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Impact of supplementation with native cerrado fruit oil on the composition of breast milk of lactating Wistar rats

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Abstract

The study aimed to evaluate the effect of baru oil (*Dipteryx alata* Vog) supplementation on maternal milk and offspring of female Wistar rats. Animals were divided into three groups $(n = 12)$, supplemented with baru oil, soybean oil, or olive oil, at a daily dose of 1,000 mg/kg during pregnancy and lactation. The body weights and food intake of rats were measured weekly and analyzed. Milk collection was performed manually from the 12th to the 21st day of lactation. After weaning, rats and pups were euthanized after a 6-h fast. Blood was collected from the animals, five sites of adipose tissue were removed from each mother rat, and the mesenteric site was removed from the pups. It was observed that breast milk from the group supplemented with baru oil had higher concentrations of polyunsaturated and monounsaturated fatty acids, such as linoleic and oleic acids. However, there was no statistical difference between the group supplemented with Baru and soybean oil or olive oil group. Supplementation of the three types of oils did not interfere with body weight gain, animal consumption, serum parameters, liver weight, and adipose tissue sites. However, it showed antiatherogenic activity.

Keywords: *Dipteryx alata* Vog; fatty acids; fruits of the Brazilian cerrado; gestation; lactation.

Practical application: Baru oil is used as an alternative for maternal supplementation with an impact on health.

1 INTRODUCTION

Breast milk is a food that has positive effects on the child's health (Sankar et al., 2015), both physical and mental (Khatun et al., 2018), in addition to reducing the incidence of many infectious diseases and hospitalizations due to infection (Pound et al., 2012). The provision of polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) in breast milk can prevent cardiovascular diseases, obesity, and associated disorders (Eckel et al., 2014).

Several types of vegetable oils are consumed daily and form part of the human diet (Freitas & Jorge, 2022). Soybean oil is rich in PUFAs and is the most used in food preparation in Brazil (Abiove, 2023; Silva, J. B. 2018). Olive oil is rich in oleic acid (Ixtaina et al., 2011; Silva, J. B. 2018) and is associated with a lower incidence of chronic cardiovascular diseases and prevention of metabolic diseases (Quitete et al., 2018; Soto-Alarcon et al., 2018).

In Brazil, several vegetable oils are commercialized, among them baru oil (*Dipteryx alata* Vog), which has a promising fatty acid profile. However, there are few clinical studies considering the ingestion of this oil involving pregnant and lactating women (Siqueira et al., 2016). Therefore, carrying out studies that can provide clinical information, as well as new data for the National Health Surveillance Agency (ANVISA), to ensure the commercialization and food safety of the population is necessary in Brazil.

In this context, the oil extracted from the baru nut (*Dipteryx alata* Vog), which is a species native to the Brazilian Cerrado widely consumed in the Midwest region, has about 50% MUFA and 30% PUFA, with emphasis on oleic acid and linoleic acid, that is, it contains nutritional characteristics that can favorably influence the mother's health and the quality of breast milk (Alves-Santos et al., 2021; Siqueira et al., 2016).

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Given the above, this study aimed to evaluate the effects of baru oil supplementation in Wistar rats during pregnancy and lactation on breast milk and offspring.

2 MATERIALS AND METHODS

2.1 Baru fatty acid profile

Baru oil samples in capsules were purchased from Akron Pharma S/A. For the analysis of the fatty acid profile, the samples were esterified according to the methodology adapted from Maia et al. (1983), and the fatty acid methyl esters were analyzed by gas chromatography (GC 2010, Shimadzu, Japan) to get your individual peaks.

To determine the atherogenicity (AI) and thrombogenicity (TI) indices, Equations 1 and 2 adapted from Ulbricht e Southgate (1991) were used:

$$
IA = [(12.0 + (4 \times 14.0) + 16.0) + 16.0]/(MUFA + PUFA \omega6 + PUFA\omega3)
$$
 (1)

$$
IT = (14.0 + 16.0 + 18.0)/[(0.5 \times MUFA) + (0.5 \times APUFA\omega6) + (3 \times APUFA\omega3) + (PUFA\omega3/PUFA\omega6)]
$$
\n(2)

where MUFA and PUFA correspond to monounsaturated and polyunsaturated fatty acids. To obtain the ω6:ω3 ratio, the ratio between ω6 concentrations and ω3 concentrations was used according to the study by N. R. Silva (2018).

