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| ORIGINAL

Composition of a maternal high fat diet rich in satured fats and omega 3 in gestation and lactation for studies with rodents

Composição de uma dieta hiperlipídica materna rica em gorduras saturadas e ômega 3 na gestação e lactação para estudos com roedores

Laura Mata de Lima SILVA[1] 0000-0002-6893-9781
Aline Maria Nunes de Lira Gomes BLOISE[1] 0000-0001-7816-6500
Danilo Augusto Ferreira FONTES[1] 0000-0002-1795-7963
Katarynna Santos de ARAÚJO[2] 0000-0002-7924-8054
Mariana Oliveira BARBOSA[2] 0000-0003-3795-9488
João Henrique COSTA-SILVA[1] 0000-0002-9794-1418

ABSTRACT

Objective

To prepare a high fat diet rich in satured fatty acids and supplemented with omega 3 for experimental studies in rodents.

[1] Universidade Federal de Pernambuco, Centro Acadêmico de Vitória, Laboratório de Nutrição, Atividade Física e Plasticidade Fenotípica. R. Alto do Reservatório, s/n., Bela Vista, 55608-680, Vitória de Santo Antão, PE, Brasil. Correspondence to: JH COSTA-SILVA. E-mail: <joao.hcsilva@ufpe.br>.

[2] Centro de Tecnologias Estratégicas do Nordeste, Grupo de Biotecnologia Branca, Central Analítica. Recife, PE, Brasil.

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Methods

Purified industrial ingredients and flaxseed oil as a source of omega 3 at a concentration of 3.5% (v/w) were used in the elaboration of the diets. Centesimal and nutritional compositions, fatty acids profile and dietary intake were evaluated. Serum levels of total protein, albumin, cholesterol and glucose in pregnant rats were verified. The offspring were assessed with regard to body mass and waist circumference. Statistical analysis was performed using the Kolmogorov-Smirnov, Anova One-Way test and Bonferroni post-test.

Results

High fat and high fat with omega 3 diets presented, respectively, 37% and 36% saturated fat, and the lipid amount was 80% higher than the American Institute of Nutrition 93G control diet. The omega 3 content was 50% higher in the high fat with omega 3 diet. There was no difference in consumption of diet types in weight (grams). The dams that received the High fat diet developed hypercholesterolemia and their High fat offspring exhibited higher body mass on the 1st day of life and increased abdominal circumference on the 30th day of life compared to the control and the high fat with omega 3 offspring.

Conclusion

The formulated diets with a higher amount of saturated fatty acids meet the nutritional requirements of the gestation and lactation period. The high fat diet with omega 3 was able to attenuate the changes observed in dams and their offspring.

Keywords: Alpha-linolenic acid. Fatty acids. Nutritional requirements. Rats.

RESUMO

Objetivo

Elaborar uma dieta hiperlipídica rica em gorduras saturadas e suplementada com ômega 3 para estudos experimentais em roedores.

Métodos

Foram utilizados ingredientes industriais e purificados na elaboração das dietas, e o óleo de linhaça como fonte de ômega 3 na concentração de 3,5% (v/m). As composições centesimal e nutricional, o perfil de ácidos graxos e o consumo das dietas foram avaliados. Verificaram-se os níveis séricos de proteínas totais, albumina, colesterol e glicose das ratas prenhas. A prole foi submetida à avaliação de massa corporal e circunferência abdominal. Na análise estatística, utilizou-se o teste de Kolmogorov-Smirnov, Anova One-Way *e o pós-teste de Bonferroni.*

Resultados

As dietas hiperlipídica e hiperlipídica com ômega 3 apresentaram, respectivamente, 37% e 36% de gorduras saturadas, sendo a quantidade de lipídios 80% superior em relação à dieta controle 93G do American Institute of Nutrition*. O teor de ômega 3 foi 50% maior na dieta hiperlipídica com ômega 3. Não houve diferença no consumo em gramas dos tipos de dieta. As mães que receberam dieta hiperlipídica tiveram hipercolesterolemia e a prole hiperlipídica apresentou maior massa corporal no 1° dia de vida e aumento de circunferência abdominal nos 30 dias de vida em relação ao grupo controle e hiperlipídica com ômega 3.*

Conclusão

As dietas formuladas atendem aos requerimentos nutricionais do período de gestação e de lactação com uma quantidade superior de ácidos graxos saturados. A dieta hiperlipídica com ômega 3 foi capaz de atenuar as alterações observadas nas mães e na prole.

