompanied by an increase in food and, consequently, caloric intake. Jahan-Mihan et al.<sup>18</sup> found similar results for the body weight of dams treated with AIN93-G diet. From the middle of the second week of treatment, the dams began to lose weight. Ong and Muhlhauser<sup>19</sup> treated breastfeeding dams with foods high in fats and sugars and observed greater weight loss in these animals from the second week of breastfeeding, as compared to the control group.

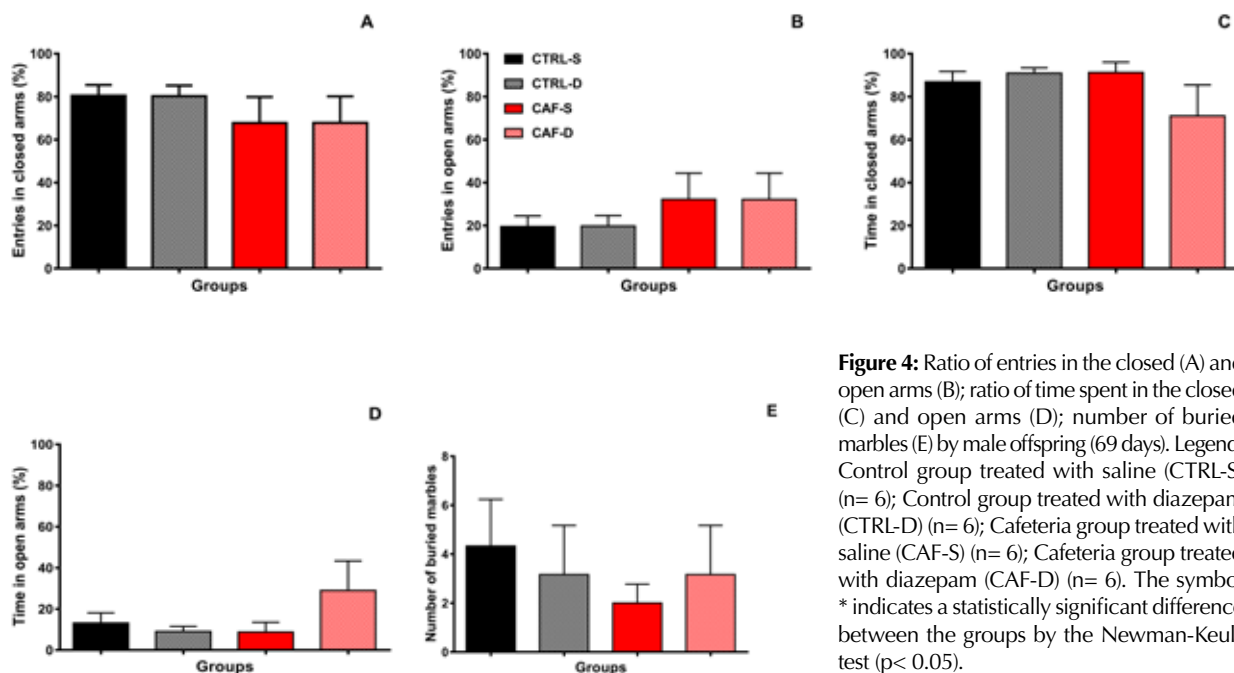
CAF dams experienced intense weight loss and had lower body weight at the end of the breastfeeding stage compared to the beginning. They also demonstrated negative weight gain and feed efficiency. This discrepancy between groups could possibly be due to the amounts of macronutrients ingested by each group. The CAF dams had an unbalanced

