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DIEGO ELIAS PEREIRA

EFEITOS DO CONSUMO MATERNO DO BARU (*Dipteryx alata* Vog.) SOBRE A MICROBIOTA FECAL, PARÂMETROS DE ESTRESSE OXIDATIVO CEREBRAL E DESENVOLVIMENTO COMPORTAMENTAL DA PROLE DE RATAS *WISTAR*

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Tese apresentada ao Programa de Pós-Graduação em Ciências e Tecnologia dos Alimentos da Universidade Federal da Paraíba, para obtenção do título de Doutor em Ciência e Tecnologia dos Alimentos.

Orientadora: Profª. Drª. Juliana Késsia Barbosa Soares

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ATA DE DEFESA DE TESE

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Aos dois dias do mês de setembro do ano de dois mil e vinte e dois, às 09h00, no Centro de Tecnologia desta Universidade, reuniu-se a Banca Examinadora composta pelas professoras: Profa. Dra. Juliana Késsia Barbosa Soares, orientadora do trabalho e presidente da Banca, Profa. Dra. Ana Carolina dos Santos Costa (Examinadora Externa à Instituição/UFRPE), Profa. Dra. Mirian Graciela da Silva Stiebbe Salvadori (Examinadora Externa/UFPB), Profa. Dra. Jailane de Souza Aquino (Examinadora Interno/UFPB) e Profa. Dra. Maria Elieidy Gomes de Oliveira (Examinador Externo/UFPB). A reunião teve por objetivo julgar o trabalho do discente **Diego Elias Pereira**, matricula nº 20181017472, sob o Título **"EFEITOS DO CONSUMO MATERNO DA AMÊNDOA DE BARU (*Dipteryx alata Vog.*) SOBRE A MICROBIOTA INTESTINAL, ESTRESSE OXIDATIVO CEREBRAL E DESENVOLVIMENTO COMPORTAMENTAL DA PROLE DE RATAS WISTAR."** Os trabalhos foram abertos pela Profa. Dra. Juliana Késsia Barbosa Soares. A seguir foi concedida a palavra ao discente para apresentação do trabalho. Cada Examinador(a) arguiu o doutorando, com tempos iguais de arguição e resposta. Terminadas as arguições, procedeu-se o julgamento do trabalho, concluindo a Banca Examinadora por sua aprovação. Atendidas as exigências da Resolução nº 15/2019/CONSEPE que regulamentam o Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, e nada mais havendo a tratar, foi lavrada a presente Ata, que vai assinada pelos membros da Banca Examinadora e pela doutoranda.

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A Deus! Pois por Ele e para Ele são todas as coisas.

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RESUMO

Este trabalho avaliou o impacto do óleo e amêndoa de baru sobre a microbiota fecal, parâmetros de estresse oxidativo cerebral e desenvolvimento comportamental da prole de ratas *Wistar* tratadas durante a gestação e lactação. As mães foram randomizadas em três grupos: Controle - receberam água destilada através de gavagem; Óleo – receberam 2.000 mg do óleo de baru/kg e Amêndoa - receberam 2.000 mg da amêndoa de baru/kg. Após o nascimento, a prole foi padronizada em ninhadas de 6 filhotes machos. Ao final da lactação, amostras do leite materno foram coletadas para análise do perfil de ácidos graxos. Na prole, foi realizada avaliação do desenvolvimento somático e ontogênese reflexa durante os primeiros 21 dias de vida. Na fase da adolescência (T45) e adulta (T90) foram realizados testes de Habituação ao Campo Aberto (OF); Reconhecimento de Objetos (TRO); Labirinto Aquático de *Morris* (LAM); Labirinto em Cruz Elevado (LCE) e Caixa Claro-escuro (CCE). O conteúdo de ácidos graxos e a análise histologica do cérebro foram avaliados em T45 e T90, assim como a coleta das fezes para análise de microbiota fecal. Tanto a administração do óleo e da amêndoa elevaram o teor de PUFAs no leite materno e no cérebro da prole adolescente e adulta, assim como, o teor de ácido docosahexaenóico e araquidônico. Observou-se aceleração da ontogenia reflexa e do desenvolvimento somático nos grupos experimentais. O grupo óleo apresentou diminuição do parâmetro de ambulação na segunda exposição ao CA, em T45. Na fase adulta, a redução ocorreu em ambos os grupos. No TRO, os grupos óleo e amêndoa apresentaram melhora da memória em curto e longo prazo, em T45. Na fase adulta, esses resultados só foram evidentes no grupo amêndoa. Os grupos experimentais apresentaram maior deambulação e *rearing* e redução do comportamento de autolimpeza e número de bolo fecal, em T45. Na fase adulta, verificou-se maior número de *rearing* e menor número de bolo fecal apenas no grupo amêndoa. No LCE, o grupo amêndoa apresentou maior número de entradas e tempo despendido nos braços abertos, em T45. O grupo amêndoa em T90, obteve maior número de entradas nos braços abertos quando comparado aos grupos óleo e controle. Na CCE, observou-se maior tempo de permanência dos animais experimentais no compartimento claro da caixa em T45 e T90. A análise histologica mostrou que a suplementação preservou as células do hipocampo e do córtex dos animais em ambas as fases da vida. Houve aumento dos níveis de glutationa e redução de MDA no cérebro da prole óleo e amêndoa, em T45. Em T90, apenas o grupo amêndoa apresentou níveis elevados de glutationa. As comunidades microbianas das fezes, assim como as vias metabólicas utilizadas pelas bactérias intestinais foram significativamente diferentes comparando os grupos experimentais ao controle em T45. Os resultados evidenciaram o efeito neuroprotetor da suplementação em diferentes fases da vida, sendo estes, associados ao impacto dos PUFAs e antioxidantes no desenvolvimento/proteção do sistema nervoso central, na remodelação da microbiota intestinal, bem como na sua produção de compostos neuroativos.

Palavras-chave: Antioxidantes; Baru; Neurodesenvolvimento; Microbiota fecal; PUFAs.

ABSTRACT

This study evaluated the impact of maternal supplementation with baru oil and almond on the fecal microbiota, brain oxidative stress and behavioral development of the offspring of Wistar rats treated during pregnancy and lactation. The mothers were randomized into three groups: Control (CG) - received distilled water by gavage; Oil – received 2.000 mg of baru oil/kg and Almond - received 2.000 mg of baru almond/kg. After birth, the offspring were standardized into litters of 6 male pups. At the end of lactation, breast milk samples were collected for analysis of the fatty acid profile. In the offspring, evaluation of somatic development and reflex ontogenesis was performed during the first 21 days of life. During adolescence (T45) and adulthood (T90) tests of Habituation to the Open Field (OF) were performed; Object Recognition test (ORT); Morris Water Maze (MWM); Elevated Plus Maze (EPM) and Light-Dark Box (LDB). Fatty acid content and histological analysis of the brain were evaluated at T45 and T90, as well as stool collection for fecal microbiota analysis. Supplementation increased the content of PUFAs in breast milk and in the brain of adolescent and adult offspring, as well as the content of docosahexaenoic and arachidonic acid ($p < 0.05$). Acceleration of reflex ontogeny and somatic development was observed in the experimental groups ($p <0.05$). The oil group showed a decrease in the ambulation parameter in the second exposure to OF, at T45. In adulthood, the reduction occurred in both groups ($p<0.05$). In ORT, the oil and almond groups showed improvement in short and long-term memory at T45. In the adult phase, these results were only evident in the almond group ($p<0.05$). The experimental groups showed greater ambulation and rearing and reduced grooming behavior and number of fecal bolus, at T45. In the adult phase, there was a higher number of rearing and lower number of fecal bolus only in the almond group ($p<0.05$). In the EPM, the almond group had the highest number of entries and time spent in the open arms, at T45. The almond group at T90 had a higher number of entries in the open arms when compared to the oil and control groups ($p<0.05$). In the LDB, a longer time spent by the experimental animals in the light compartment of the box was observed at T45 and T90 ($p<0.05$). Histological analysis showed that the supplementation preserved the cells of the hippocampus and cortex of the animals in both life stages. There was an increase in glutathione levels and a reduction in MDA in the brain of the oil and almond offspring, at T45. At T90, only the almond group showed high levels of glutathione ($p<0.05$). The microbial communities of faeces, as well as the metabolic pathways used by intestinal bacteria were significantly different comparing the experimental groups to the control at T45. The results showed the neuroprotective effect of supplementation at different stages of life, which are associated with the impact of PUFAs and antioxidants in the development/protection of the central nervous system, in the remodeling of the gut microbiota, as well as in its production of neuroactive compounds.

Keywords: Baru; PUFAs; Phytocomplexes; gut-brain axis; neurodevelopment.

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ACA	Abertura do Conduto Auditivo
AG's	Ácidos graxos
AGE	Ácidos graxos essenciais
ALA	Ácido graxo linolênico
AOL	Abertura dos Olhos
AP	Aversão ao Precipício
APA	Abertura do Pavilhão Auricular
APE	Aparecimento dos Pelos Epidérmicos
ARA	Ácido araquidônico
ATP	Adenosina trifosfato
BHE	Barreira hematoencefálica
BNDF	Fator neurotrófico derivado do cérebro
GC	Grupo controle
CES	Centro de Educação e Saúde
CEUA	Comissão de Ética no uso de Animais
CFT	Compostos fenólicos totais
CPV	Colocação Espacial Desencadeada Pelas Vibrissas
CTDR	Centro de Tecnologia e Desenvolvimento Regional
DHA	Ácido docosahexaenóico
EII	Erupção dos dentes incisivos inferiores
EIS	Erupção dos dentes incisivos superiores
EPA	Ácido eicosapentaenoico
EQ	Equivalentes de catequina
EROs	Espécies reativa de oxigênio
FRAP	Atividade redutora férrica
FABPm	Proteína de ligação a ácidos graxos associada à membrana
FATP1 e 4	Proteínas de transporte presentes na membrana da BHE
GAE	Equivalentes de ácido gálico
GN	Geotaxia negativa
IL1-β	Interleucina 1 beta
LABROM	Laboratório de Bromatologia

LANEX	Laboratório de Nutrição Experimental
LMCA	Laboratório Multiusuário de Caracterização e Análise
LAFQ	Laboratório de Análise Físico-química
LPNB	Laboratório de Produtos Naturais Bioativos
MDA	Malonaldeído
MUFA	Ácido graxo monoinsaturado
Mfsd2a	Transportador para ácidos graxos ω-3
OF	Objeto familiar
ON	Objeto novo
TN	Tempo de exploração do objeto novo
TF	Tempo de exploração do objeto familiar
PUFAs	Ácido graxo poliinsaturados
PP	Prensão palmar
RDQL	Recuperação do decúbito em queda livre
RPD	Recuperação postural de decúbito
RS	Resposta ao susto
SAT	Ácido graxo saturado
SN	Sistema nervoso
SNC	Sistema nervoso central
T45	45 dias de vida
T90	90 dias de vida
TNF-ɣ	Fator de necrose tumoral gama
TRO	Teste de Reconhecimento de Objetos
UFCG	Universidade Federal de Campina Grande
UFPB	Universidade Federal da Paraíba
UFRN	Universidade Federal do Rio Grande do Norte
UFRPE	Universidade Federal Rural de Pernambuco

SUMÁRIO

1 INTRODUÇÃO

O período de gestação e lactação é considerado crítico para o neurodesenvolvimento e fatores como a nutrição materna são imprescindíveis para a formação inicial da microbiota intestinal da prole e neurogênese adequada (BUSS *et al*., 2012; BORDELEAU *et al*., 2021). Uma possível via mecanicista estabelece uma interface entre mãe/feto e a formação de uma microbiota inicial nesta fase da vida (DASH *et al*., 2022). Durante o período pós-natal a remodelação da microbiota intestinal é decorrente do aleitamento materno (SELMA-ROYO *et al*., 2021). Tanto no período intrauterino quanto no extrauterino a deficiência e/ou ingestão excessiva de nutrientes materno influenciam a regulação do eixo intestino-cérebro na prole (JANTSCH *et al*., 2022).

Microrganismos intestinais contribuem para o neurodesenvolvimento, participando da formação da barreira hematoencefálica (BHE), neurogênese, maturação da microglia e mielinização (CERDÓ *et al*., 2020; DASH *et al*., 2022; PARKER *et al*., 2020). A relação homeostática entre os filos *Firmicutes e Bacteroidetes,* assim como a abundância relativa de microrganismos como *Akkermansia, Lachnospiraceae, Bifidobacterium , Roseburia e Lactobacillus* são cruciais para a produção de compostos neuroativos, que por sua vez, impactam a neuroquímica, neurofisiologia e anatomia do tecido cerebral (FU *et al*., 2021; ONISHI *et al*., 2017). Ademais, a ingestão materna de gorduras poli-insaturadas e antioxidantes auxiliam o neurodesenvolvimento, por promoverem a abundância de bactérias intestinais benéficas e de vias metabólicas, assim como de compostos neuroativos importantes para a gênese e manutenção do sistema nervoso central (SNC) (DUEÑAS *et al*., 2015; FU *et al*., 2021; ROBERTSON *et al*., 2017).