Palavras-chaves: *Ácido alfa-linoleico. Ácidos graxos. Necessidades nutricionais. Ratos.*

INTRODUCTION

Consumption of high fat diets in pregnancy and lactation is associated with cardiometabolic diseases in adulthood [1,2]. Fat content in high fat diets varies among experimental studies, reaching 65% of Total Energy Value (TEV) fat in some diets [1,3,4]. High fat rodent diets can be classified into:

high lipid diets with 30% to 50% TEV and very high lipid diets with over 50% TEV [5]; such values are well above those recommended by the American Institute of Nutrition (AIN), which advocates the supply of 7% lipids in the concentration of the soybean-based experimental diet, which corresponds to 17% TEV, as safe for meeting the needs of essential fatty acids. during growth, reproduction and lactation in rodents [6,7].

Pregnancy is a physiological state that requires adequate intake of essential fatty acids, particularly Docosahexaenoic Acid (DHA), for the development of the fetal central nervous system [8,9]. Supplementation of 200mg DHA from safe sources such as fish rich in this nutrient (herring, tuna and salmon) or from seaweed-based nutritional supplements is recommended for pregnant women to prevent heavy metal contamination such as mercury contamination [9]. Maternal consumption of DHA may also reduce the risk of premature birth in high-risk pregnancies [10]. On the other hand, the use of omega-3 enriched diets during pregnancy and lactation may decrease the expression of genes related to increased adiposity in offspring [11]. A decrease in omega 3 intake with an increased omega 6:3 ratio in the diet may also influence body composition at birth, increasing adiposity. [12].

Fatty acid profile in high fat diets during pregnancy and/or lactation may be a factor related to the development of metabolic disorders in adulthood [13]. Consumption of a diet rich in saturated fatty acids was associated with the development of hepatic steatosis with a higher degree of hepatic impairment, unlike a diet rich in polyunsaturated fatty acids [14]. The excess of saturated fatty acids resulted in altered endocrine pancreas morphometry with lower density of insulin-containing islets, which was not observed in offspring of rats that received the flaxseed high fat diet [3].

Nutritional manipulation with high fat diets during the critical period may imply morphological and functional changes in organs important for endocrine regulation with risk for the development of metabolic diseases in adulthood [3,4,14]. The increased nutritional demands of rodents regarding macronutrients (carbohydrates, proteins and lipids) and micronutrients (vitamins and minerals) during pregnancy, lactation and growth [6,7] sometimes is not considered in the elaboration of experimental diets or in the supply of food to animals by their breeders, especially when using commercial feed and this fact can lead to potential deficiencies.

In the search for a high fat diet pattern with or without omega 3 supplementation, different diets are available, either formulated or commercial diets. The high fat diet model used depends on the type of experimental design, study objectives, research line (*e.g.*, fetal programming or phenotypic plasticity), among others. Nevertheless the diet must meet the demand of different nutrients, considering the life stage of the animal. Conservation and presentation of diets should also be considered. Reduction of dietary intake due to palatability may jeopardize animal growth and development, resulting in lower birth weight and lower pregnancy rates among females [15].

Westernized high fat diet formulation should consider the nutritional requirements of rodents, increasing lipid supply without hampering other nutrients. Diet development also involves steps, such as: selection of ingredients, preparation and pelletization, which may impair quality and acceptance of the feed by the animal, especially regarding the proper texture, jeopardizing the development of research. Westernized experimental diets used in a study with rodents, the Cavalcante *et al.* [16] diet, based on data from the Family Budget Survey of the *Instituto Brasileiro de Geografia e Estatística* (Brazilian Institute of Geography and Statistics/Brazil 2002-2003) and the recommendations of AIN 93G [6,7], demonstrates the consumption pattern of the Brazilian population, whose highest caloric intake comes from refined cereals, simple sugars, vegetable oils and animal fats. The diet composition is 49.3% carbohydrate, 31.5% lipid and 19.9% protein, rich in saturated fat. The authors observed

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that the consumption of this diet during pregnancy and lactation promoted an early maturation in puppies' physical characteristics and neural reflexes.