**Table 2.** Nutritional assessment and relative organ weight of male offspring.

Variables	CTRL	CAF
	Nutritional assessment	
Total food intake (g)	1079.45 ± 76.73	723.81 ± 103.47*
Total caloric intake (kcal)	3702.10 ± 263.16	3430.88 ± 490.43
Carbohydrate intake (kcal)	2110.12 ± 150.00	1363.09 ± 194.85*
Protein intake (kcal)	895.08 ± 63.63	467.58 ± 66.84*
Fat intake (kcal)	696.57 ± 49.51	1599.92 ± 228.70*
Weight gain (g)	288.35 ± 19.54	202.66 ± 16.82*
Feed efficiency (g/g)	0.27 ± 0.01	0.28 ± 0.04
Body length (cm)	24.20 ± 0.56	22.91 ± 0.53*
Lee index	0.29 ± 0.00	0.27 ± 0.00*
Tibia length (cm)	37.13 ± 0.96	34.92 ± 1.03*
Femur length (cm)	32.31 ± 0.92	30.79 ± 0.87*
Total mineral content of bones (%)**	36.47 ± 3.30	40.23 ± 2.14*
	Relative organ weight	
Heart (%)	0.26 ± 0.01	0.31 ± 0.01*
Kidney (%)	0.38 ± 0.05	0.33 ± 0.02*
Liver (%)	2.88 ± 0.20	2.82 ± 0.25
Spleen (%)	0.21 ± 0.02	0.22 ± 0.03
Adrenal glands (%)	0.010 ± 0.002	0.009 ± 0.002
Abdominal adipose tissue (%)	4.37 ± 0.85	6.45 ± 1.02*

Legend: Control group (CTRL) (n= 12); and Cafeteria group (CAF) (n= 12). The symbol \* indicates a statistically significant difference between the groups by the Newman-Keuls test (p< 0.05).

\*\*tibia and femur



**Figure 4:** Ratio of entries in the closed (A) and open arms (B); ratio of time spent in the closed (C) and open arms (D); number of buried marbles (E) by male offspring (69 days). Legend: Control group treated with saline (CTRL-S) (n= 6); Control group treated with diazepam (CTRL-D) (n= 6); Cafeteria group treated with saline (CAF-S) (n= 6); Cafeteria group treated with diazepam (CAF-D) (n= 6). The symbol \* indicates a statistically significant difference between the groups by the Newman-Keuls test (p< 0.05).



diet, high in lipids and low in proteins and carbohydrates, consistent with the study by Pomar et al.<sup>5</sup>.

Agnoux et al.<sup>20</sup> reported the importance of adequate protein intake for maternal health during the breastfeeding phase. The consumption of protein below the required amount can have consequences not only for the offspring through a change in the composition of the breast milk, but also for the mother herself, since she will have more pronounced weight loss during this period.

Despite consuming fewer calories over the experimental period and having a greater weight loss at the end of 21 days of breastfeeding, CAF dams demonstrated a large accumulation of abdominal adipose tissue. The higher caloric intake of fats may have contributed to this result.

CAF dams presented a high-risk nutritional state. The results indicate that energy expenditure promoted by breastfeeding, associated with high fat and low protein intake, may have led dams in this group to lose lean mass and accumulate adipose tissue.

Similar results were previously reported by Pomar et al.<sup>5</sup> using Wistar rats receiving a varied cafeteria diet of 9 different foods during breastfeeding. At the end of breastfeeding phase, the dams had a higher percentage of fat mass and less lean mass in relation to the control group.

Maternal intake of high-fat foods from the CAF group also led to changes in litter weight. Bautista et al.<sup>4</sup> indicated that hyperlipidic diets can promote changes in the composition of breast milk, and consequently, in the weight and body composition of the offspring.

Pomar et al.<sup>5</sup> found that dams treated with hypercaloric foods produced milk with a high concentration of lipids and a low protein content, similar to the composition of the cafeteria diet used in this study. A similar experiment was carried out by Agnoux et al.<sup>20</sup>, who demonstrated that a diet with a low protein content not only lowered the protein concentration in breast milk, but also reduced the essential amino acid levels and increased the free fatty acid concentrations.