Além da contribuição da microbiota intestinal para o neurodesenvolvimento, os PUFAs e compostos antioxidantes dietéticos podem ser disponibilizados diretamente ao feto, através de mecanismos que envolvem a transferência placentária e aleitamento materno DEMMELMAIR *et al*., 2016). Estes por sua vez, cruzam a BHE e se depositam no tecido cerebral da prole, contribuindo para a neurogênese, migração neural, apoptoses, sinapses e mielinização (INNIS, 1991). Por não serem sintetizados endogenamente, os PUFAs necessitam serem ingeridos para um suprimento materno e fetal adequado (HARAYAMA; SHIMIZU, 2020; MORGANE *et al*., 1993). A dieta materna rica em ácidos graxos poli-insaturados (PUFAs) e antioxidantes está relacionada com a

aceleração do desenvolvimento reflexo e somático, melhor cognição e aprendizagem (MELO *et al*., 2019), além de redução do comportamento tipo ansioso na prole (SILVA *et al*., 2021).

Outros ácidos graxos como os saturados e monoinsaturados também contribuem para o bom desenvolvimento do sistema nervoso (SN). O ácido monoinsaturado oleico, por exemplo, é o principal constituinte da mielina, enquanto o ácido graxo saturado palmítico participa dos processos de gliogênese, sinaptogênese e mielinização (FUKATA; FUKATA, 2010; GONZÁLEZ; VISENTIN, 2016; SONG *et al*., 2019).

As recomendações dietéticas sugerem que a gordura deve representar cerca de 20% a 35% da ingestão energética e o tipo de gordura ingerida é fundamental para a prevenção de diversas doenças (LAITINEN, 2021). Uma nutrição materna inadequada durante a gestação e lactação pode resultar em disbiose, comprometimento da neurogênese, aumento do estresse oxidativo cerebral, maior probabilidade de déficit de aprendizagem, declínio cognitivo e comportamentos do tipo ansiogênico e depressivo na mãe e na prole (DELPECH *et al*., 2015; FURMAN; AXELSEN, 2019; LEYROLLE *et al*., 2021; TANG *et al*., 2016).

A amêndoa de baru (*Dipteryx alata* Vogel.) é considerada um alimento com potencial efeito funcional por conter uma variedade de nutrientes essenciais com ação farmacológica (LIMA *et al*., 2022). Fruto do barueiro, pertencente à família da Leguminosae Faboideae, nativa das regiões do cerrado brasileiro apresenta em sua composição elevado conteúdo lipídico, destacando-se pelo conteúdo de ácido graxo saturado palmítico (C16:0), monoinsaturados (MUFA) oleico (C18:1, ω-9) e poliinsaturados (PUFAs) linoleico (C18:2, ω-6) e linolênico (C18:3, ω-3), este último em menor quantidade (OLIVEIRA-ALVES *et al*., 2020; BORGES *et al*., 2022). Além dos lipídios, a amêndoa de baru apresenta alto teor de proteínas (40%) e é fonte de todos os aminoácidos essenciais (OLIVEIRA *et al*., 2011). Apresenta quantidades consideráveis de minerais, fibras e compostos fenólicos com alto poder antioxidante (SILVA *et al*., 2020; LEMOS *et al*., 2012). Estudos experimentais com modelos animais evidenciam efeitos benéficos da amêndoa e do óleo de baru, com foco nos seus componentes lipídicos e antioxidantes, na melhora do perfil bioquímico e redução de gordura corporal (FERNANDES *et al*., 2015), na redução de marcadores do estresse oxidativo (SOUZA *et al*., 2019), na melhora da função vascular e redução da agregação plaquetária (SILVA-LUIS *et al*., 2022).

Devido a importância nutricional da amêndoa de baru, seus benefícios para a saúde e a escassez de estudos na literatura investigando o impacto de seu consumo durante a fase perinatal sobre a microbiota intestinal e neurodesenvolvimento da prole, com o presente trabalho objetivou-se avaliar o impacto da suplementação materna com óleo e amêndoa de baru sobre a microbiota intestinal, estresse oxidativo cerebral e desenvolvimento comportamental da prole de ratas *Wistar* tratadas durante a gestação e lactação.

2 REFERENCIAL TEÓRICO

2.1 ÁCIDOS GRAXOS ESSENCIAIS PARA O NEURODESENVOLVIMENTO

O desenvolvimento do cérebro é um evento dinâmico sequencial, caracterizado por estágios de crescimento e maturação geneticamente programados (CLANDININ *et al*., 1980; MORGANE *et al*., 1993). Sua formação inicia-se na fase embrionária, por volta da terceira semana de gestação e compreende processos de neurulação, proliferação, migração celular, diferenciação, sinaptogênese, poda sináptica e mielinização Kolb *et al*. (2014). Até os 3 primeiros anos pós-natal, a quantidade de conexões realizadas entre as células do sistema nervoso (SN) é bem maior do que em qualquer outro momento da vida (TIERNEY; NELSON, 2009). Durante esta fase, dita como crítica do desenvolvimento, a arborização dendrítica resulta no desenvolvimento e/ou maturação de processos biológicos relacionados a características sensoriais, visuais e auditivas (KRETCHMER *et al*., 1996). Insultos nutricionais neste período podem implicar na má formação do SN e no comprometimento cognitivo a curto e longo prazo (PRADO; DEWEY, 2014; SMITH; REYES, 2017; ZHANG *et al*., 2014)

Durante a fase crítica do desenvolvimento, o excesso ou escassez de nutrientes essenciais impactam a neuroanatomia, neuroquímica e neurofisiologia do cérebro BUCHHORN, 2016). Pesquisas anteriores reportam que a ingesta materna inadequada implica no comprometimento de eventos ontogenéticos, devido a ocorrência de má formação de estruturas como hipocampo, corpo estriado, córtex visual e auditivo ALAMY; BENGELLOUN, 2012; NAIK *et al*., 2015; WINICK; ROSSO, 1973). Ademais, estudos com roedores submetidos a dietas deficientes em nutrientes essenciais durante o período de gestação e lactação demonstraram prejuízos em relação a maturação do SN, aprendizagem espacial e memória de seus descendentes (BORDELEAU *et al*., 2021; DECOEUR *et al*., 2022; PÉREZ-GARCIÁ *et al*., 2016). Dentre os nutrientes essenciais, os lipídios dietéticos estão em destaque devido aos inúmeros estudos que demonstram sua importância sobre a neurogênese, síntese e atividade de neurotransmissores, mecanismos cognitivos e de aprendizagem (CUSTERS *et al*., 2022; MORLEY; BANKS, 2010).

O SN é constituído majoritariamente por lipídios e intermediários lipídicos, representando cerca de 60% do peso seco do cérebro (UAUY *et al*., 2001) . Estes, desempenham papeis importantes para a integridade estrutural e biofísica das células

nervosas, além de atuarem como moléculas sinalizadoras bioativas e fonte de combustível para o metabolismo mitocondrial (CERMENATI *et al*., 2015; BRUCE *et al*., 2017; BONETTO; DI SCALA, 2019). Dentre os lipídios que compõem o tecido cerebral estão os ácidos graxos essenciais (AGE) (SINGH, 2005). Apesar de não serem sintetizados pelo organismo, estes podem ser obtidos através de fontes alimentares (HARAYAMA; SHIMIZU, 2020). Os principais AGE envolvidos no desenvolvimento cerebral são o α-linolênico (18:3 ω-3) e o α-linoléico (18:2 ω-6), precursores dos ácidos graxos araquidônico - ARA (20:4 ω-6), eicosapentaenoico - EPA (20:5 ω-3) e docosahexaenóico - DHA (22:6 ω-3), sendo estes sintetizados no retículo endoplasmático liso, especialmente no fígado, através de biorreações de alongamento da cadeia de carbono e dessaturação (KOREN, 2015), conforme descrito na Figura 1.

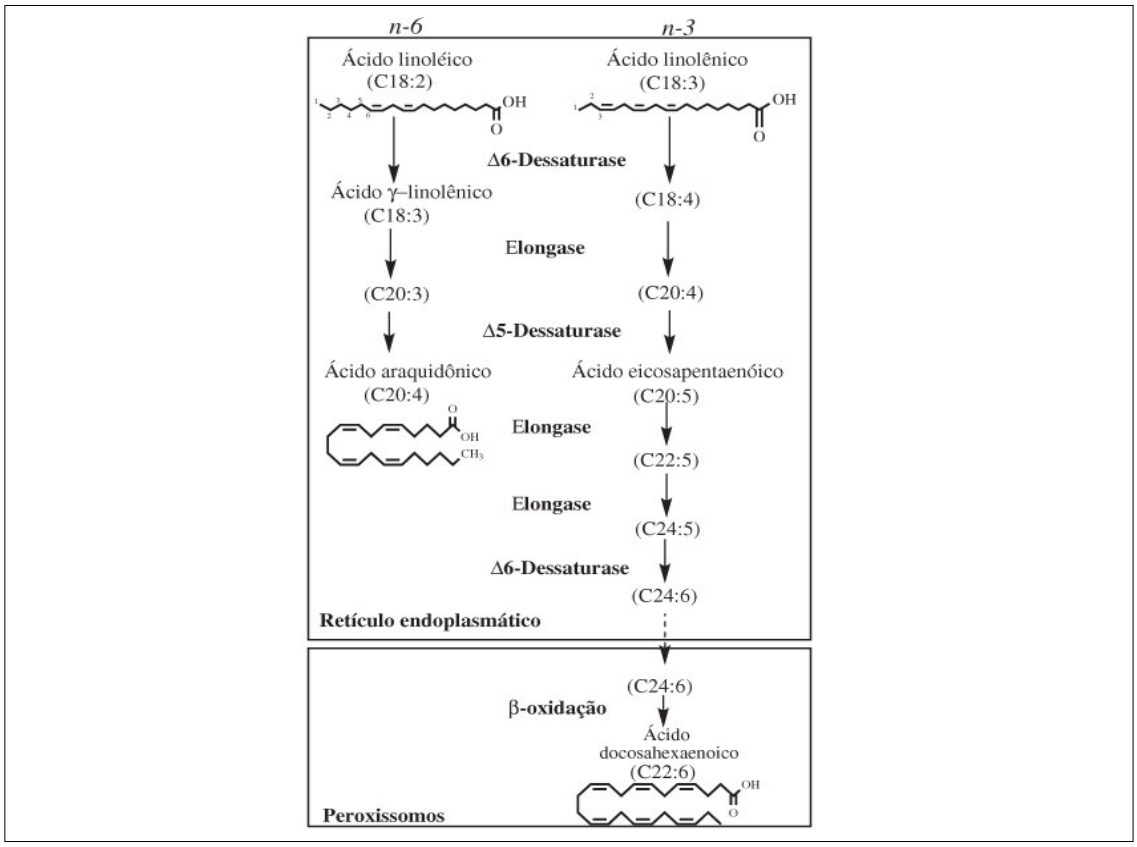


Figura 1 - Esquema do metabolismo dos ácidos graxos da família ω3 e ω6**. Fonte:** Appolinário *et al*., (2011).

Embora alguns AG's possam ser obtido pela síntese de novo, o acúmulo de ácidos graxos poliinsaturados (PUFAs) no tecido cerebral do feto é dependente do suprimento materno pré-fetal (através de transferência placentária via proteína de ligação a ácidos graxos associada à membrana - FABPm) e pós-fetal (através do aleitamento materno)

(KOLETZKO *et al*., 2000). Os mecanismos pelos quais os ácidos graxos cruzam a barreira hematoencefálica (BHE) e se depositam nas células neuronais do feto ainda não estão totalmente elucidados, porém, diversos autores postulam que esta ação seja decorrente da atividade de proteínas de transporte presentes na membrana da BHE (FATP1 e FATP4); da atividade da ácido graxo translocase/CD36 que promove o transporte de AG's através de células endoteliais dos microvasos do cérebro e também pela ação do transportador para ácidos graxos ω-3 (Mfsd2a) no endotélio da BHE MITCHELL *et al*., 2011; WONG *et al*., 2016).

No cérebro os PUFAs são primariamente esterificados a fosfolipídios cerebrais, principalmente na forma de fosfatidiletanolamina e fosfatidilserina, porém, podem ser liberados da membrana neuronal transformando-se em moléculas biologicamente ativas e assim regular processos como transdução de sinais intracelulares, ativação de fatores de transcrição e modulação da expressão gênica (BAZINET & LAYÉ, 2014; BENTSEN, 2017; SCHVERER *et al*., 2020). O ácidos araquidônico - ARA (ω-6) e o docosahexaenóico - DHA (ω-3) são os PUFAs mais abundantes no cérebro, representando cerca de 30% dos ácidos graxos totais e, em menores concentrações devido ao seu extenso metabolismo está o ácido eicosapentaenóico - EPA (ω-3) (SAMBRA *et al*., 2021). Ensaios clínicos envolvendo roedores e seres humanos tem apoiado a hipótese de que o suprimento adequado de PUFAs durante a gestação e lactação impactam a expansão de células gliais, axônios e dendritos dos neurônios, além de influenciar o desenvolvimento e função dos sistemas monoaminérgicos (vias serotoninérgicas, dopaminérgicas e noradrenérgicas) e a plasticidade sináptica (CHALON *et al*., 2001; UAUY *et al*., 2001).