Considering the repercussions of high fat diet in pregnancy and lactation on offspring and the scarcity of studies that address the need for standardization of diets, the present work proposed to elaborate a high fat diet rich in saturated fats, which could reflect the consumption of the Brazilian population and a diet of saturated fats with omega 3, meeting the nutritional requirements of rodents and acknowledging the importance of developing strategies that can reduce the effects of a high fat diet, supporting further studies with animal models.

METHODS

The high saturated fatty acid diet with omega 3 (HFw3) was prepared using the basic ingredients of the high fat diet (HF) described above. Flaxseed oil was used as a source of omega 3 (LinoOil®, Cisbra, *Rio Grande do Sul*, Brazil), hich was previously analyzed for determination of the lipid profile in the *Laboratório de Fitoquímicos e Processos da Central Analitica* (LAFIP/CEAN, Phytochemistry and Processes Laboratory/Analytical Center) of the *Centro de Tecnologias e Estratégias do Nordeste* (CETENE, Northeast Center for Strategic Technologies) (*Recife, Pernambuco*, Brazil). The HFw3 diet also included in its composition soybean oil, besides flaxseed oil, with a total content of 7% vegetable oil, similar to AIN 93G. Initially a formulation with 2.5% flaxseed oil had been proposed, but after analysis of the dietary fatty acid profile, it was observed that a concentration of 3.5% flaxseed oil and 3.5% soybean oil supplied the required amounts of omega 6 indicated in AIN 93G. The preparation of the diet followed the same procedure described above for the HF diet.

The dried ingredients were mixed and sieved three times. Then the fat-sourcing ingredients were added, mixing and sieving again three times. Hot water was added to the end-product to obtain a homogeneous solid mass. After this step, the dough was cut into pellets and dried at 70ºC for 48 hours in a heating and ventilation oven. The diet was prepared at room temperature at 24ºC at the Dietetic Technique Laboratory of the *Centro Acadêmico de Vitória* (CAV) at the *Universidade Federal de Pernambuco* (UFPE).

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The determination of the centesimal composition was performed in triplicate using the Association of Official Analytical Chemists (AOAC) moisture, protein, lipid and ash determination methods [17]. Results were expressed as g/100g diet according to the average of three sample repetitions. The carbohydrate fraction was determined by the difference of the values found for moisture, ether extract, proteins and ashes in 100g of the product, without dissociation between total carbohydrates and fiber content [17]. The analysis was performed at CAV/UFPE Bromatology Laboratory.

The analysis was performed at the CETENE, LAFIP/CEAN Laboratory. The preparation of methyl esters followed the AOAC methodology [17]. After obtaining the methyl esters, the fatty acid profile was determined by Gas Chromatography with Flame Ionization Detector (GC-FID), containing a capillary column: DB-5ms (dimensions 30m longx250µm diameterx0.25µm, FAME Supelco™ mix C4-C24, Bellefonte, PA, USA). Chromatograph operating conditions were: 1.00mL/min column flow; detector temperature 300°C; 300°C injector temperature, 150°C oven temperature for 4 minutes, 150-280°C (4°C/min); The carrier gas used was helium, and a 1µL aliquot of the samples was injected into the apparatus. Fatty acid identification was performed by comparing sample peak retention times with standard peak retention times. The results obtained were in % of area.

Primiparous albino rats (*Rattus norvegicus*) (n=20) Wistar strain, weighting 220 to 250 grams and at 90 days of life were placed for mating at a *ratio* of 1:3 (male:female). Pregnancy was determined by observing the presence of sperm in a vaginal swab, defining the first day of pregnancy. On the first day of gestation until the 21st day of lactation, female rats were separated and placed in individual cages, randomly allocated to their relevant diets, with water and ration *ad libitum*, composing the following groups: Control Diet (n=5), High Fat Diet (HF) (n=9) and Omega 3 High Fat Diet (HFw3) (n=6).

Temperature and moisture were kept within the range of 22 to 24°C and 55 to 65%, respectively, with 12h light and dark (lights on from 6 am to 6 pm). The offspring were reduced to eight pups per litter for each dam. This reduction should occur after the first dark cycle from birth so that the dam does not reject the remaining offspring and until the 3rd day of life so as not to interfere with milk production.