Bautista et al.<sup>4</sup> found comparable results treating lactating rats with a hyperlipidic diet (20% lard addition). Dams fed the high fat diet demonstrated similar total caloric intake, but increased fat intake and accumulation at the end of the breastfeeding period. This feeding promoted a greater weight gain and adiposity in the pups.

Thus, the greater weight gain observed in the CAF litter in the present study may be related to an increase in adipose tissue in these animals, derived from the substantial amount of lipids in breast milk.

Mice treated for 4 weeks with a lard-added diet (39%) obtained an anxiolytic effect, proven by a lower mean number of spheres hidden in relation to the control group in the marble burying test<sup>21</sup>. Ohland et al.<sup>22</sup> also found decreased anxiety in mice treated with a diet rich in sugars and fats for 3 weeks.

Approximately 8-12% of human mothers in the post-gestational period develop some anxiety disorder<sup>23</sup>. The

literature confirms anxiogenic effects in mothers during breastfeeding in Wistar rats<sup>24</sup>. This effect is associated with negative outcomes for the offspring, including decreased breastfeeding and interaction between mother and offspring; inadequate physical and nutritional development for the offspring; negative offspring temperament; atypical neurodevelopment and, later, emotional and behavioral problems for the offspring<sup>23,25</sup>.

Foods like chocolate and peanuts are sources of tryptophan, which in turn plays a key role in the cascade of serotonin synthesis events. Thus, the ingestion of these palatable foods, also popularly called "comfort foods", can stimulate serotonergic production, leading to increased pleasure and producing the anxiolytic effects observed in CAF dams in marble burying test<sup>22,26</sup>.

Browne et al.<sup>27</sup> indicate that diets with high tryptophan content may induce behavioral improvements that are associated with the serotonergic system. Similarly, Stamatakis et al.<sup>24</sup> also correlate the decrease in anxiety with changes in the amount of 5-HT<sub>1A</sub> type receptors and the improvement in maternal behavior. Thus, the results of CAF dams demonstrate a decrease in anxiety, which, in contrast to the deleterious results that were presented for this group in the nutritional evaluation, suggests a positive effect of the cafeteria diet on maternal behavior.

### Weaning to adulthood (D21-D70)

After weaning, male offspring increased their food and caloric intake by the fifth week, due to the growth phase they were in. Regarding the CTRL group, CAF offspring did not present a higher food/caloric intake at any time until adulthood (D70).

Souza et al.<sup>28</sup> used a cafeteria diet similar to the present work (chow, chocolate, peanuts and biscuits) in rats and found similar results. From the third week of their study, the CAF offspring group showed lower food and energy consumption than the group that received the standard diet.

Disturbances in the energetic metabolism of the offspring were shown in other studies using hypercaloric diets during the breastfeeding phase<sup>5,8</sup>. In the present study, it was shown that although the dietary intake of the CAF offspring remained below the CTRL group during the experimental period, in the last week of treatment these animals showed a higher average caloric intake than the CTRL.

The lower food intake presented by the CAF offspring in relation to the CTRL is related to the higher energy density of the cafeteria diet (474.00 kcal) in comparison to the lab chow (342.96 kcal). These results were also presented by Souza et al.<sup>28</sup>, when working with the same diets.

Nevertheless, the highest mean caloric intake in the last week of the CAF offspring group indicates a possible change in the energy metabolism of these animals, tending to consume a greater amount of energy from the beginning of adulthood. Several studies have linked hypercaloric diets during breastfeeding with changes in breast milk including increased fat and hormones such as leptin, and decreased



amounts of protein, leading to changes in energetic metabolism of offspring in adulthood<sup>5,8,9,10</sup>.

There was an initial hypothesis that the model used (cafeteria diet during and after breastfeeding until adulthood) could lead CAF offspring to a higher caloric intake from the early stages of weaning, since Bayol et al.<sup>10</sup> identified a preference for foods high in sugar and fat when mothers were treated with cafeteria diet during breastfeeding. However, this effect on caloric intake seems to begin in the adult stage, and therefore, a protocol covering a longer period is needed to verify how this alteration in energy metabolism would develop.