2.2 Experimental test

All procedures reported in this study were approved by the Ethics Committee on Animal Research/CEUA of the Universidade Federal do Mato Grosso do Sul under protocol number 1,263/2022. The experiment was carried out in the experimentation room of the UFMS Central Animal Facility, where the animals (female mothers and offspring) were housed in individual maternity cages made of polypropylene and covered with wood shavings under controlled conditions (temperature 22 ± 1°C, humidity 65%, and 12-h light/dark cycle). Throughout the experiment, Wistar rats received commercial chow (Nuvilab®, Brazil) and water was provided ad libitum and cared for in accordance with the principles of the Guide for Care and Use of Experimental Animals.

A total of 36 female Wistar rats were used in this experiment, considering the first gestational cycle, aged between 70 and 80 days, and body weight between 150 and 200 g. The estrus cycle was performed, and the females were placed in the males' boxes with the dirty shavings (upon exposure to male odors or male rats urine soaked-bedding) 48 h before mating (Whitten effect). For the mating procedure, one male and two females remained in each box. For the confirmation of pregnancy, the female rats were evaluated visually by observing the vaginal tampon.

The pregnant rats were housed in individual maternity cages and divided into three groups ($n = 12$ /group) with different dietary: supplemented with soybean oil (soy group), olive oil (olive oil group), and baru oil (baru experimental group). Rats were then divided into three weight-matched groups, that is, the average initial weight of the rats (*n* = 36) was used so that the groups would have similar weights.

Oil supplementation in Wistar rats was performed via gavage, with doses of 1,000 mg/kg body weight/d, during the 21 days of pregnancy and 21 days of lactation. During supplementation, the rats were weighed weekly. Pups were removed from their mothers 21 days after delivery. Litters with 10 pups (5 males and 5 females) remained with their mothers until the end of the study. Litters were weighed weekly until weaning.

2.3 Methods used to collect milk from rats

Milk collection from the rats started on the 12th day of lactation and was performed on alternate days until the 21st day (weaning). To perform the milk collection, each rat was separated from her pups 2 h before the procedure and kept in a thermal blanket at an approximate temperature of 32°C. In addition, 0.4 mL (2UI) of oxytocin was administered, and after 10 min, animals were anesthetized using an inhaler device.

An amount of 0.1–0.5 mL of milk from the rats was collected by manual massage and placed in cryotubes, which were stored in an ultrafreezer (-80°C).

2.4 Animal euthanasia

At the end of the 21 days of lactation, the rats and pups were fasted for 6 h and then anesthetized with isoflurane with exsanguination through the inferior vena cava.

From each mother rat, five sites of adipose tissue were removed (epididymal, mesenteric, retroperitoneal, perirenal, and omental), and from the pup, the mesenteric tissue was removed (Chau et al., 2014), considering that the small size of the other sites made weighing impossible. The livers of both pups and mothers were also weighed on an analytical electronic scale (Bel Diagnóstica® model MG214AI), with values expressed in units of milligrams.

2.5 Serum parameters

Fasting blood glucose tests were performed using a colorimetric enzymatic kit, and serum total cholesterol (TC), high- -density lipoprotein (HDL-c), non-HDL-c, and triglycerides (TG) concentrations were measured using the commercial kit LabTest Diagnóstica®, Brazil.

2.6 Statistical analyses

Results were expressed in mean ± standard deviation. Parametric data were analyzed by the one-way ANOVA test followed by Tukey's post-test. For non-parametric data, the Kruskal-Wallis test was used followed by the Dunn post-test. Dunn's post hoc tests are carried out on each pair of groups. A significance level of *p* < 0.05 was considered. All analyses were performed

with the SigmaStat program, version 3.5 (Systat Software Inc., San Jose, CA, USA).

3 RESULTS AND DISCUSSION

3.1 Fatty acid profile of baru, soybean, and olive oils

The fatty acid profiles of baru, soybean, and olive oils are shown in Table 1. The fatty acid profile of baru oil was predominantly MUFAs (48.11%), followed by PUFAs (35.65%) and saturated fatty acids (SFA) (14.44%). According to the data shown in Table 1, soybean oil mostly contains PUFA (62.3%), followed by MUFA (23.4%) and SFA (14.33%). In olive oil, MUFA predominates (69.5%), followed by SFA (11.65%) and PUFA (6%).