Feed intake was assessed between the second and third gestational week on alternate days at the beginning of the light period (at eight o'clock in the morning), calculating the difference between the amount offered on the previous day and the cage leftover. Data from some animals were excluded due to difficulties during the food intake assessment test and therefore, only data from nine dam rats were taken into account in this evaluation, three from each group. On the 21st day of life, offspring were weaned and they began to receive a standard Presence® commercial ration (*Grupo Neovia, São Paulo*, Brazil), which contained 25.4% protein, 2.8% lipids, 54.5 % carbohydrates, 8.5% ash and 8.8% moisture per 100g diet. The caloric supply of this diet was 3.44 Kcal per gram of diet; 29.4% of the calories originated from protein, 7.6% from fat and 63% from carbohydrates.

At 19 days pregnancy, the rats were submitted to 12hour fasting (overnight). After this period, the dams were anesthetized with ketamine (80mg/Kgi.p) and xylazine (10mg/Kgi.p) to collect blood samples (1.0mL) through retro orbital plexus rupture. After coagulation, the blood was centrifuged at 3500 RPM for 10 minutes to obtain the serum, which was stored at -20°C in an Eppendorf tube until biochemical analyses could be performed using the Automated Biochemical Analyzer (version.4, Pioway Medical Lab Equipment Co. Nanjing, China). Blood glucose, total cholesterol, total protein and albumin were analyzed. Male offspring from each group of dams were submitted to murinometric measurements of body mass on the 1st, 21st and 30th day of life and abdominal circumference on the 21st and 30th day of life.

Data were analyzed using the GraphPadPrism Software (GraphPad Software Corporation, version 5.0, San Diego, CA Unided States, 2007). The normal distribution of the variables was verified by the Kolmogorov-Smirnov Test. After analysis of the normal distribution, the comparison between the groups was performed using the Anova One-way test, followed by the Bonferroni post test. Results were expressed as mean ±SEM (Standard Error of The Mean). The significance level considered was $p<0.05$.

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RESULTS

The amount of casein was equivalent between diets. The main sources of fat in the high fat diets were: butter, vegetable oil and lard. Monosodium glutamate was included as an ingredient, as shown in Table 1. The concentration of Butylated Hydroxytoluene antioxidant was increased in high fat diets due to changing fat sources. The HFw3 diet composition contains the ingredients in equal amounts to the HF diet, except for flaxseed and soybean oils, which together make up 7% vegetable oil according to the dietary composition of AIN 93G.

The analysis of centesimal composition, described in Table 2, showed a 20% increase in protein content in the HF diet and 13% in HFw3 in relation to the AIN 93G control diet. There was a 178% and 165% increase in lipid content in the HF and HFw3 diets, respectively. The amount of carbohydrates was reduced by approximately 20% in high fat diets. The percentage energy from fat was 80% higher in the HF diet and 74% in the HFw3 diet according to the nutritional composition analysis, also shown in Table 2.

The Chromatography Qualitative Analysis (Table 3) indicated that fatty acids C16:0, C18:0, C18:1, C18:2 and C18:3 were present in the three diets reviewed. Only the AIN 93G diet contained no medium chain fatty acids (C8:0, C10:0 and C12:0). The quantitative analysis showed that the three samples presented a lipid profile predominantly composed of unsaturated fatty acids, but with different proportions of saturated, monounsaturated and polyunsaturated fatty acids between the samples. The AIN 93G diet exhibited higher content of polyunsaturated fatty acids, followed by

Table 1. Composition of ingredients used in the formulation of experimental diets. *Vitória de Santo Antão* (PE), Brazil, 2018.

Ingredient in g per 100g of diet	AIN 93G	High fat diet	Omega 3 high fat diet with flaxseed oil 3.5%
Corn starch	39.7000	15	15
Dextrinized starch	13.2000	-	-
Wheat flour	-	12	12
Cornmeal cookie	-	7	7
Soy flour	-	6	6
Lard	-	2	2
Butter	-	8	8
Casein (>85%)	20	20	20
Guar gum	-	0.500	0.500
Sucrose	10	18	18
Flaxseed oil	-	-	3.500
Soybean oil	7	7	3.500
Fiber (cellulose)	5	0.300	0.300
Vitamins	1	0.700	0.700
Minerals mix	3.5000	2.500	2.500
DL-Methionine	0.3000	0.250	0.250
Choline Bitartrate	0.2500	0.250	0.250
Butylated hydroxytoluene	0.0014	0.014	0.014
Monosodium glutamate (12.3%)	-	0.200	0.200
Sodium Chloride	-	0.300	0.300
Total (g)	100	100	100

Note: The AIN-93G diet was adapted as recommended by Reeves *et al.* [6,7]. The high-fat diet was adapted from the Cavalcante *et al*. study [16].