O acúmulo de ARA, DHA e EPA no cérebro é mais intenso durante o terceiro trimestre da gravidez e os dois primeiros anos após o nascimento, quando ocorre o platô . O aporte de ARA durante esta fase é considerado fundamental, uma vez que atua sobre as vias de sinalização envolvidas na divisão celular e na síntese de ácido adrenico (C22:4 n-6), componente crucial para a neurogênese e enriquecimento de lipídios mielínicos (CHEN *et al*., 2020). A atividade do ARA sobre o processo de neurogênese em parte, deve-se a sua capacidade de ativar a proteína de membrana neuronal (sintaxina-3) envolvida no crescimento e reparo de neuritos e na transmissão sináptica (HADLEY *et al*., 2016). Outros estudos demonstram o envolvimento de ARA na ativação de proteínas quinases e canais iônicos que impedem a reciclagem de neurotransmissores, controlando

e modulando a transmissão sináptica e excitabilidade neuronal (ANGELOVA; MÜLLER, 2009).

No que diz respeito ao DHA, este é o ácido graxo poliinsaturados de cadeia longa (LCPUFA) mais abundante no cérebro, podendo ser encontrado esterificado em fosfatidiletanolamina, fosfatidilserina e em fosfatidilcolina nas membranas celulares que formam a substância cinzenta (O'BRIEN *et al*., 1965). Apesar dos neurônios não sintetizarem DHA a partir de ácido linolênico, este pode ser produzido pelas células gliais (especialmente os astrócitos) através de reações de dessaturação e alongamento de ALA, sendo posteriormente transferido aos neurônios (SAMBRA *et al*., 2021). Evidências cientificas apontam a sua importância frente a ao crescimento, migração e maturação neuronal [conforme revisado em MELDRUM; SIMMER, 2016]. Devido a sua estrutura química e insaturações, confere flexibilidade e fluidez à membrana neuronal, ampliando a eficiência na transdução de sinal (STILLWELL *et al*., 2005). Além disso, é capaz de modular a expressão de genes relacionados à geração de energia neuronal requisito fundamental para a excitabilidade elétrica da célula (FELTHAM *et al*., 2020).

A importância do aporte de EPA durante o período crítico de desenvolvimento pode ser justificado pela capacidade deste ácido graxo em regular a neurogênese, proteger as células neuronais contra danos oxidativos, estimular as proteínas mielínicas, além de melhorar as funções vasculares e endoteliais (BAZINET *et al*., 2020; PATAN *et al*., 2021). Os níveis de EPA no tecido cerebral, parece também estarem relacionados a modulação da concentrações de citocinas (interleucina 1 beta - IL1-β e o Fator de necrose tumoral gama - TNF-ɣ) e de moléculas essenciais para o controle do mecanismo neurotrófico (proteína de ligação ao elemento de resposta ao AMPc - CREB e Fator neurotrófico derivado do cérebro - BNDF) (BAZINET *et al*., 2020).

Dietas deficientes em PUFAs implicam em alterações significativas no desenvolvimento neural adequado da prole, tanto em termo anatômicos quanto bioquímicos, a citar: redução do volume do hipocampo, maior susceptibilidade ao estresse oxidativo, redução dos níveis de BNDF, interrupção da maturação de oligodendrócitos e integridade da mielina, diminuição da neurogênese na região do giro denteado do hipocampo e alterações do sistema glutamatérgico e serotoninérgico (TANG *et al*. (2016). Por outro lado, a suplementação materna de PUFAs está relacionada a efeitos na progênie, como aceleração do neurodesenvolvimento, redução do estresse oxidativo no tecido cerebral e melhora de desordens neuropsiquiátricas, como sintomas de ansiedade e depressão (BENTSEN, 2017).

Outros AG's não essenciais também estão envolvidos em processos importantes no que diz respeito ao desenvolvimento do SN. O ácido graxo saturado palmítico por exemplo, participa de processos de palmitoilação que regula o tráfego, função e sinalização das proteínas neuronais, auxiliando no desenvolvimento neuronal e na plasticidade sináptica (FUKATA; FUKATA, 2010). Ademais, outras pesquisas têm evidenciado a participação deste AG em atividades relacionadas a gliogênese e mielinização (GONZÁLEZ; VISENTIN, 2016). No que diz respeito ao ácido monoinsaturado oleico (18:1 n-9), este é um dos principais constituintes dos fosfolipídios de membrana e é altamente concentrado em mielina, além disso, atua como fator neurotrófico promovendo o crescimento axonal e dendrítico, aumentando a migração e agregação neuronal, além de facilitar a formação de sinapses (SONG *et al*., 2019). De acordo com Shi *et al*., (2022), o ácido oleico pode atuar sobre a proteína TLX, que é receptor nuclear capaz de aumentar a proliferação celular e a neurogênese no hipocampo. Importa ressaltar, que assim como os AGE, o ácido palmítico e oleico utilizam dos mesmo mecanismos (via placenta/leite materno) para serem depositados no cérebro da prole (HAMILTON; BRUNALDI, 2007).

2.2 EFEITO NEUROPROTETOR DOS COMPOSTOS ANTIOXIDANTES

O tecido cerebral humano apresenta uma alta taxa de atividade metabólica oxidativa, consumindo cerca de mais de 20% do oxigênio basal total (MAGISTRETTI; ALLAMAN, 2015). A produção de Espécies reativa de oxigênio (EROs) neste tecido é decorrente da atividade das mitocôndrias, que reduzem o oxigênio basal a H_2O a fim de apoiar a síntese de adenosina trifosfato (ATP). Porém, quanto maior a concentração de oxigênio menos eficiente é a cadeia transportadora de elétrons em relação ao vazamento de EROs (TURRENS, 2003). EROs são moléculas instáveis extremamente reativas capazes de interagir com lipídios e outras biomoléculas alterando sua estrutura e função (CADET; DAVIES, 2017). Apesar da alta concentração de EROs ser prejudicial para o funcionamento normal do cérebro, dentro dos níveis adequados estes atuam como molécula de sinalização em vários processos biológicos, incluindo a neurotransmissão (PAMBUK; MUHAMMAD, 2018).

A síntese de radicais de oxigênio altamente reativos é decorrente de interações entre vários metais de transição e seus equivalentes redutores (PISOSCHI; POP, 2015). Como o tecido cerebral é enriquecido de ferro, cobre, zinco e manganês, a produção de

EROs se torna mais efetiva devido a reação de Fenton (LEE *et al*., 2020). As células neuronais contam com um complexo sistema antioxidante capaz de reduzir diferentes substâncias químicas (SHIM; KIM, 2013). A superóxido dismutase, catalase e glutationa peroxidase fazem deste sistema enzimático e atuam prevenindo a geração de EROs, bloqueando e capturando os radicais gerados (IGHODARO; AKINLOYE, 2018). Devido ao abundante conteúdo lipídico e as altas demandas energéticas, por vezes, esse sistema se mostra ineficiente, tornando o cérebro um alvo fácil para insultos oxidativos excessivos (SALIM, 2017).

Atualmente, o uso de antioxidantes derivados de matrizes vegetais para reduzir os danos causados pelo estresse oxidativo às células nervosas tem sido bem documentado (ALBARRACIN *et al*., 2012; CUI *et al*., 2020). Antioxidantes como polifenóis, vitaminas, alcalóides, polissacarídeos e peptídeos ativos ajudam a manter a estrutura e a função dos neurônios e prolongam seu estado saudável (KAMAT *et al*., 2008). Durante a gestação e lactação estes componentes são disponibilizados ao feto via placenta e leite materno (AYCICEK *et al*., 2006; SEBASTIANI *et al*., 2022). Algumas dessas moléculas atravessam a barreira hematoencefálica (BHE) de forma intacta e se depositam no cérebro da prole, enquanto outras necessitam primeiramente serem biotransformadas ou metabolizados pela microbiota intestinal, por exemplo (FILOSA *et al*., 2018; VELÁSQUEZ-JIMÉNEZ *et al*., 2021). Uma vez dentro do SNC, os antioxidantes mantém a integridade molecular, celular, estrutural e funcional do cérebro (LÜ *et al*., 2010).

Vários estudos apoiam as habilidades neuroprotetoras dos polifenóis (REBAS *et al*., 2020; SPAGNUOLO *et al*., 2016; SZWAJGIER *et al*., 2017). Estes podem ser subclassificados em flavonóides, ácidos fenólicos e taninos, dependendo de sua estrutura química (RASOULI *et al*., 2017). A ingestão destes componentes tem sido frequentemente associada a maior proteção neuronal e redução de estresse oxidativo, neurotoxinas e neuroinflamação, além de promover melhora da memória, aprendizado e transtorno de humor (CARUSO *et al*., 2022).

Os flavonóides correspondem à classe fenólica de maior importância e variedade entre as matrizes de origem vegetal (PANCHE *et al*., 2016). Compartilham uma estrutura comum que consiste em dois anéis aromáticos, sendo estes unidos por três átomos de carbono, originando um heterociclo oxigenado (KUMAR; PANDEY, 2013). Os flavonóides podem alcançar a circulação sistêmica e outros tecidos logo após serem absorvidos no intestino (BAKY *et al*., 2021). A chegada dos flavonóides ao cérebro

depende de sua lipofilicidade, sendo a captação dos 0-metilados maior, devido a estes serem menos polares (SPENCER, 2009). No tecido cerebral induzem a secreção de células gliais e do fator de crescimento, além de prevenir a degradação de neurônios dopaminérgicos na substância negra (JAEGER *et al*., 2018); protegem contra a ruptura da mielina (ZHAO *et al*., 2013); interagem com cascatas críticas de sinalização de proteínas e lipídios quinases promovendo a sobrevivência neuronal e plasticidade sináptica; induzem efeitos benéficos no sistema vascular cerebral promovendo a angiogênese e neurogênese; além de reduzir efetivamente os fatores inflamatórios (fator-α, interleucina-6, interleucina-1β, NO e PGE2) e aumentar as propriedades neuroprotetoras da micróglia e dos astrócitos (BAKHTIARI *et al*., 2017).

Os ácidos fenólicos são outra classe de antioxidantes que compreendem uma ampla família de moléculas com um ou mais anéis fenólicos. Estão presente nas matrizes vegetais como compostos fisiológicos essenciais (KIOKIAS *et al*., 2020). Podem exercer neuroproteção através de inúmeros mecanismos, incluindo a capacidade de diminuir os níveis de pró-oxidantes e mediadores pró-inflamatórios, elevar a produção e/ou a atividade antioxidantes e anti-inflamatórios, promover o aumento da concentração e liberação de fatores neurotróficos, prevenir a agregação de proteínas, elevar os níveis de mediadores anti-apoptóticos, ativar vias de sinalização pró-sobrevivência bloqueando vias de sinalização de morte celular, além de diminuir a atividade de astrócitos e micróglia reativos, Figura 2 (SZWAJGIER *et al*., 2017).

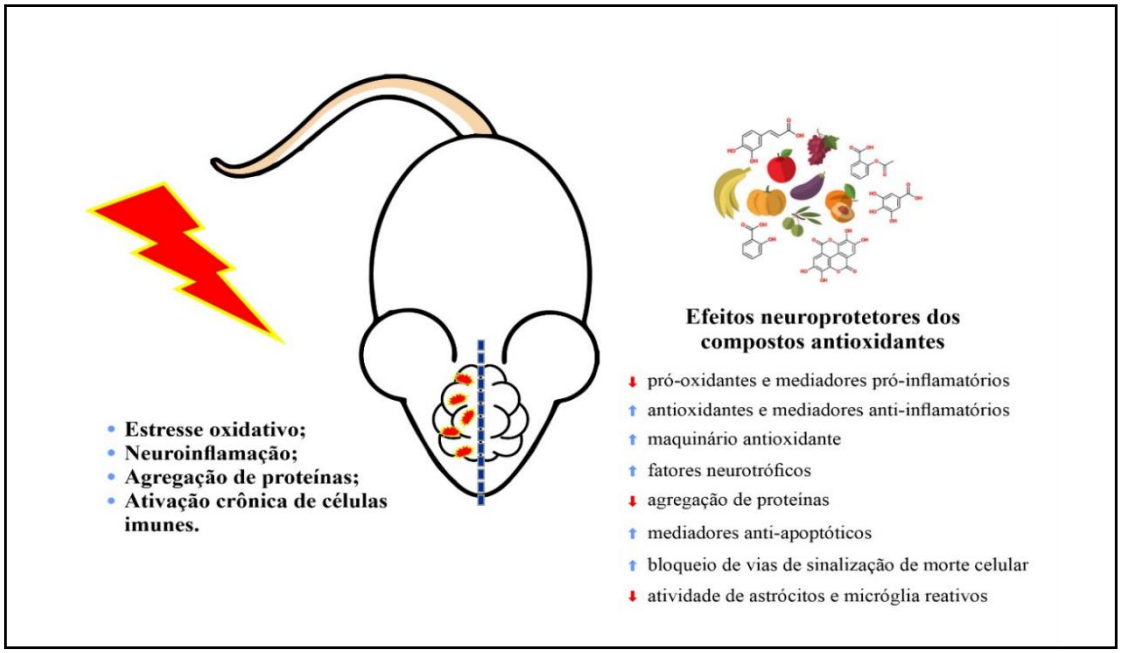


Figura 2 – Atividade neuroprotetora dos compostos antioxidantes. **Fonte:** adaptado de Szwajgier *et al*., (2017).