Body weight of the CAF offspring was always below the CTRL group, as well as the weight gain of the cafeteria diet group, which was also lower. This may be directly related to the lower food/caloric intake of the CAF group, which result in the lower weight gain.

The low protein intake not only during the breastfeeding phase, but also during the early stages of life, may have provided these animals with developmental delays far more severe than those demonstrated by Pomar et al.<sup>5</sup>. This is proven by the shorter length of the body, tibia and femur, and the lower weight of the kidneys of the male CAF offspring, which collectively shows that these animals did not undergo adequate growth, also resulting in a lower body weight.

Although the effect was greater in CAF offspring, dams also experienced a greater accumulation of abdominal adipose tissue due to a high energy intake from a fatty diet. The accumulation of adipose tissue in the abdominal region, as seen in the CAF offspring is a risk factor for several comorbidities. Excess abdominal fat can cause metabolic changes such as type 2 diabetes mellitus, cardiovascular diseases such as atherosclerosis and acute myocardial infarction<sup>29,30,31</sup>.

The visceral adipose tissue is directly related to the risk of smaller ectopic fat deposits, as in the pericardial and periaortic regions. The increase in relative heart weight in CAF offspring may be due to a deposition of fat on the myocardial tissue, which is associated with a higher risk of developing cardiovascular diseases<sup>32</sup>.

Chaplais et al.<sup>33</sup> performed a meta-analysis, including humans aged 10 to 17 years, assessing the state of bone tissue as a function of the nutritional status of these individuals. The authors demonstrated that in approximately 70% of the evaluated studies there was a correlation between a higher fat mass in children and adolescents and a greater quantity and density of the bone minerals.

However, even with increased bone mineral density, obesity is linked to an increased risk of fractures. A possible mechanism to explain such a relationship could be the absorption of calcium. Free fatty acids consumed in the diet may form complexes with calcium, making them difficult to absorb<sup>34</sup>. Therefore, because of the high fat intake, and although they would have obtained a higher amount of bone minerals in the tibia and femur, CAF offspring may be at a higher risk of developing osteoporosis and fractures.

Another explanation for the greater amount of bone minerals in the CAF group could be through a mechanism involving leptin. This hormone acts on bone mass, functioning as a growth factor for chondrocytes and stimulating growth centers of the skeleton. However, leptin also indirectly acts on the alteration of bone microstructure, which can lead to an increased risk of fractures<sup>35</sup>. As CAF animals had a diet with high fat content and an accumulation in abdominal adipose tissue, it is suggested that the leptin levels of these animals would be elevated, also promoting a greater bone mineralization.

Some studies have already shown that palatable diets can promote anxiolytic states, as well as attenuate anxiogenic conditions originating from stressors in the prenatal and postnatal stages in rodents<sup>36,37</sup>. In a study by Pini et al.<sup>38</sup>, male Wistar rats who were treated with a cafeteria diet from weaning until adulthood showed lower anxiety levels than the control diet group.

The mechanisms responsible for anxiety are complex and involve several systems. However, changes in neurotransmitter circuits have been well documented<sup>39</sup>. Therefore, a low dose of diazepam was administered to the offspring to evaluate the effects of this drug against possible alterations in the neurotransmitter systems of CAF animals. Nevertheless, no results were obtained for the behavioral evaluation against the application of diazepam. The use of drugs with action in other neurotransmitter systems (e.g. serotonergic) and also in older animals is suggested for other studies.

## CONCLUSIONS

CAF dams had higher fat and lower protein intake, which resulted in a high risk change in body composition, with weight loss and accumulation of adipose tissue during breastfeeding. The CAF diet provided an anxiolytic effect in the dams.

In CAF offspring, the cafeteria diet also led to accumulation of abdominal adipose tissue and severe delays in body development but did not promote a hyperphagic state in the offspring.

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