Table 2. Centesimal and nutritional composition of formulated experimental diets. *Vitória de Santo Antão* (PE), Brazil, 2018.

Nutrient	AIN 93G	High fat diet	Omega 3 high fat diet with flaxseed oil 3.5%
Moisture (g/100g)	3.50	4.00	3.8
Proteins (g/100g)	18.60	22.30	21
Lipids (g/100g)	6.10	17	16.20
Carbohydrates (g/100g)	68.60	53.20	55.90
Ash (g/100g)	3.30	3.50	3.10
Kcal/g	3.69	4.52	4.51
Total fat (%TEV)	18,60	33.60	32.30
Proteins (%TEV)	20.20	19.60	18.20
Carbohydrates (%TEV)	61	46.80	49.40

Note: The omega 3 high fat diet was made with 3.5% flaxseed oil in the composition of ingredients. The analysis of centesimal composition was performed at the Bromatology Laboratory of the Academic Center of *Vitória de Santo Antão* of the Federal University of *Pernambuco*, following the Association of Official Analytical Chemists determination methodology of moisture, proteins, lipids and ashes [17]. The amount of carbohydrate present in the sample was obtained by difference. TEV: Total Energy Value; The nutritional composition regarding the amount of calories and caloric percentage of fats, proteins and carbohydrates was determined from the centesimal analysis of diets performed at the Bromatology laboratory of the Academic Center of *Vitória de Santo Antão* of the Federal University of *Pernambuco*.

monounsaturated and saturated fatty acids; the HF diet had a higher amount of saturated fatty acids, followed by polyunsaturated and monounsaturated; HFw3 presented higher monounsaturated fatty acids content, followed by saturated and polyunsaturated fatty acids.

There was a 100% increase in saturated fatty acids in high fat diets compared to the AIN 93G control diet (Table 3). Palmitic acid was 90.0% and 81.5% higher in the HF and HFw3 diets, respectively. The percentage of monounsaturated fatty acids was similar between the AIN 93G and HF diets, and the HFw3 diet had a 33.0% increase in monounsaturated fats. The HF and HFw3 diets had a lower content of polyunsaturated fatty acids, namely 37.0% and 53.0%, respectively. Although the AIN 93G diet had a higher percentage of omega 3, the HFw3 diet presented a 50.0% higher content in 100g of the sample when compared to the other diets, which contained similar amounts of this nutrient. The HFw3 diet also had lower omega 6:3 *ratio*. The omega 6 content was higher in the HF diet, which also presented higher omega 6:3 *ratio*, being 1.7 times higher than the AIN 93G diet.

In the evaluation of consumption by pregnant rats, shown in Table 4, there was no significant difference between the amount of diet consumed by the rats receiving the AIN 93G diet and the ones receiving the high fat diets (AIN 93G=14.3±2.3g; HF=13.1±1.9g; HFw3=10.2±1.8g, p=0.0697). Table 4 also shows that there were no significant differences in total protein, albumin and serum glucose values. However, the cholesterol of pregnant rats that received the HFw3 diet was significantly lower when compared to those receiving the HF diet alone, but these had values significantly higher than the control.

In the murinometric evaluation of offspring, higher body mass was observed on the first day of life in offspring receiving the HF diet, without significant difference between the offspring whose mothers received the control diet and HFw3. However, at 21 days of life, HFw3 offspring presented higher body mass in relation to control and HF offspring. At 30 days of life, there was no difference between the groups, as shown in Table 5. The measurement of abdominal circumference showed that at the end of the lactation period (21st day) there was no difference between the groups, but after this period (on the 30th day of life), the offspring which maternal diet during pregnancy and lactation was HF presented increased abdominal circumference when compared to the offspring of maternal control diet and HFw3.

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Table 3. Fatty acid composition in diets regarding the presence of double bond in the carbon chain. *Vitória de Santo Antão* (PE), Brasil, 2018.