Dentre os ácidos fenólicos o ácido ferúlico é um composto comumente encontrado nas folhas e sementes de plantas. Sua estrutura conjugada pode formar um radical fenoxi relativamente estável capaz de bloquear a reação em cadeia do radical livre, protegendo os neurônios de roedores contra o estresse oxidativo através da inibição da ativação da p38 MAPK (proteínas quinases ativadas por mitógenos), caspase-3 (marcador de morte celular programada), COX-2 (prostaglandin-endoperoxide synthase 2) e hinos (óxido nítrico sintase induzível) (LI *et al*., 2021). Sua ingestão está associada a maiores níveis de serotonina e norepinefrina no hipocampo e córtex frontal (regiões especificas relacionadas a memória e ansiedade), aumento da atividades de superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GSH-Px), além de redução da peroxidação lipídica (TBARs) no cérebro de camundongos (CHENG *et al*., 2008). Outras pesquisas reportam a capacidade do ácido ferúlico em regular de forma positiva o gene do fator neurotrófico derivado do cérebro (BDNF), em inibir interleucinas pró-inflamatória (IL-6, IL-1), restaurar os níveis de glutamato e resgatar neurônios dopaminérgicos na área da substância negra e no corpo estriado (SZWAJGIER *et al*. (2017).

O ácido gálico, composto pertencente ao grupo dos fenólicos, também apresenta propriedades antioxidantes, anti-inflamatórias e neuroprotetoras bem documentadas [conforme revisado por Shabani *et al*. 2020]. Estudos com roedores evidenciaram que a ingestão crônica deste composto foi capaz de prevenir déficits cognitivos e comprometimento sináptico atenuando a liberação de citocinas pró-inflamatórias (IL-1β, IL-6 e TNF-α), reduzindo a neuroinflamação e aumentando a atividade neurotrófica (SARKAKI *et al*., 2015; KIM *et al*., 2011). Em pesquisas utilizando modelos de declínio cognitivo e neurotoxicidade induzido por estreptozotocina (STZ); ciclofosfamida e arsenito de sódio, respectivamente, pode-se observar que a suplementação do ácido gálico foi capaz de restaurar o nível aumentado das citocinas pró-inflamatórias IL-6 e TNF-α, bem como o nível reduzido de proteína quinase Akt e pAkt no hipocampo, além de diminuir a peroxidação lipídica e elevar a atividade de GPx, CAT e SOD em diferentes regiões cerebrais (NAGHIZADEH; MANSOURI, 2015; GOUDARZI *et al*., 2019).

O ácido cumárico é uma molécula também pertencente ao grupo dos polifenólicos, possui atividade neuroprotetora evidenciada tanto em estudos in vitro quanto in vivo (GUVEN *et al*., 2015; UEDA *et al*., 2019). Em ratos, sua administração esteve correlacionada a redução de estresse oxidativo e a degeneração axonal (GUVEN *et al*., 2015). Em pesquisas que utilizaram modelos isquêmicos a suplementação diminuiu de

forma significativa o fator 1-alfa (HIF1α), fator nuclear kappa B (NF-κB), a atividade da acetilcolina (AChE) e malondialdeído (MDA) no cérebro dos roedores, nesse mesmo modelo a administração do ácido cumárico promoveu atividade antiapopitótica, evidenciada através da redução da caspase-3 (DAROI, 2021; DAROI *et al*., 2022; EKINCI-AKDEMIR *et al*., 2017).

A neuroproteção do ácido elágico e homovanílico também tem sido frequentemente investigados. O ácido elágico é um composto fenólico orgânico heterotetracíclico capaz de reduzir de forma significativa os níveis de MDA e aumentar a atividade de GSH-Px e CAT, além do mais, a suplementação deste ácido esteve relacionada a redução de marcadores pró-inflamatórios (proteína ácida fibrilar glial - GFAP e proteína C-reativa - CRP), redução dos níveis de AchE e aumento da concentração de sinaptofisina e preservação da arquitetura neural (JHA; PANCHAL; SHAH, 2018). Quanto ao ácido homovanílico, também conhecido como ácido 3-metoxi-4-hidroxifenilacético, este esteve relacionado a redução de sintomas depressivos e de humor em humanos, por modular a atividade dopaminérgica e plasticidade neuronal (STUERENBURG; GANZER; MÜLLER-THOMSEN, 2004). Todos esses achados parecem repercutir de forma direta ou indireta no desempenho de roedores frente a testes cognitivos e comportamentais (CARUSO *et al*., 2022).

2.3 ALIMENTOS FONTES DE LIPÍDIOS E COMPOSTOS ANTIOXIDANTES NA MEMÓRIA E ANSIEDADE

Os PUFAs desempenham papel crucial na neurofisiologia e bioquímica do tecido cerebral, protegendo a estrutura e as funções da membrana neuronal e assim, reduzindo o risco de distúrbios do sistema nervoso central e declínio cognitivo (KOREN; FACMT, 2012). Diversas pesquisas envolvendo humanos e roedores tem apontado a deficiência de PUFAs como um dos principais mecanismos envolvidos no comprometimento do aprendizado e memória a curto e longo prazo, além do risco aumentado para o desenvolvimento do comportamento de ansiedade (DECOEUR *et al*., 2022; GONZÁLEZ; VISENTIN, 2016; MÜLLER *et al*., 2015).

Como já discutido, os PUFAs além de conferir fluidez a membrana neuronal, agem como moléculas sinalizadoras sob diversos processos fundamentais para o funcionamento adequado do SN (BOURRE, 2009) . Um desequilíbrio entre os estímulos pró-oxidantes e as defesas antioxidantes contribuem para o estresse oxidativo no tecido

cerebral (ARUOMA *et al*., 2003). A ação das EROs sobre as membranas neuronais (ricas em PUFAs) concorre para o processo de peroxidação lipídica, atraso no desenvolvimento neurológico e pior desempenho em testes neurocognitivos (SHICHIRI, 2014). O impacto dos antioxidantes endógenos na prevenção dos declínios cognitivos tem se tornado alvo de investigação (SURVESWARAN *et al*., 2007). Roedores suplementados com PUFAs e antioxidantes durante o início da vida apresentaram aumento da atividade de enzimas antioxidantes, redução do estado pró-inflamatório, aceleração do neurodesenvolvimento e melhor desempenho frente aos testes de memória e ansiedade, conforme demonstrado no Quadro 1.

Quadro 1- Visão geral dos estudos que investigaram a relação do consumo de alimentos fontes de lipídios e antioxidantes com memória e comportamento de ansiedade em roedores.

REFERÊNCIA	OBJETIVO DO ESTUDO	INTERVENÇÃO	AVALIAÇÃO DE INTERESSE	TESTE UTILIZADO	RESULTADOS
Melo *et al*. (2019).	Avaliação da memória da prole de ratas *Wistar* tratadas durante a gestação e lactação com óleo (AO) e polpa de abacate (PA).	3.000 mg/kg de óleo e polpa de abacate (gavagem – 42 dias).	Memória a curto e longo prazo da prole adolescente – T45 e adulta – T90.	Campo aberto (CA) e Reconhecimento de objeto (ORT)	Os animais AO e PA apresentaram diminuição do parâmetro de deambulação na segunda exposição ao Teste CA, em T45 e T90 ($p < 0,05$). No ORT, a prole PA e AO apresentaram melhor desempenho no teste de memória a curto e longo prazo quando comparados ao GC, nas fases adulta e adolescente ($p < 0,05$).
Silva *et al*. (2021).	Avaliação dos efeitos da semente de macaíba na ansiedade, memória e estresse oxidativo no cérebro de ratos *Wistar* sadios e dislipidêmicos.	1.000 mg/kg da semente de macaíba (gavagem – 21 dias).	Memória a curto e longo prazo e comportamento de ansiedade.	Campo aberto (CA); Reconhecimento de objeto (ORT) e Labirinto em Cruz Elevado (LCE)	O grupo macaíba apresentou comportamento ansiolítico expressos através do maior número de *rearing* e menor número de *grooming* no teste de CA ($p < 0,05$). No LCE os animais macaíba e dislipidemia + macaíba apresentaram maior tempo de permanência nos braços abertos e na área central do aparato ($p < 0,05$). O consumo da semente de macaíba reduziu a taxa de exploração pelos animais macaíba e dislipidemia + macaíba no ORT ($p < 0,05$).
Melo *et al*. (2017).	Avaliação dos efeitos do consumo de castanha de caju na memória da prole ratas *Wistar* tratadas durante a gestação e lactação.	Dieta normolipídica (NL) - 7% de lipídios derivados da castanha de caju; Dieta Hiperlipídica (HL) - 20% de lipídios derivados da castanha de caju. (dieta *Ad. Libitum* – 42 dias).	Memória a curto e longo prazo da prole adulta.	Campo aberto (CA) e Reconhecimento de objeto (ORT)	NL apresentou melhor memória no teste de habituação em campo aberto; o NL e HL mostraram melhora da memória de curto prazo no ORT, mas danos de longo prazo no HL ($p < 0,05$).
Batool *et al*. (2017).	Avaliação do potencial protetor da amêndoa e nozes contra amnésia induzida por escopolamina em ratos.	400mg/kg de suspenção de amêndoa e nozes (gavagem – 28 dias).	Memória espacial e memória a curto e longo prazo.	Labirinto em cruz elevado (LCE), Labirinto aquático de Morris (MWM) e	A administração de amêndoas e nozes reverteu significativamente o efeito da escopolamina melhorando a memória dos animais. No teste do MWM os animais apresentaram redução significativa ($p<0,01$)

				Reconhecimento de objeto (ORT)	da latência de transferência e escape, bem como aumento do índice de reconhecimento do quadrante da plataforma ($p<0,01$); No ORT os animais apresentaram maior taxa de exploração pelo objeto novo ($p<0,01$). No LCE, verificou-se aumento na aquisição de aprendizagem e retenção de memória ($p<0,01$) em relação aos grupos controle.
Wang *et al*. (2019).	Avaliação dos efeitos do óleo de Noz na memória espacial de ratos através da modulação da expressão de genes de canais iônicos sensíveis a ácido, Asic2a e Asic4 .	1,1, 2,2, 11 g/kg de óleo de noz (gavagem – 30 dias).	Memória espacial	Labirinto aquático de Morris (MWM)	A suplementação com óleo de noz nas doses de 2,2 e 11 g kg^{-1} dia^{-1}melhorou significativamente o aprendizado e a memória ($p < 0,05$).
Bem *et al*. (2020).	Avaliar o efeito do extrato de semente de açaí na ansiedade induzida pela separação materna periódica (SM) em ratos machos adultos.	200 mg/kg do extrato da semente de açaí (gavagem – 34 dias).	Comportamento de ansiedade.	Testes de campo aberto (CA) e nado forçado (NF)	O tratamento com extrato da semente de açaí apresentou resultados semelhantes ao do grupo fluoxetina, aumentando significativamente ($p \leq 0,05$) o tempo gasto na área central e diminuiu o tempo gasto na área periférica pelos ratos expostos à SM. Os tratamentos com extrato da semente de açaí obtiveram tempo de imobilidade maior quando comparados aos demais grupos frente ao teste de NF.
Haider *et al*. (2012)	Examinar o impacto do consumo de azeite de oliva (AO) na ansiedade, atividade locomotora e memória espacial de ratos *Wistar*.	4 ml/kg de azeite de oliva (gavagem – 10 dias).	Comportamento de ansiedade e memória espacial.	Teste de campo aberto (CA), Caixa Claro-escuro (CCE); Labirinto aquático de Morris (MWM)	Os resultados mostraram que o grupo AO apresentou diminuição significativa da atividade locomotora no teste de CA. A memória de trabalho espacial de ratos tratados com azeite foi significativamente melhorada no teste do MWM, enquanto o tempo gasto na CCE foi significativamente aumentado, indicando o efeito ansiolítico do azeite.