The oil extracted from the baru nut has antioxidant action, preventing cardiovascular diseases and neoplasms, anti-rheumatic action, controlling hypercholesterolemia, and acting as a hormone regulator (Pinto, 2018).

3.2 Profile of fatty acids in breast milk of experimental groups and breast milk of non-supplemented Wistar rats

As shown in Table 2, the profile of fatty acids in breast milk from the group supplemented with baru oil showed a higher concentration of SFA (56.025% \pm 6.566), followed by PUFA $(23.102\% \pm 0.523)$ and MUFA $(17.397\% \pm 2.254)$. The groups supplemented with soybean and olive oils also showed a decreasing order of fatty acid concentrations similar to the baru group, with the soybean oil group presenting $53.713\% \pm 12.598$ (SFA), 22.832% ± 0.231 (PUFA), and 14.682% ± 0.132 (MUFA) and the olive oil group $45.178\% \pm 0.119$ (SFA), $21.880\% \pm 0.549$ (PUFA), and $21.620\% \pm 0.160$ (MUFA). In relation to breast milk of non-supplemented Wistar rats, the highest concentration was SFA (63.88%), followed by PUFA (19.22%) and MUFA (15.03%).

Azagra-Boronat et al. (2020) analyzed unsupplemented Wistar rat milk and found the following composition. The palmitic (28.22% ± 0.89), myristic (12.47% ± 0.57), lauric (9.53% ± 0.37), capric (7.90% \pm 0.42), stearic (3.23% \pm 0.06), and arachidic

Table 1. Fatty acid profiles of baru, soybean, and olive oils (%).

 $(0.12\% \pm 0.01)$ acids showed the highest concentrations among the SFA in the maternal milk and also, with the exception of capric acid, showed higher concentrations in relation to the baru, soy, and olive oil groups. Among the MUFAs in rat breast milk, oleic acid was the majority (13.69% \pm 0.85), followed by palmitoleic acid (1.11% \pm 0.23), which has a higher concentration in relation to the rat oil groups, baru, soy, and olive oils and, among PUFAs, linoleic (16.09% \pm 0.40), arachidonic (0.93% \pm 0.05), and alpha-linolenic acids (0.90% \pm 0.05). The concentrations of gamma-linolenic acid (0.34% ± 0.02), 8,11-eicosadienoic acid $(0.39\% \pm 0.07)$, and docosahexaenoic acid $(0.13\% \pm 0.01)$ were not significant between the PUFAs, but their concentrations were higher in relation to the baru, soy, and olive oil groups.

According to studies, the presence of oleic acid in breast milk can act as a protective factor, increasing HDL-c and preventing cardiovascular diseases (Nogoy et al., 2020). Furthermore, linoleic acid is important for brain development during fetal and postnatal development (Mennitti et al., 2018; Schuchardt et al., 2010) and can act as a messenger and regulate gene expression (Priego et al., 2013) and prevent cardiovascular diseases (Raposo, 2010).According to the results, supplementation with oils rich in MUFAs and PUFAs (baru and olive oil groups) decreased the concentrations of some SFAs such as palmitic and myristic acids as observed in non-supplemented rat breast milk. In fact, studies demonstrate that high consumption of PUFAs and MUFAs can prevent cardiovascular diseases, obesity, and associated disorders (Eckel et al., 2014).

The atherogenicity index (AI) and the thrombogenicity index (TI) assess the quality of fatty acids and their effects on lipoprotein metabolism; therefore, these indices are applied to assess the nutritional quality of oils. Although these indexes do not have recommended values, the lowest values express a ratio of fatty acids that are more favorable to health; on the contrary, the highest values of these indexes suggest that the consumption of the studied oil can cause damage to health (Ulbricht & Southgate, 1991).

In view of the results obtained in this study, the milk of rats supplemented with baru, soy, and olive oils has antiatherogenic activity because they have low atherogenicity and thrombogenicity rates (Table 2), which are satisfactory for health.

Data are presented as mean±standard deviation; 'Aguila and Mandarim-de-Lacerda (2010); ²Siqueira et al. (2016); ³Figueiredo et al. (2022); ⁴Zambiazi et al. (2007).

Data are presented as mean ± standard deviation. Different letters on the same line represent statistical differences between groups (*p* < 0.05); 'Kruskal-Wallis/Dunn's; ²ANOVA/Tukey;
³Azagra-Boronat et al. (2020), ∑N ³Azagra-Boronat et al. (2020). ∑ NIFA: sum of unidentified fatty acids.