Fatty acid	Fatty acid percentage composition Diets		
	AIN 93G	High fat diet	Omega 3 high fat diet with flaxseed oil 3.5%
Saturated			
Octanoic acid (C8:0)	0	0	0
Decanoic acid (C:10)	0	0.85	0.74
Lauric acid (C12:0)	0	1.16	1.15
Myristic acid (C14: 0)	0	4.22	4.37
Pentadecanoate Acid (C15:0)	0	0.47	0
Palmitic acid (C16:0)	11.97	22.78	21.73
Heptadecanoate Acid (C17:0)	0	0	0
Stearic acid (C18:0)	4.62	7.53	8.07
Arachidic acid (C20:0)	0.36	0	0
Behenic acid (C22:0)	0.37	0	0
Total	17.32	37.01	36.06
Monounsaturated			
Myristoleic acid (C14:1)	0	0.46	0
Palmitoleic acid (C16:1)	0	0.95	0.87
Heptadecanoic acid (C17:1)	0	0	0
Oleic acid (C18:1)	29.93	28.32	38.47
Total	29.93	29.73	39.34
Polyunsaturated			
Linolenic acid (C18:3)	3.89	1.64	2.32
Linoleic acid (C18:2)	48.87	31.63	22.28
Total	52.76	33.27	24.60
Omega 3 (g/100g diet)	0.27	0.27	0.40
Omega 6 (g/100g diet)	3.42	5.73	3.78
Omega 6:3 ratio	12.60	21.20	9.40

Note: Fatty acids were identified according to external standard (FAME Supelco™ mix C4-C24, Bellefonte, PA, United States and the percentage (%) calculated according to peak area normalization by the gas chromatography method at the Phytochemical Laboratory and processes of the Northeast Center for Technologies and Strategies. From the percentage determination of linolenic (omega 3) and linoleic (omega 6) acids, the amounts (g/100g of diet) of these fatty acids were calculated and the omega *ratio* was obtained. 6/3.

DISCUSSION

In the formulation of the high fat diet, the amount of ingredients was modified to obtain a diet rich in saturated fatty acids, with butter as the main source. The fatty acid profile of butter is 73.5% saturated fatty acids, 19.8% monounsaturated and 3.9% polyunsaturated, differing from margarine that contains 21.2% saturated fat, 23.8%. monounsaturated and 49.0% polyunsaturated fatty acids [14]. Lard was kept in the formulation of the high-fat diet, since it is an ingredient that is frequently found in the composition of high-fat diets in other studies [3,4,18]. Monosodium glutamate has been added as it is a substance used in the development of experimental models of neuroendocrine obesity [19].

Table 4. Average food intake and serum levels of biochemical parameters at 19 days of gestation of Wistar rats fed with AIN 93G, high fat diet and high fat diet supplemented with omega 3. *Vitória de Santo Antão* (PE), Brazil (2018).

Variables	Group									*p*
	AIN 93G			HF			HFw3			
	M	±	SEM	M	±	SEM	M	±	SEM	
Food consumption										
g/day	14.2	±	1.4	13.1	±	1.1	10.1	±	0.9	0.0697
kcal/day	52.7	±	5.0	59.3	±	4.9	45.9	±	4.0	0.1784
Carbohydrates/day	8.4	±	0.8[a]	6.9	±	0.6[a]	5.6	±	0.4[b]	0.0424*
Protein/day	2.5	±	0.24	2.9	±	0.24	2.1	±	0.2	0.0944
Lipids/day	0.9	±	0.1[a]	2.2	±	0.2[b]	1.6	±	0.1[b]	0.0022**
g/kg	48.6	±	6.3	45.4	±	2.2	34.5	±	2.2	0.0648
kcal/kg	180.0	±	23.1	205.3	±	10.2	156.0	±	10.1	0.1210
Biochemical Parameters										
Total protein (g/dL)	6.6	±	0.3	6.3	±	0.1	6.0	±	0.1	0.1138
Albumin (g/dL)	4.8	±	0.1	4.8	±	0.1	4.6	±	0.2	0.5708
Glucose (mg/dL)	120.8	±	4.1	129.4	±	6.80	109.0	±	10.6	0.1943
Total cholesterol (mg/dL)	73.3	±	1.7[a]	90.0	±	3.3[b]	64.3	±	4.7[a]	0.0005**

Note: [a,b]Values with equal letters in the same line do not differ after statistical analysis; Pregnant rats received 18% lipid control diet (AIN-93G), 33% lipid high fat diet (HF) or omega 3 supplemented high fat diet (HFw3) with 33% lipids with 3,5% flaxseed oil, according to the experimental group, during pregnancy. Values were expressed as mean ± SEM. Values with equal letters in the same line do not differ after statistical analysis (*p<0.05 and **p<0.01, One-Way ANOVA and Bonferroni post test: control [N=3], HF [N=3], HFw3 [N=3] in the food intake assessment and control [N=5], HF [N=9], HFw3 [N=5-6] for biochemical assessment).