Bahaeddin *et al*. (2016).	Avaliar o consumo da avelã sem pele como terapia para o comprometimento cognitivo induzido por injeção de Aβ intra hipocampal.	800 mg/kg de avelã sem pele (gavagem - 16 dias).	Comportamento de ansiedade e memória.	Teste do labirinto em Y, Aprendizagem de evitação passiva e Labirinto em Cruz Elevado (LCE)	Os resultados deste estudo mostraram que a alimentação com avelã melhorou a memória dos roedores (examinada usando o teste do labirinto em Y e a aprendizagem de evitação passiva) e reduziu o comportamento relacionado à ansiedade, onde os animais suplementados apresentaram maior número de entradas e tempo de permanência nos braços abertos do LCE, configurando um efeito ansiolítico da suplementação.
Frausto-González *et al*. (2021).	Avaliar os efeitos do extrato da castanha do Pará sobre o comportamento do tipo ansiolítico e redução de lipídios em camundongos recebendo uma dieta hipercalórica.	3 e 300 mg/kg do extrato de castanha do Pará (gavagem – 40 dias).	Comportamento de ansiedade.	Campo aberto (CA), Hole-board e Labirinto em Cruz Elevado (LCE)	Uma diminuição significativa na exploração comportamental, induzida por ambas as doses do extrato da castanha do Pará foi observada no teste do CA e no Hole-board. Os animais suplementados com 300 mg/kg da castanha do Pará apresentaram maior tempo de permanência nos braços abertos do LCE.
Rabiei *et al*. (2016)	Avaliar os efeitos do óleo da semente de uva sobre o comportamento de ansiedade e coordenação motora de ratos *Wistar* machos.	50, 100, 200 mg/kg de óleo da semente de uva (gavagem – 5 dias)	Comportamento de ansiedade.	Labirinto em Cruz Elevado (LCE)	O número de entradas no braço aberto no LCE aumentou significativamente comparado ao grupo controle. Além disso, a gavagem de óleo de semente de uva na dose de 200 mg/kg aumentou significativamente o tempo de permanência dos animais nos braços abertos do aparato.

No estudo de Melo e colaboradores (2019), a administração materna da polpa e do óleo de abacate (*Persea americana* Mill.) repercutiu em melhor desempenho da prole no teste de habituação em campo aberto, sendo o efeito da suplementação traduzido na redução do parâmetro de deambulação na segunda exposição dos animais ao aparato. Além disso, no teste de reconhecimento de objetos, observou-se maior taxa de exploração do objeto novo pelos animais dos grupos experimentais tanto a curto quanto a longo prazo ($p < 0,05$). Estes resultados, foram atribuídos aos componentes nutricionais presentes no abacate (fonte de PUFAs e compostos antioxidantes) capazes de afetarem as funções cerebrais durante a fase de desenvolvimento promovendo efeitos permanentes. Ainda de acordo com os autores, os PUFAs estão envolvidos em diferentes mecanismos que afetam a memória animal como: expressão de fator neurotrófico derivado do cérebro (BNDF), síntese do receptor N-metil-D-aspartato (NMDA), indução de LTP (potencial de longo prazo), liberação de glutamato em funções glutamatérgicas, regulação do sistema de neurotransmissão colinérgica e do sistema regulador GABA/Glu diminuindo o dano oxidativo e a apoptose celular. Quanto aos antioxidantes presentes no abacate (fenólicos, flavonóides e carotenóides), estes participam do desenvolvimento/manutenção do sistema nervoso central, reduzindo estresse oxidativo, atividades pró-inflamatórias e garantindo a integridade estrutural e funções da membranas neuronais, processos imprescindíveis ao mecanismo adequado de memória e aprendizado.

Pesquisa desenvolvida por Silva *et al*. (2021), cujo objetivo foi avaliar os efeitos da semente de macaíba no comportamento de ansiedade e memória de ratos *Wistar* dislipidêmicos, demonstrou que a suplementação da semente de macaíba, reduziu os danos oxidativos no cérebro dos animais pertencentes aos grupos saudáveis e dislipidêmicos, além de melhorar o comportamento de ansiedade. Na presente pesquisa, os autores apontam a relação da dislipidemia com comportamentos ansiogênicos, devido ao estresse oxidativo e processo inflamatório ocasionado pela ingestão de grandes quantidades de gorduras, principalmente saturadas. No teste do campo aberto, os animais suplementados com a semente de macaíba apresentaram maior número de *rearing* e menores comportamentos de *grooming*. No labirinto em cruz elevado, estes animais permaneceram por mais tempo nos braços abertos e na área central do aparato, indicando um efeito ansiolítico. Tais dados foram justificados pela presença de fenólicos e carotenóides na semente da macaíba que contribuiu para redução do estresse oxidativo e melhor proteção as membranas neurais. Os autores apontam ainda a atividade dos flavonóides na supressão da liberação de citocinas pró-inflamatórias (IL-1β e TNF-α),

além de regular a atividade de fatores de transcrição clínicos e terapêuticos relacionados à oxidação (óxido nítrico e NADPH oxidase). Por outro lado, a administração da semente de macaíba em ratos saudáveis e dislipidêmicos levou ao comprometimento de memória no teste de reconhecimento de objetos (TRO), podendo este fato ser explicado pelas quantidades reduzidas de ácidos graxos mono e poliinsaturados na semente da macaíba, fundamentais para fluidez e sinalização da membrana neural, para a regulação dos sistemas glutamatérgico, dopaminérgico, noradrenérgico e serotoninérgico, imprescindíveis a neurogênese (hipocampo e córtex) e plasticidade sináptica.

A ingestão de dieta normolipídica e hiperlipídica a base de castanha de caju durante a gestação e lactação influencia o neurodesenvolvimento e a memória da prole. No estudo de Melo *et al*., (2017), ao submeterem os animais ao teste de habituação ao campo aberto observou-se que o grupo normolipídico apresentou redução de deambulação na 2ª exposição ao aparato. Quanto ao teste de reconhecimento de objetos, a razão de exploração do objeto novo foi maior em NL e HL em comparação com CONT no curto prazo. Para o longo prazo, o tempo de exploração foi diminuído no grupo hiperlipidico em comparação ao grupo controle e normolipídico. Esses resultados foram associados a avaliação do perfil de ácidos graxos no cérebro da prole estudada, onde as quantidades de DHA e MUFA foram semelhantes entre controle e normolipídico no final do experimento, mas menores no grupo hiperlipidico, demonstrando que tanto os níveis de DHA quanto os de MUFA interferem diretamente no desenvolvimento da memória animal e que ambos os PUFAs modulam a plasticidade sináptica, regulam os níveis de BNDF no hipocampo e respostas inflamatórias no SNC.

Batool *et al*., (2017), levantaram a hipótese de que a suplementação de 400 mg/kg/dia de amêndoas e nozes poderia reverter os danos ocasionados a função neuronal de roedores expostos a 50mg/kg/semana de cádmio, durante 4 semanas. No teste do labirinto aquático de Morris foi revelado que o cádmio não afetou a latência de escape durante a sessão de teste, mostrando a falha de retenção de memória. Por outro lado, a suplementação de amendoa e nozes reduziu a latência de escape dos roedores após 24 horas da sessão de treinamento. Quanto a avaliação do campo aberto os animais amêndoa e nozes obtiveram redução significativa no parâmetro de deambulação e tempo de *rearing*. No TRO, os animais amêndoa e nozes apresentaram maior taxa de exploração em relação ao objeto novo. Os efeitos verificados, foram atribuídos a nutrientes essenciais como colina e polifenóis presentes na amêndoa e nas nozes, sendo estes, capazes de aumentar os níveis de acetilcolina no hipocampo e córtex frontal dos roedores, além de

reduzir o estresse oxidativo neste tecido, corroborando para um bom desempenho cognitivo como evidenciado nos testes comportamentais.

O óleo de noz rico em MUFAs, PUFAs e vitamina E também tem sido associado com a melhorara da função cognitiva, devido a capacidade que estes compostos possuem em modular a biofísica da membrana neuronal, canais iônicos localizados no sistema nervoso central e periférico, além de reduzir os produtos da peroxidação lipídica. Wang *et al*., (2019), objetivaram avaliar os efeitos do óleo de noz na memória espacial de ratos através da modulação da expressão de genes de canais iônicos sensíveis a ácido Asic2a e Asic4. A suplementação do óleo de noz nas concentrações de 2,2 e 11 g kg/dia reduziu a latência de escape e aumentou o número de cruzamento dos animais no quadrante da plataforma no teste do labirinto aquático de Morris. Estes resultados estiverem correlacionados ao aumento da expressão de Asic2a e Asic4, acetilcolinesterase, superóxido dismutase e óxido nítrico sintase no hipocampo dos roedores, sendo estes marcadores positivamente associada ao bom desempenho cognitivo.

Muitas evidências sugerem um papel protetor dos compostos fenólicos nos transtornos do humor. Diante disso, Bem *et al*. (2020) avaliou o efeito do extrato de semente de açaí (fonte de antioxidantes) na ansiedade induzida por separação materna periódica (SPM) em ratos machos adultos. A administração de 200mg/Kg (34 dias após a indução de estresse) da semente de açaí aumentou o tempo gasto pelos animais na área central do LCE. Além disso, a suplementação esteve associada ao aumento de receptores glicocorticóides no hipotálamo, dos receptores de tropomiosina quinase B (TRKB) no hipocampo, dos níveis de nitrito e atividade antioxidante no tronco encefálico dos roedores. O estudo propõe o efeito ansiolítico da matriz por reduzir a reatividade do eixo hipotálamo-hipófise-adrenal, aumentar a via NO-BDNF-TRKB e a defesa antioxidante no cérebro do grupo experimental.

O azeite de oliva também apresenta atividade ansiolíticas e cognitiva, devido ser fonte de antioxidantes, MUFAS, PUFAs. De acordo com Haider *et al*. (2012), a ingestão de 4 mL/kg de azeite de oliva durante 10 dias resultou na redução da atividade de locomoção dos roedores no teste de campo aberto; redução da latência de escape no labirinto aquático de Morris e maior tempo gasto no compartimento claro da caixa claro-escuro. De acordo com os autores as propriedades antioxidantes e o teor de MUFA foram capazes de modular o sistema nervoso central dos roedores proporcionando um melhor desempenho cognitivo e um comportamento do tipo ansiolítico, não evidenciando os mecanismos de fato.

O efeito da avelã na neurotoxicidade induzida pela injeção de Aβ foi avaliado por Bahaeddin *et al*., (2016). De acordo com os autores, a injeção de Aβ provoca comprometimento da memória, ansiedade, neuroinflamação e apoptose. Os resultados da presente pesquisa mostraram que a administração oral de avelã melhorou o comprometimento da memória e reduziu comportamento relacionado à ansiedade em ratos injetados com Aβ. Ademais. Análises de Western blotting mostrou que a avelã reduziu a COX-2, IL-1β, TNFα, Bax, Bcl-2 e caspase-3, minimizando assim, a neuroinflamação, apoptose celular, comprometimento da memória e comportamento do tipo ansiogênico. Os resultados observados foram atribuídos ao efeito sinérgico dos nutrientes presentes na avelã, que é fonte de MUFAs, PUFAs, vitaminas, magnésio e selênio, sendo estes nutrientes classificados com propriedades anti-inflamatórias, neuroprotetoras, antioxidantes e ansiolítica.

A castanha do Pará é outra oleaginosa fonte de componentes nutricionais com ação farmacológica, a citar: lipídios, minerais e fitoquímicos com ação anti-inflamatória e antioxidante. Frausto-González *et al*. (2021), buscou avaliar os efeito neurofarmacológico do extrato da castanha do Pará sobre o comportamento de ansiedade em roedores submetidos a um modelo experimental de obesidade induzida por dieta. Os resultados evidenciaram atividade ansiolítica da castanha do Pará que foi traduzida na redução da deambulação dos roedores no teste de CA e no Hole-board, e pelo aumento do tempo de permanência dos roedores nos braços abertos do LCE. OS autores propõe uma relação entre os ácidos graxos presentes na oleaginosa com a modulação do receptor $GABA_A$, evidenciada através da avaliação de resposta do tipo hipnótica induzida pelo pentobarbital sódico, onde o extrato da castanha do Pará produziu efeito semelhante.

Rabiei *et al*., (2016), demonstraram que o óleo da semente de uva também apresenta atividade ansiolítica semelhante ao pentobarbital sódico. 200 mg/kg do óleo da semente de uva e 1 mg/kg do fármaco obtiveram efeitos semelhantes quanto ao número de entradas e tempo de permanência dos animais no braço aberto no LCE. Os efeitos ansiolíticos evidenciados na presente pesquisa são atribuídos aos polifenóis e proantocianidinas, potentes antioxidantes, capazes de atenuar as espécies reativas de oxigênio e peroxidação lipídica, além de aumentar a atividade de enzimas antioxidantes no cérebro de roedores.