3.3 Food intake and weekly body weight of female rats supplemented with baru, soybean, and olive oils during pregnancy and lactation and their offspring

During the gestational period, metabolic changes occur which increase the formation of maternal and fetal tissue, as well as the accumulation of fat reserves to favor a continuous supply of energy during the first months of lactation. During this period, reserves are mobilized to release fatty acids that are transferred to the milk (Butte & King, 2005; Demmelmair et al., 2016).

During the lactation period, energy expenditure and nutrient segregation in breast milk intensify, making it necessary to readjust nutritional intake to meet the infant's needs (Park & Eicher-Miller, 2014; Romano et al., 2014).

The results obtained in our study showed that there was no significant difference in food consumption and weight of rats supplemented with baru, soy, and olive oils during pregnancy and lactation (Table 3). However, in week 2, with the exception of the soy group during the lactation period, there was a reduction in the food consumption of the rats during pregnancy and

lactation; however, the weight continued to increase. Intake remained constant during the lactation period, with weeks of lower acceptance and periods of higher consumption.

Regarding the body weight of the litter of puppies at weeks 1, 2, and 3, it was found that there was no statistically significant difference between the groups (Table 4).

Our findings on the body weight of rat pups corroborate the findings of Bidô (2018), who evaluated the effects of maternal supplementation with oil and baru nuts during the critical period of gestational development and during lactation of rats and found that the body weight continued to increase throughout the development of the offspring, with no variation between the studied groups.

The results obtained in our research confirm that the experimental model did not cause damage to the development of the offspring, despite the milk extraction, that is, the maintenance of the cranial mammary glands without milk extraction and the time away from the mothers for milking guarantee the good development of its offspring; therefore, it is a functional model for the objectives foreseen here.

3.4 Serum parameters

According to the data presented in Tables 5 and 6, there was no significant difference between the glycemia of the rats supplemented with baru oils, as well as those supplemented with soy and olive oil, compared with the glycemia of the pups at the end of the breastfeeding period.

The assessment of blood glucose is important because diabetes mellitus, which is a disorder characterized by an increase in the level of glucose in the blood, is associated with the occurrence of several pathologies, such as cardiovascular diseases (Sposito et al., 2007) and gestational diabetes. Gestational diabetes

Table 3. Food consumption and weekly body weight of rats supplemented with baru, soybean, or olive oil, during pregnancy and lactation.

		Pregnancy				Lactation				
Weeks	Baru group	Soy group	Olive oil group	p-value	Baru group	Soy group	Olive oil group	p-value		
Food consumption (g)										
Week 11	73.92 ± 30.46	65.42 ± 25.38	$75.17 \pm 3.2.97$	0.686	63.25 ± 44.83	37.50 ± 40.91	43.25 ± 4.62	0.205		
Week 2^2	32.58 ± 17.31	30.58 ± 16.27	27.42 ± 15.25	0.738	36.00 ± 20.34	44.17 ± 19.15	25.58 ± 15.90	0.062		
Week 32	29.75 ± 12.91	34.25 ± 15.36	33.75 ± 8.02	0.630	40.50 ± 7.05	48.40 ± 11.70	66.29 ± 40.85	0.576		
Weeks	Baru group	Soy group	Olive oil group	p-value	Baru group	Soy group	Olive oil group	p-value		
Body weight (g)										
Week 11	176.33 ± 16.42	188.75 ± 19.62	178.75 ± 18.95	0.230	245.58 ± 22.07	246.00 ± 35.78	242.75 ± 23.45	0.740		
Week 22	209.17 ± 20.36	198.50 ± 25.13	203.50 ± 19.421	0.494	270.45 ± 30.50	265.33 ± 30.98	269.33 ± 21.15	0.897		
Week 32	220.92 ± 13.10	220.25 ± 24.18	221.42 ± 20.309	0.990	285.22 ± 20.17	270.58 ± 26.68	281.50 ± 20.54	0.313		

Results are presented as mean ± standard deviation; ¹Kruskal-Wallis; ²ANOVA.

Table 4. Body weight (g) of rat pups supplemented with baru, soybean, and olive oils.