Table 5. Body mass and abdominal circumference of offspring of Wistar rats fed AIN 93G diets, high fat diet and high fat diet supplemented with omega 3 during pregnancy and lactation. *Vitória de Santo Antão* (PE), Brazil (2018).

Age/Variables	Group									*p*
	AIN 93G			HF			HFw3			
	M	±	SEM	M	±	SEM	M	±	SEM	
1st day										
Body mass (g)	6.5	±	0.2[a]	7.4	±	0.2[b]	6.8	±	0.1[a]	0.0029**
21st day										
Body mass (g)	53.9	±	0.7[a]	54.4	±	0.8[a]	57.6	±	0.7[b]	0.0023**
Abdominal circumference (cm)	10.2	±	0.1	10.2	±	0.1	10.1	±	0.1	0.9707
30th day										
Body mass (g)	93.3	±	2.3	97.0	±	2.3	100.2	±	2.6	0.1316
Abdominal circumference (cm)	12.0	±	0.1[a]	12.7	±	0.2[b]	11.8	±	0.2[a]	0.0046**

Note: [a,b]Values with equal letters in the same line do not differ after statistical analysis; Pregnant rat received a 18% lipid control diet, a 33% lipid high-fat (HF) diet, or an omega 3 supplemented high-fat diet (HFw3) with 33% lipids added with 3.5% flaxseed oil, according to the experimental group during pregnancy. Values were expressed as mean ± SEM. Values with equal letters in the same line do not differ after statistical analysis (*p<0.05 and **p<0.01, One-Way ANOVA and Bonferroni post test: control [N=31-35], HF [N=25-27], HFw3 [N=23-27]).

The HFw3 diet was developed by partially replacing the fat source so as not to compromise the omega 6 supply or cause its deficiency. Deficiency of essential fatty acids and particularly of omega 6 is associated with low weight and decreased adipose tissue and a change of both plasma lipid and

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plasma membrane of the adipocytes, with an increase in omega 9:6 ratio and alteration. of lipolytic and lipogenic response in the adipose tissue [20]. The composition of the HFw3 diet provides the amount of omega 6 as recommended by AIN 93G [6] with the aim of offering a higher omega 3 diet but ensuring adequate amounts of other essential fatty acids.

Alpha-Linolenic Acid (ALA) from the omega 3 series is found mainly in flaxseed (*Linun usitatissimum L.*), both in the seed and in the oil obtained by processing the seeds. Flaxseed oil (*Linun usitatissimum L.*) contains 55 to 57% of ALA and 15 to 18% of alpha-linoleic (omega 6) acid [21], with no difference between golden and brown flaxseed oil [22] and is a low-cost product. and easily found. The use of golden flaxseed oil is justified by the fact that such flaxseed is grown without pesticides; therefore, it is organic. Studies show the beneficial effects of using flaxseed oil in high fat experimental diets in the critical period of development [3,4]. In young animals exposed to the high-fat diet, omega 3 supplementation from flaxseed decreased hepatic inflammation and prevented steatosis [23]. Data published by our research group show that the omega-3 high-fat diet was associated with lower triglycerides values at 21 days of life in the offspring, and the high-fat diet was associated with elevated Alanine Aminotransferase levels during the same period [24].

Studies using high fat diets to evaluate the effect of metabolic programming must meet the nutritional requirements for rodents, ruling out possible biases due to misinterpretation of results. According to some authors, studies to better evaluate the efficacy of a particular compound are well developed when using a high fat diet over a very high fat diet [5,25]. High fat diets, about 60% TEV, exceed nutritional recommendations, both for rodents and humans, making it difficult to extrapolate data beyond experimental research [25]. Given the macronutrient recommendations for the reproduction and growth phase [6,7], very high fat diets should not be used in studies of fetal programming and phenotypic plasticity, as this type of diet jeopardizes the necessary nutritional intake, especially protein, and may cause perinatal malnutrition and potential results misinterpretation. Control and high fat diets should ensure availability of the essential nutrients for development so that the difference between them is only in terms of lipid content [25].