2.4 PUFAs, COMPOSTOS ANTIOXIDANTES E SUA RELAÇÃO COM O EIXO INTESTINO-CÉREBRO

O eixo intestino-cérebro é um sistema de comunicação multimodal onde a microbiota influencia o fenótipo do hospedeiro de forma direta ou indireta, através do contato íntimo com as células intestinais ou mediante a produção de compostos metabolitos bacterianos, como ácidos graxos de cadeia curta (AGCCs) e mediadores imunológicos (quimicocinas), que por sua vez, alteram a fisiologia do sistema nervoso, imunológico, endócrino e entérico, via nervo vago (CARABOTTI *et al*., 2015). Um intestino saudável é caracterizados por um microbiota diversificada e por mecanismos de proteção que incluem a junção de oclusão entre as células, receptores Toll-Like (TLR, do inglês Toll-Like receptors) e o muco (FAN; PEDERSEN, 2020). SINGH *et al*. (2017), evidenciam que hábitos alimentares inadequados podem fragilizar estes mecanismos, devido a redução da diversificação dos microrganismos e aumento da concentração de bactérias nocivas produtoras de toxinas e componentes inflamatórios. Por outro lado, a exposição a uma dieta adicionada com PUFAs e antioxidantes a longo prazo tem sido associada a restauração da composição da microbiota saudável, melhora da memória, cognição e comportamento de ansiedade em roedores (FU *et al*., 2021; DUEÑAS *et al*., 2015; DASH *et al*., 2022).

2.4.1 PUFAs

Os PUFAs podem alterar a diversidade e a abundância do microbioma intestinal em humanos e roedores (PUSCEDDU *et al*., 2015). A intervenção com ômega-3 foi associada a um aumento reversível da abundância de vários gêneros, incluindo *Akkermansia, Lachnospiraceae, Bifidobacterium , Roseburia e Lactobacillus*, microrganismos produtores de AGCCs, capazes de influenciar a síntese e/ou produção e liberação de neurotransmissores (FU *et al*., 2021). *Firmicutes e Bacteroidetes* são os dois principais filos predominantes da microbiota intestinal humana. Uma ingestão desequilibrada de PUFAs aumenta particularmente a relação F/B, o que leva a alterações comportamentais, cognitivas, além de outras doenças neurológicas (ONISHI *et al*., 2017). Robertson *et al*., (2017), observaram que ácidos graxos ômega-3 regulam criticamente o desenvolvimento da microbiota intestinal, restaurando a relação F/B e aumentando a abundância fecal de *Bifidobacterium e Lactobacillus*. De acordo com a pesquisa, a

intervenção com n-3 PUFAs e as modificações ocorridas na abundância relativa de bactérias intestinais esteve também relacionada a alterações comportamentais benéficas na prole a curto e longo prazo. Por outro lado, dietas deficientes em n-3 PUFAs estão intimamente associadas a disbiose, redução da atividade do eixo HPA e inflamação, por promover crescimento de bactérias nocivas produtoras de mediadores pró-inflamatórios como endotoxinas e interleucina 17 (IL17) (ROBERTSON *et al*., 2017; REEMST *et al*., 2021; FU *et al*. 2021).

Microrganismos intestinais desempenham um importante papel no neurodesenvolvimento, desde a formação da barreira hematoencefálica (BHE), perpassando pelos processos de neurogênese, maturação da microglia e mielinização (DASH *et al*., 2022; PARKER *et al*., 2020; CERDÓ *et al*., 2020). Vários componentes dietéticos liberados do intestino são cruciais para a maturidade das células neuronais e funções adequadas (PARKER *et al*., 2020). A disbiose parece ser umas das principais causas para o comprometimento da homeostasia do tecido cerebral, devido a produção entérica de metabolitos inflamatórios capazes de cruzar a BHE (ALMEIDA *et al*., 2020). Os PUFAs aumentam a produção de compostos anti-inflamatórios, além de mediadores lipídicos pró-resolução especializados a partir do ácido araquidônico (AA) eicosapentaenóico (EPA), docosahexaenóico (DHA) e docosapentaenóico (DPA). Estes inibem a produção de citocinas pró-inflamatórias, promovem a regeneração da mucosa intestinal e a abundância de bactérias produtoras de ácidos graxos de cadeia curta (AGCC) (ALMEIDA *et al*., 2020).

Pesquisas que avaliaram o impacto da suplementação de PUFAs sobre a microbiota obtiveram como resultado o aumento significativo de *Blautia* , *Bacterioides* , *Roseburia* e *Coprococcus*, táxons produtores do AGCC como butirato (KUMAR *et al*., 2022; LOUIS; FLINT, 2009; LOUIS; FLINT, 2017). Outros estudos revelam a ação do butirato sobre a restauração de células neuronais em um modelo experimental neonatal de hipóxia-isquemia; sobre a perda neuronal no cérebro de camundongos submetidos a um modelo de lesão cerebral traumática; além de melhorar a memória e aprendizado em roedores; reduzir a neuroinflamação e o estresse oxidativo em células gliais e neuronais (JAWORSKA *et al*, 2019; LI *et al*., 2016; GOVINDARAJAN *et al*., 2011; RODE *et al*., 2021).

O propionato e acetato também são AGCC com importância para a neuroproteção e estudos que utilizaram a suplementação de PUFAS em roedores, detectaram aumento de baterias intestinais produtoras destes componentes (COSTANTINI *et al*., 2017). O

aumento dos níveis de propionato e acetato melhoraram a disfunção cerebral induzida por procedimento de ligadura e punção cecal (LIU *et al*., 2021). Ambos os AGCC apresentam ação anti-inflamatória sobre o tecido nervoso, regulando as vias NF-κB (fator de transcrição envolvido no controle da expressão de genes ligados à resposta inflamatória), JNK (proteína quinase), e produção de TNF-α (VINOLO *et al*., 2011; SILVA *et al*., 2020).

Notavelmente, há evidências de que a microbiota intestinal exerça efeitos distintos sobre os neurotransmissores, produzindo-os ou consumindo-os, isso inclui a dopamina, norepinefrina, serotonina ou ácido gama-aminobutírico (GABA) (CHEN *et al*., 2021). Microrganismos como por exemplo *Klebsiella, Serratia, Bacillus* e *Staphylococcus* apresentam capacidade de síntese de dopamina (HOLM *et al*., 2016; ÖZOĞUL, 2004; SHISHOV *et al*., 2009). A serotonina pode ser sintetizada a partir da atividade metabólica de *Lactobacillus, Streptococcus, Lactococcus lactis* e *Morganella* ÖZOĞUL, 2004; SHISHOV *et al*., 2009). Já entre os microrganismos capazes de sintetizar GABA estão: *Bifidobacterium, Lactobacillus* e *Streptococcus* (BARRETT *et al*., 2012; KOMATSUZAKI *et al*., 2005; POKUSAEVA *et al*., 2017; YANG *et al*., 2008). Como relatado anteriormente, os PUFAs apresentam capacidade de modular a abundância relativa das bactérias supracitadas, o que consequentemente interfere de modo direto nos níveis de neurotransmissores (FU *et al*., 2021).

Algumas bactérias intestinais apresentam ainda propriedade para codificar genes para enzimas específicas, que por sua vez, catalisam a conversão de substratos em neurotransmissores ou precursores correspondentes. Além disso, os metabólitos produzidos por esses microrganismos podem também atuar como moléculas sinalizadoras para a síntese e liberação de neurotransmissores pelas células enteroendócrinas (CHEN *et al*., 2021). Assim, a suplementação de PUFAs se torna uma estratégia para a diversificação dos microrganismos intestinais envolvidos no metabolismo e regulação dos neurotransmissores ou de seus precursores (MARRONE; COCCURELLO, 2019). Além de serem substratos para a produção de outros metabolitos bacterianos neuroprotetores (DRUART *et al*., 2015).

É importante ressaltar que a atividade dos neuro metabolitos sintetizados a partir dos PUFAs pelas bactérias intestinais, decorrem da capacidade de comunicação destes com o sistema autônomo simpático e parassimpático, que conduz os sinais aferentes, provenientes do lúmen para as vias entéricas, espinais e vagais até alcançarem de fato o sistema nervoso central (CARABOTTI *et al*., 2015).

2.4.2 Compostos antioxidantes

Até aqui, a maioria dos estudos acerca da ação dos compostos antioxidantes no organismos de humanos e roedores estiveram direcionados a sua capacidade antioxidante e anti-inflamatória, porém, seu papel sobre a colonização do microbioma intestinal, bem como, sua relação com o eixo intestino-cérebro ainda é limitada. Pesquisas objetivando avaliar a relação dos polifenóis junto a microbiota intestinal permitiu compreender a característica probiótica deste composto (SINGH *et al*., 2019; MILUTINOVIĆ *et al*., 2021). Dueñas *et al*. (2015), por exemplo, observaram o efeito dos polifenóis no aumento da abundância relativa de *Bifidobacterium* e *Lactobacillus*, com consequente redução dos patógenos *C. perfringens e C. histolyticum.* Já no estudo de Hervert-hernández e Goñi (2011) e Man *et al*., (2020), a ingestão dos polifenóis reduziu a concentração de *Salmonella* e *Helicobacter* e elevou as proporções de bactérias Gram-positivas em decorrência as Gram-negativas. As catequinas apresentam atividade sobre o crescimento de *Clostridium histolyticum, Eubacterium rectale* e *E. coli* (TZOUNIS *et al*., 2008). Proantocianidinas provenientes do extrato de vinho tinto influenciou o aumento de *Bacteroides , Bifidobacterium* e *Lactobacillus spp* (TOMBOLA *et al*., 2003). Quanto o resveratrol proveniente da uva, este aumentou a contagem de células fecais de *Lactobacillus* e *Bifidobacterium spp.* (LARROSA *et al*., 2009).

De acordo com o peso molecular alguns polifenóis podem ser absorvidos de forma direta no intestino delgado (5 a 10%), porém, estruturas mais complexas necessitam da ação de microrganismos intestinais para serem transformados em compostos metabólicos (via hidroxilação, desmetilação, descarboxilação e desidroxilação) e assim, contribuírem para a homeostasia do lúmem intestinal, reduzindo estresse oxidativo, quelando compostos pró-inflamatórios e aumentando a abundância de bactérias produtoras de neurotransmissores, a exemplo das *Bifidobacterium, , Escherichia e Enterococcus* spp. envolvidas na regulação dos níveis plasmáticos de triptofano e metabolismo da serotonina, além dos *Lactobacillus e Bifidobacterium* que influenciam na síntese do GABA (KAWABATA *et al*., 2019; STRANDWITZ, 2018; SOCAŁA *et al*. (2021)

Além da atividade dos compostos bioativos no lúmen intestinal, estes podem alcançar a circulação sistêmica e cruzar a BHE (FIGUEIRA *et al*., 2019; SINGH *et al*. 2019). No cérebro, os polifenóis apresentam potencial para proteger os neurônios contra lesões induzidas por neurotoxinas e redução/supressão neuroinflamação (VAUZOUR,

2012). Pesquisa *in vivo* demonstrou a atividade dos polifenóis na ativação de vias celulares de resposta ao estresse, resultando em expressão do fator neurotrófico derivado do cérebro (BDNF) (RAMIREZ *et al*., 2005). Polifenóis regulam também o fator de transcrição NF- κB reduzindo a expressão de citocinas inflamatórias (HUANG *et al*., 2005). Ademais, os polifenóis podem ainda modular fatores de transcrição da família forkhead (FoxO), regulando genes que codificam enzimas antioxidantes e outras proteínas de resposta ao estresse no cérebro (MICHAN; SINCLAIR, 2007; KNUTSON; LEEUWENBURGH, 2008).

2.5 AMÊNDOA DE BARU FONTE DE NUTRIENTES ESSENCIAIS

A amêndoa de Baru (Dipteryx alata Vog.) é fruto do barueiro, árvore da savana brasileira, nativa do bioma do cerrado. É classificada, botanicamente como sendo uma semente comestível com características similares as das nozes verdadeiras, porém pertencente à família *Leguminosae,* Figura 3 (CZEDER *et al*., 2012). Apresenta características nutricionais significativas e potencial farmacológico, sendo utilizada na medicina alternativa por possuir ação antirreumática, antiespasmódica, reguladora do ciclo menstrual e do tônus muscular (LIMA *et al*., 2022) . Apesar de ser fonte de fatores antinutricionais a torrefação inativa o inibidor da tripsina, permitindo assim, o seu consumo, além do mais, o tratamento térmico melhora a digestibilidade das proteínas e confere a amêndoa sabor e odor característico sem alterar de forma significativa seu conteúdo lipídico e de antioxidantes (LEMOS *et al*., 2012).

Figura 3 – Escala fotográfica do fruto do baru. **A -** Fruto do baru (*Dipteryx alata* Vog); **B-C** – Endocarpo; **D-** amêndoa.

Fonte: Appolinário *et al*., (2011).

Em termos de composição química a oleaginosa apresenta 11,10 g.100g^{-1} de carboidrato com predominância de fibras insolúveis em torno de 12% (SILVA *et al*., 2022). Possuí elevado teor de proteínas (238–281 g kg $^{-1}$), fornecendo todos os aminoácidos essenciais da dieta humana, a citar: histidina (23,4), isoleucina (37,5), leucina (77,8), lisina (48,4), metionina (22,0), fenilalanina (77,2), treonina (44,9), triptofano (20,2) e valina (51,8) [mg aminoácido g^{-1} proteína]. Quanto aos aminoácidos não-essenciais, na oleaginosa podem ser encontrados a alanina (46,1), arginina (91,44), asparagina (109,33), glutamina (204,38), glicina (48,86), prolina (54,22) e serina (42,81), sendo a razão líquida relativa de proteína da amêndoa de baru aproximadamente 74% (FERNANDES *et al*., 2010).