	Body weight (g)							
Groups	Baru group	Soy group	Olive oil group	p-value				
Week 1	142.64 ± 60.26	146.66 ± 49.17	138.09 ± 45.65	0.923				
Week 2	168.89 ± 84.58	188.58 ± 42.24	179.10 ± 20.31	0.754				
Week 3	195.67 ± 89.20	231.80 ± 50.17	192.90 ± 84.78	0.414				

Results are presented as mean ± standard deviation.

Results are presented as mean ± standard deviation; ¹Kruskal-Wallis; ²ANOVA.

Results are presented as mean \pm standard deviation; 'Kruskal-Wallis; 2ANOVA.

Results are presented as mean ± standard deviation.

can cause miscarriages, perinatal mortality, birth defects, fetal body fat, macrosomia, hyperinsulinemia, and hypoglycemia (Makwana et al., 2017).

The glucose concentration of the rats in the baru group $(214.37 \pm 47.90 \text{ mg/dL})$ and olive oil $(214.67 \pm 79.93 \text{ mg/dL})$ was close to the reference value (200.00 ± 26.85 mg/dL) from the Central Animal Facility of UFMS (Silva, 2015). However, the glucose level of the litter of puppies is lower than the reference value of the UFMS Central Animal Facility. It should be noted that the reference values of serum parameters for rodents may vary according to the breeders, as they may be influenced by factors such as sex, strain, and genotype (Barril et al., 2019; Roberto et al., 2018).

The mean values of cholesterol, triglycerides, HDL-c, and non-HDL-c in the serum of the rats supplemented in the experiment, and their pups at the end of the breastfeeding period, did not show significant differences ($p < 0.05$).

According to studies, the combination of MUFAs and PU-FAs can reduce TC and LDL-c cholesterol and may increase HDL-c cholesterol, suggesting that these compounds have an important cardioprotective action and may act in the prevention of cardiovascular disease (Bidô, 2018; Nogoy et al., 2020; Pereira, 2018), including also avoiding possible chronic diseases such as obesity, diabetes, and dyslipidemia in the offspring (Bidô, 2018).

In addition, the experimental studies by Bidô (2018) and Pereira (2018) evaluated the effects of maternal supplementation in Wistar rats, using baru nut oil (with a dosage of 2,000 mg/ kg/day), during the critical period of development (pregnancy and lactation), and observed that there is a change in the lipid profile, raising HDL-c cholesterol and reducing serum levels of triglycerides and TC.

In our study, it was observed that MUFAs and PUFAs predominate in baru oil supplemented in rats, with emphasis on oleic (16.111% \pm 2.272) and linoleic (20.555 \pm 0.604) acids. However, baru oil (1,000 mg/kg) did not interfere with the lipid profile of these animals and their offspring. Similar results were obtained in the study by Reis (2016), who evaluated the effects of baru oil on markers of cardiovascular risk and liver and kidney functions in normal and dyslipidemic rats, in which they were supplemented with said oil (1.0 g/kg weight/day) and fed during pregnancy and lactation with commercial feed.

According to Mensink et al. (2003) and Sposito et al. (2007), SFAs have an effect on serum cholesterol levels, act in opposite directions to PUFAs, and may increase serum levels of TC and triglycerides.

In other studies, such as the one carried out by Reis (2016), baru oil did not interfere with the lipid profile; therefore, this may be associated with the various fatty acids that compose it and also the lower concentration of PUFAs, when compared with other oils, as small differences in their chemical structures can cause significant changes in the body's metabolic responses.

3.5 Liver weight and adipose tissue sites

There was no statistical difference in the average weight of the liver and fat sites of the females belonging to the baru, soybean, and olive oil groups (Table 7). Likewise, it did not statistically alter the weight of the liver and mesenteric site of the pups at the end of the suckling period.

4 CONCLUSION

The results obtained in this study indicate that the profile of fatty acids found in the breast milk of the group supplemented with baru oil has significant concentrations of PUFAs followed by MUFAs, with emphasis on linoleic and oleic acids.

Supplementation with baru oil did not induce changes in weight gain and food consumption of the animals, nor did it cause changes in serum parameters, liver weight, and adipose tissue sites. In addition, such supplementation showed antiatherogenic activity demonstrating to be beneficial to human health.

The results obtained in our study reinforce the use of baru oil as a functional nutritional food of great relevance for human health and may be an alternative and regional option closer to olive oil.

The knowledge from this study can be used in new investigations related to the use of higher doses of baru oil, supplementation of rats from pre-conception, and toxicological studies, which help in new registrations of oils and collaborate with regional development and their safe commercialization.

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