In the assessment of food intake, there was no significant difference between the groups, although lower food intake was observed in pregnant rats of the HF and HFw3 groups. It is reported that animals receiving high fat diet during pregnancy exhibit lower food intake compared to those receiving normolipidic diet. Carvalho *et al.* [26] found a 30% reduction in the consumption of high fat diet with higher saturated fat content when compared to the standard diet. Increasing the caloric density of the diet reduces food intake, because in the *ad libitum* regime animals consume a constant amount of energy [14]. The composition of high fat diets includes lower carbohydrate content and higher lipid content in relation to AIN 93G, which contributed to the reduction in carbohydrate consumption in dams fed with the HFw3 diet and higher lipid consumption between the two groups in relation to the control group. Cavalcante *et al.* [16] also showed a higher lipid consumption in detriment of lower carbohydrate consumption in pregnant animals that were on Western diet in the last week of gestation.

In the present study, no difference was observed in serum total proteins and albumin was observed. This fact is considered a biomarker of nutritional status and low levels are associated with energy-protein malnutrition [27]. Animals submitted to perinatal malnutrition with a low protein diet show lower levels of albumin [28]. Normal albumin values may infer adequate dietary intake without nutritional impairment.

Dams on HF diet had high cholesterol levels, which was not observed in those on the HFw3 diet. Omega 3 fatty acids can improve lipid profile by lowering triglycerides, total cholesterol, and LDL cholesterol fraction levels. even in animals consuming the high-fat diet [29]. Some mechanisms are proposed by which omega 3 decreases lipid levels such as: reduction of hepatic lipogenesis by inhibiting Sterol Regulatory Element-Binding Proteins and expression of enzymes responsible for the synthesis of cholesterol, fatty acids and triglycerides; inhibition of key enzymes for hepatic triglyceride synthesis such as the phosphatidic acid phosphatase and diacylglycerol acyltransferase, and increased lipoprotein lipase expression for uptake of triglycerides by circulating lipoproteins, such as very low density lipoprotein and chylomicrons [30].

The HF offspring presented higher body mass on the 1st day of life and larger abdominal circumference at 30 days of life. Consumption during pregnancy of a saturated fatty acid-rich high fat diet has effects on offspring's body composition, such as weight gain, increased visceral fat, and long-term adipocyte hypertrophy [13].

A meta-analysis concluded that consumption of a high-fat diet during pregnancy is associated with increased body fat, hyperleptinemia, hyperglycemia, hyperinsulinemia, dyslipidemia, and arterial hypertension in the offspring, with risk of developing metabolic syndrome [31]. In addition, HFw3 offspring also exhibited increased body mass at weaning, which demonstrates the overwhelming effect of a high fat diet early in life, even with omega 3 supplementation. Up to 42 days of life, considered as early adolescence in animals, there may be changes in body composition deriving from the nutritional environment, with repercussions on health in adulthood [32].

CONCLUSION

The formulated high fat diet may be indicated for experimental studies which objective is to evaluate the effect of diets rich in saturated fatty acids when manipulated during pregnancy and lactation, but that meet the recommended nutritional recommendations for rodents. Protein intake remained ideal for the growth and reproduction phase, as well as carbohydrates, which represent the main energy source, similar to the control diet, although in a lower percentage. Monounsaturated fats were in the same proportions of the control diet. In contrast, the percentage of saturated fatty acids was high, without impairing the supply of omega 3 and omega 6. The high fat diet with omega 3 ensures the essential nutrients for growth and development of rodents from commercial flaxseed oil as a source of alpha-linolenic acid, considering the feasibility, but with lower omega 6 content. The adequate supply of nutrients with modification only in the lipidic component allows the reduction of biases by other types of nutritional imbalances. It was observed that there was no statistical difference in the consumption analysis between diets, suggesting that the high-fat diet and the high fat diet with omega 3 may be well accepted, so that the nutritional requirements are met. The high-fat diet changed the dams' cholesterol levels, which was not observed with omega 3 supplementation. In addition, the offspring which dams received this diet exhibited higher body mass on the first day of life and increased abdominal circumference after weaning, which demonstrates that the high-fat diet has short- and long-term effects on the mother and offspring, and omega 3 supplementation can mitigate these changes.

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CONTRIBUTORS

LML SILVA and JH COSTA-SILVA contributed to study conception and design, data analysis and interpretation, manuscript preparation and writing, and approval of the final version. AMNLG BLOISE, DAF FONTES, KS ARAUJO, and MO BARBOSA contributed to data analysis and interpretation, article review and approval of the final version.

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