Os lipídios representam a fração majoritária dos nutrientes presentes na amêndoa de baru (40%), onde 80% do seu conteúdo lipídico é composto por ácidos graxos insaturados (BORGES *et al*., 2012). Da amêndoa de baru é possível ainda extrair o óleo, subproduto oriundo da prensagem a frio, com alto grau de insaturação (81,2%) e concentração de ácido oléico (C18:1) em torno de 45% e linoléico (C18:2) em torno de 28,0%, conforme descrito no Quadro 2 (PINELI *et al*.,2015; BORGES *et al*., 2022; LEMOS *et al*., 2012).

Quadro 2. Perfil de ácidos graxos da amêndoa e do óleo de baru.

Ácidos Graxos (AG)	Nº de carbono	Fórmula química	Amêndoa de baru (%)	Óleo de baru (%)
AG tetradecanóico	C14:0	$C_{14}H_{28}O_2$	0.17	0.04
AG palmítico	C16:0	$C_{16}H_{32}O_2$	7.56	6.37
AG heptadecanóico	C17:0	$C_{17}H_{34}O_2$	-	0.08
AG esteárico	C18:0	$C_{18}H_{36}O_2$	4.34	5.28
AG araquídico	C20:0	$C_{20}H_{40}O_2$	1.24	1.38
AG behênico	C22:0	$C_{22}H_{44}O_2$	2.51	3.90
AG Lignocérico	C24:0	$C_{24}H_{48}O_2$	2.96	4.79
AG palmitoleico	C16:1	$C_{16}H_{30}O_2$	-	0.04
AG cis -10-heptadecenóico	C17:1	$C_{17}H_{32}O_2$	-	0.21
AG oleico	C18:1	$C_{18}H_{34}O_2$	44.58	45.83
AG linoleico	C18:2	$C_{18}H_{32}O_2$	30.92	28.93
AG linolênico	C18:3	$C_{18}H_{30}O_2$	2.19	0.16
AG eicosenoico	C20:1	$C_{20}H_{38}O_2$	2.55	2.69
AG SATURADOS totais			**18.61**	**21.84**
AG MUFAs Totais			**47.13**	**48.77**
AG PUFAs Totais			**33.11**	**29.09**

Fonte: Pineli *et al*. (2015)**;** Borges *et al*. (2022); Lemos *et al.* (2016).

Quanto aos teores de minerais, Campidelli e colaboradores (2020), identificaram na amendoa de baru *in natura* a presença de boro (2.4), enxofre (380), potássio (1810), cálcio (240), magnésio (330), cobre (2.8), manganês (6.4) e ferro (6,5) [mg 100 g^{-1}]. De acordo com a pesquisa, o processo de torrefação utilizando o binômio tempo-temperatura de 65°C e 105ºC por 30 minutos não produziram impactos significativos no teor de minerais. Porém, outros estudos demonstram que esses valores podem variar de acordo com a região e o modo de cultivo (SILVA., *et al*., 2020; MARIN *et al*., 2009)

A amendoa de baru é considerada também uma ótima fonte de nutrientes antioxidantes (SIQUEIRA *et al*., 2012). Na pesquisa desenvolvida por Lemos *et al*., (2012), foi possível identificar e quantificar os compostos bioativos da amêndoa de baru,

bem como, avaliar o efeito da torra sobre os compostos e capacidade antioxidante. De acordo com os autores, o conteúdo de fenólicos e antocianinas totais apresentaram valores médios em torno de 111,3 mg GAE/100g e 1,20 mg cianidina-3-glicosídeo /100g, respectivamente, superando o conteúdo de outras castanhas populares consumidas no brasil. Quanto a capacidade antioxidante, foi observado valor de 13,9 μmol/L TE/100g na amêndoa submetida a torrefação. O teor de compostos fenólicos foram determinados pelos autores utilizando cromatografia líquida de alta eficiência (HPLC), os resultado estão expressos no Quadro 3.

Quadro 3. Teor de compostos fenólicos da amêndoa de baru torrada.

Compostos Fenólicos	Amendoa de baru (mg/100 g)
ácido p-cumárico	3.8
Ácido elágico	4.9
Ácido cafeico	2.3
ácido gálico	170.9
Ácido hidroxibenzóico	0.6
Catequina	45.6
Ácido ferúlico	17.5
Epicatequina	4.8

Fonte: Lemos *et al*. (2012).

Diante aos inúmeros nutrientes que apresenta e principalmente pela concentração de antioxidantes e PUFAs, a amêndoa de baru vêm ganhando destaque no cenário científico, principalmente pelo seu potencial farmacológico. Estudos experimentais tem demonstrado a relação da ingestão do óleo e amêndoa de baru com atividade antioxidante, anti-inflamatória, anti-dislipidemica e neuroprotetora (CAMPIDELLI *et al*., 2022; SIQUEIRA *et al*., 2012; BENTO *et al*., 2014).

3 MATERIAL E MÉTODOS

3.1 LOCAL DE EXECUÇÃO DA PESQUISA E DELINEAMENTO EXPERIMENTAL

Esta pesquisa foi desenvolvida utilizando modelo animal experimental. Os procedimentos foram conduzidos no Laboratório de Nutrição Experimental (LANEX), Laboratório de Bromatologia (LABROM) e Tecnologia de Alimentos (LTA) do Centro de Educação e Saúde (CES) da Universidade Federal de Capina Grande (UFCG); no Laboratório de Avaliação de Produtos de Origem Animal da Universidade Federal da Paraíba (UFPB); Laboratório de Análise Físico-química (LAFQ) do Centro de Tecnologia e Desenvolvimento Regional (CTDR) da Universidade Federal da Paraíba (UFPB); Laboratório de Farmacologia – III da Universidade Federal do Rio Grande do Norte (UFRN) e no Laboratório de Produtos Naturais Bioativos (LPNB) do Departamento de Química (DQ) da Universidade Federal Rural de Pernambuco (UFRPE).

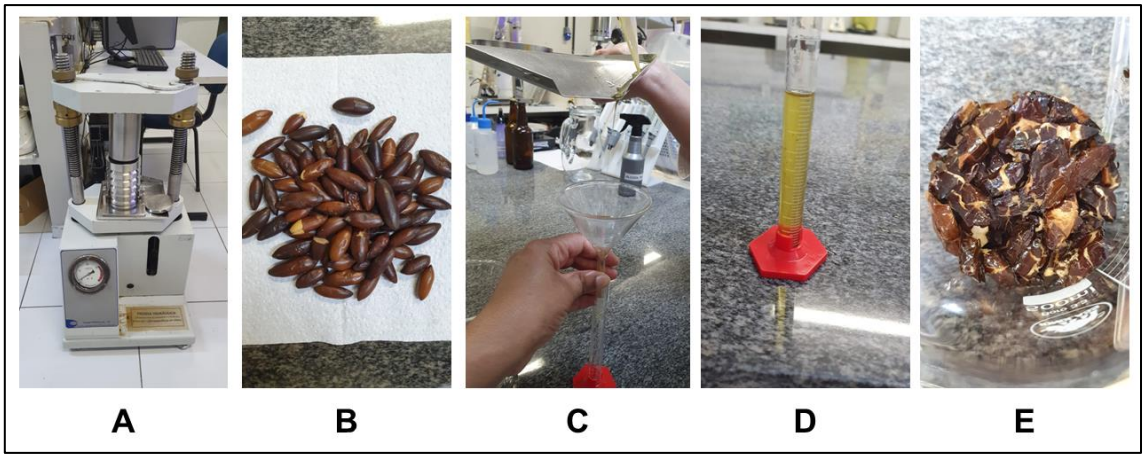
3.2 AMOSTRAS

A amêndoa de baru (20 kg) utilizada no experimento pertencia a espécie *Dipteryx alata* Vog. da cidade de Goiânia/GO, Brasil: latitude -16º40`43``S, longitude -49º15`14``W e 749 m de altitude. As amêndoas foram acondicionadas em embalagens laminadas a vácuo, de material escuro para proteção contra a luminosidade e, em seguida, foram conduzidas ao Laboratório de Tecnologia de Alimentos da Universidade Federal de Campina Grande (LTA – UFCG). Parte da amêndoa (11 kg) foi utilizada para extração do óleo, através da técnica de extração a frio, utilizando-se prensa hidráulica SL 10, de marca SOLAB® (Piracicaba, Brasil), com capacidade para esmagamento de 680 kg/dia. A prensagem foi realizada no laboratório de Físico-química do Centro de Tecnologia e Desenvolvimento Regional – CTDR da Universidade Federal da Paraíba – UFPB. Foram prensados 1 kg de amêndoa por vez sob uma pressão de 12 bar, sendo este procedimento realizado em condições de iluminação reduzida e com as amêndoas previamente congeladas, visando evitar o processo de oxidação. O óleo extraído da amêndoa foi acondicionado em vidro âmbar e armazenado a -20 °C, Figura 4. O rendimento da extração foi calculado levando em consideração a massa do produto (11 kg da amêndoa

de baru) e o volume de óleo obtido após processo de prensagem (1000 mL), utilizando a seguinte equação: $n = \frac{v}{m}\ x\ 100$. De acordo com os cálculos, obteve-se um rendimento de 9,09% para a extração do óleo.

A outra parte das amêndoas (9 kg restantes) foi utilizada para a preparação da solução de baru. Para isso, as amêndoas foram trituradas, pesadas e misturadas com água destilada em uma proporção de 2:1. As condições de manipulação seguiram os mesmos critérios de iluminação e temperatura utilizados para extração do óleo. A solução de baru era preparada sempre cinco minutos antes da administração nos animais.

Figura 4 - Esquema ilustrativo do processo de extração do óleo de baru. **A** - Prensa hidráulica SL 10 SOLAB® utilizada para extração do óleo de baru. **B** – Amêndoa de baru *in natura*. **C** – Coleta do óleo extraído da amêndoa de baru *in natura*. **D** – Extração completa do óleo de baru. **E** – Torta da amêndoa de baru.



3.3 IDENTIFICAÇÃO E QUANTIFICAÇÃO DOS ÁCIDOS GRAXOS DA AMÊNDOA E DO ÓLEO DE BARU

Para análise do perfil de ácidos graxos da amêndoa e do óleo de baru, inicialmente realizou-se a extração dos lipídeos conforme metodologia descrita por Folch, Less e Stanley (1957). O procedimento de transesterificação das amostras foi realizado segundo Hartman e Lago (1973). Os ácidos graxos foram analisados através de um cromatógrafo gasoso (VARIAN 430-GC, Califórnia, EUA), acoplado com coluna capilar de sílica fundida (CP WAX 52 CB, VARIAN, Califórnia, EUA) com dimensões de 60 m x 0,25 mm e 0,25 μm de espessura do filme. Foi utilizado o hélio como gás de arraste (vazão de 1mL/min). A temperatura inicial do forno foi de 100 °C, com programação para atingir

240 °C, aumentando 2,5 °C por minuto, permanecendo por 30 minutos, totalizando 86 minutos de corrida. A temperatura do injetor foi mantida em 250 °C e a do detector em 260 °C. Alíquotas de 32 1,0 μL do extrato esterificado foram injetadas em injetor tipo Split/Splitless. Os cromatogramas foram registrados em software tipo Galaxie Chromatography Data System. Os resultados dos ácidos graxos foram quantificados por integração das áreas dos ésteres metílicos e expressos em percentual de área.

3.4 DETERMINAÇÃO DO TEOR DE COMPOSTOS FENÓLICOS TOTAIS, FLAVONÓIDES TOTAIS E ATIVIDADE ANTIOXIDANTE *IN VITRO* DO ÓLEO E DA AMÊNDOA DE BARU

3.4.1 Extração

Para determinação do teor de polifenóis totais, flavonoides totais e da atividade antioxidante *in vitro* (FRAP e ABTS), o óleo e a amêndoa de baru foram submetidos à extração dos constituintes bioativos.

A extração dos constituintes do óleo do baru foi realizada conforme metodologia descrita por Parry *et al*. (2005), com algumas modificações. Inicialmente, foram pesados 1 g de óleo em um tubo de ensaio e adicionou-se 3 mL de metanol a 80%. Em seguida o tubo com a mistura foi submetido a agitação em vórtex por 1 min e, posteriormente, centrifugado (Centribio 80-2B) a 4000 rpm por 5 minutos. Após, o sobrenadante foi recolhido em frasco âmbar e armazenado em Freezer (CHB53EBANA, Consul, Rio Claro – SP) a -18 °C até o momento das análises. Para extração dos constituintes bioativos da amêndoa de baru foi pesado um grama de polpa em um tubo de ensaio e adicionado etanol a 80% na proporção 1:10 (g/v). Posteriormente, o tubo com a mistura foi coberto com papel alumínio, deixado em repouso (maceração) por 60 min em temperatura ambiente (23± 0,5 °C). Após, a mistura foi filtrada sendo o volume completado para 10 mL com o solvente de extração. O extrato foi recolhido em frasco âmbar e armazenado em freezer a -18 °C até o momento das análises. Todas as extrações (óleo e amêndoa) foram realizadas em triplicatas.

3.4. 2 Determinação do Teor de Compostos Fenólicos Totais

Para determinar o teor de compostos fenólicos totais do óleo e da amêndoa de baru utilizou-se metodologia descrita por Liu *et al*. (2002) com algumas modificações. Resumidamente, 250 µL de cada extrato (óleo e amêndoa) foram misturados em tubo de ensaio com 1250 µL do reagente Folin-Ciocalteau a 10%. As soluções foram agitadas em vórtex e armazenadas em temperatura ambiente (23 ±0,5 °C) na ausência da luz por 6 minutos. Após, foram adicionados 1000 µL da solução de carbonato de sódio a 7,5%. A mistura foi levada ao banho maria a uma temperatura de 50 °C, durante 5 min. Após, a absorbância foi medida a 765 nm utilizando espectrofotômetro (BEL Photonics, Piracicaba, São Paulo, Brasil). Também foi realizado um branco com a ausência dos extratos para zerar o espectrofotômetro. O conteúdo de compostos fenólicos totais das amostras foi determinado utilizando uma curva padrão preparada com ácido gálico. Os resultados foram expressos em mg equivalentes de ácido gálico (EAG) por cem gramas de amêndoa de baru com base no peso seco (mg EAG/100 g). Para o óleo os resultados foram expressos em mg equivalentes de ácido gálico (EAG) por cem gramas de óleo de baru (mg EAG/100 g).

3.4.3 Determinação de flavonóides totais

O teor de flavonoides totais foi determinado de acordo com o método proposto por Zhishen, Mengcheng e Jianming (1999). Uma alíquota de 0,5 mL dos extratos do óleo e da amêndoa de baru foram adicionadas a 2 mL de água destilada em um tubo de ensaio. Em seguida, adicionou-se 150 µL de nitrito de sódio a 5%. Após 5 min, 150 µL de cloreto de alumínio a 10% foram adicionados e, após 6 min, 1 mL de hidróxido de sódio a 1 M, seguido pela adição de 1,2 mL de água destilada. A absorbância da amostra foi medida a 510 nm usando um espectrofotômetro (BEL Photonics, Piracicaba, São Paulo, Brasil) contra um branco na ausência dos extratos. O teor de flavonoides totais dos extratos foi determinado usando uma curva padrão de equivalentes de catequina (EC). Os resultados foram expressos em mg equivalentes de catequina (EC) por cem gramas da amêndoa de baru com base no peso seco (mg EC/100 g). Para o óleo os resultados foram expressos em mg equivalentes de catequina (EC) por cem gramas de óleo de baru com base no peso (mg EC/100 g).

3.4.4 Atividade antioxidante - método FRAP

Para determinação da atividade antioxidante por meio da redução do ferro (FRAP) foi utilizada metodologia descrita por Benzie e Strain (1999), adaptada por Pulido, Bravo e Saura-Calixto (2000). O reagente FRAP foi preparado somente no momento da análise, através da mistura de 11 mL de tampão acetato (0,3M, pH 3,6), 1,1 mL de solução TPTZ (10 mM em HCl a 40 mM) e 1,1 mL de solução aquosa de cloreto férrico (20 mM). Para a análise, 200 µL dos extratos (óleo e amêndoa de baru) foram adicionados a 1800 µL do reagente FRAP em um tubo de ensaio e levados ao banho maria a 37 °C por 30 minutos. Para cada extrato foi realizado um branco, sem adição do extrato. Após, as absorbâncias foram medidas em espectrofotômetro (BEL Photonics, Piracicaba, São Paulo, Brasil) a 593 nm. Para determinar a atividade antioxidante (FRAP) dos extratos do óleo e da amêndoa foi utilizada curva de calibração com Trolox e os resultados foram expressos em µmol de trolox/g de amostra.

3.4.5 Atividade antioxidante - método ABTS

O método ABTS foi realizado de acordo com a metodologia de Surveswaran *et al*. (2007), com algumas modificações. Inicialmente, formou-se o radical ABTS através da reação da solução $ABTS^{\bullet+}$ a 7 mM com a solução de persulfato de potássio 140 mM incubados a temperatura de 25 °C, no escuro durante 12-16 horas. Uma vez formado o radical, o mesmo foi diluído em água destilada até obter o valor de absorbância de 0,800 (±0,020) a 734 nm. A partir de cada extrato (óleo e amêndoa de baru) foram preparadas quatro diluições diferentes, em triplicatas. Em ambiente escuro foi transferido para um tubo de ensaio uma alíquota de 100 µL dos extratos e adicionado 500 µL do radical $ABTS^{\bullet+}$. Após, os tubos de ensaio foram mantidos na ausência de luz por 6 minutos. Em seguida, realizou-se a leitura a 734 nm em espectrofotômetro (BEL Photonics, Piracicaba, São Paulo, Brasil). Também foi feita uma solução "controle" que consistiu em uma alíquota de 100 µL do solvente extrator dos extratos adicionada de 500 µL do radical $ABTS^{\bullet+}$. A solução "branco" foi o solvente extrator de cada extrato, utilizada para zerar o espectrofotômetro. Como referência, foi utilizado o Trolox e os resultados expressos em µM trolox/g de amostra. Também calculou-se o IC_{50} através da equação da reta plotada através dos resultados contendo os valores de concentração (mg/mL) utilizadas no eixo X e os percentuais de proteção encontrados no eixo Y.

3.5 ENSAIO BIOLÓGICO

3.5.1 Animais e Dietas Experimentais

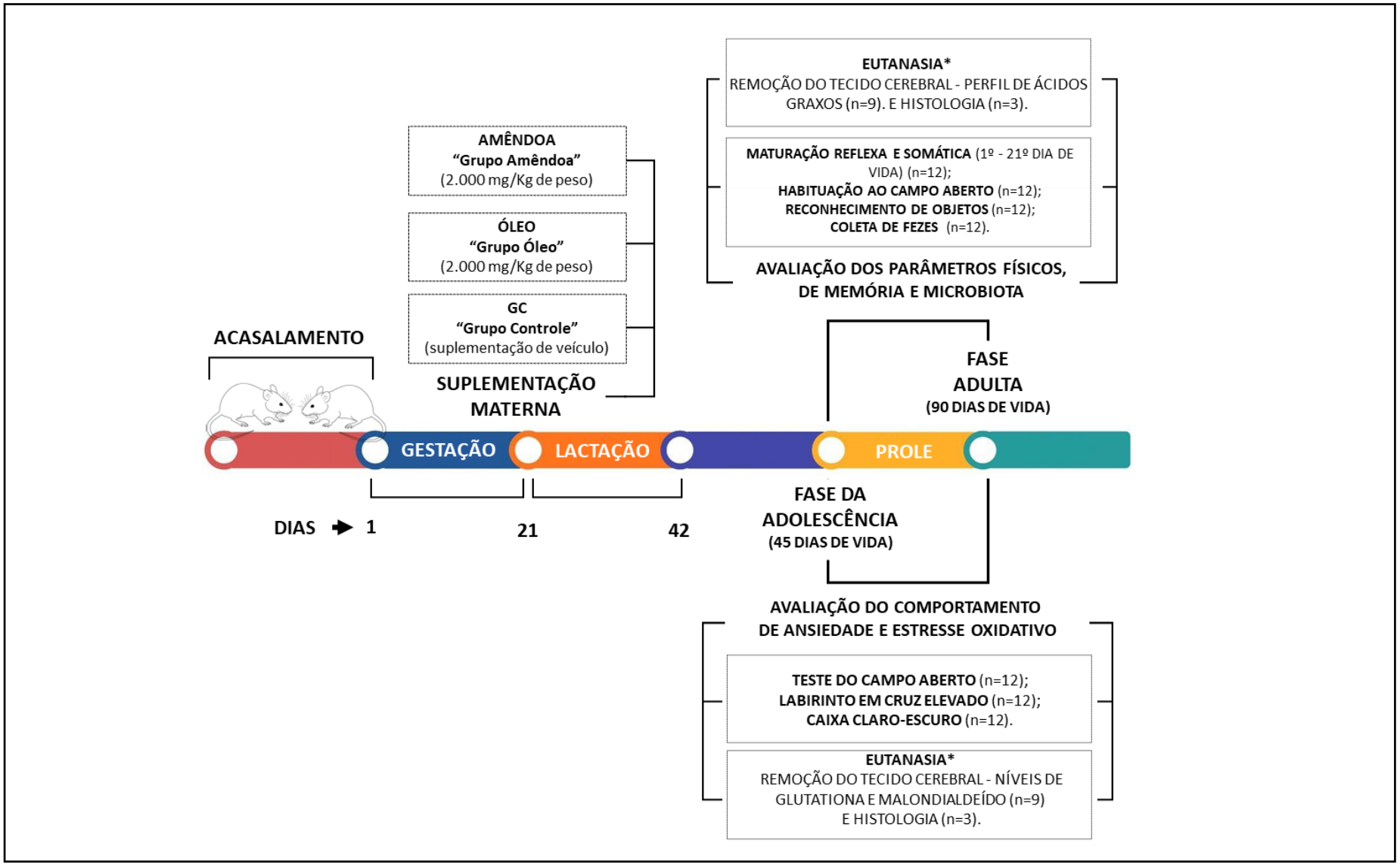
Fêmeas primíparas da linhagem Wistar (90 dias de vida/250 ±50 g de peso) foram obtidas do Laboratório de Nutrição Experimental do Departamento de Nutrição da Universidade Federal de Campina Grande - LANEX/UFCG e utilizadas para obtenção de 45 ratos neonatos. Após confirmação de prenhez, as ratas foram alojadas em gaiolas-maternidade individuais de polipropileno, em condições-padrão do laboratório (temperatura média de 22 ± 1 °C, umidade 55 ± 5%, ciclo claro/escuro de 12/12 horas – luz artificial das 6:00 as 18:00 horas). As ninhadas foram padronizadas em 6 (seis) filhotes machos (PARRA-VARGAS *et al*., 2020).

Vinte e quatro mães foram divididas de forma randomizada em três grupos: Controle (GC) - recebeu água destilada; Óleo - tratadas com 2.000 mg de óleo de baru/kg de peso de animal; e Amêndoa - tratadas com 2000 mg de da solução da amêndoa de baru/kg de peso de animal. A gavagem foi administrada a partir do 7º dia de gestação até o 21º dia lactação. Foram fornecidos ração padrão (Presence®) e água *ad libitum*. Após o desmame, com 21 dias de vida, a prole passou a receber a mesma ração padrão ofertada as mães e esta foi fornecida até a fase adulta. A descrição dos grupos e do protocolo experimental estão ilustrados na figura 5.

A dose administrada foi definida a partir da avaliação de estudos elaborados por: Reis *et al*. (2018), que verificaram efeito hepatoprotetor e antioxidante ao administrar uma dose de 1 g/kg/dia do óleo de baru em um modelo pré-clínico de lipotoxicidade e dislipidemia e Siqueira e colaboradores (2012), que observaram efeito preventivo do consumo da amêndoa de baru no estresse oxidativo induzido por ferro em ratos.

Todos os métodos experimentais foram previamente aprovados pelo Comitê de Ética para Uso Animal – CEUA da UFCG sob certificação de nº 104-2017, em atendimento às normas estabelecidas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA, Brasil) por meio da Lei nº 11.794/2008 (Lei Arouca), e com as diretrizes de Pesquisa Animal: Relato de Experimentos In Vivo (ARRIVE) 2.0 para experimentos in vivo com animais (DU SERT *et al*., 2020). A amêndoa utilizada na suplementação foi registrada no SisGen, sob protocolo de nºA071A68. (Anexo).

Figura 5- Descrição dos grupos e protocolo experimental.



Fonte: Próprio autor.

3.6 ONTOGENIA REFLEXA E MATURAÇÃO SOMÁTICA DOS NEONATOS

A consolidação de respostas reflexas e somáticas foram pesquisadas diariamente, no horário entre 6 e 8 horas da manhã, do 1º ao 21º dia pós-natal. A resposta foi considerada consolidada quando a reação esperada se repetiu por três dias consecutivos, sendo considerado o dia da consolidação o 1º dia do aparecimento. O tempo máximo de observação considerado foi de 10 segundos, cronometrados por cronômetro digital de mão Kenko (modelo KK-2808, São Paulo, Brasil).

3.6.1 Ontogenia Reflexa

Os reflexos pesquisados seguiram o modelo experimental estabelecido por Smart e Dobbing (1971). Foram avaliados os seguintes reflexos: a) Desaparecimento da Preensão Palmar (PP) e Aparecimentos das seguintes respostas: b) Recuperação Postural de Decúbito (RPD), c) Colocação Espacial Desencadeada Pelas Vibrissas (CPV), d) Aversão ao Precipício (AP), e) Geotaxia Negativa (GN), f) Resposta ao Susto (RS) e g) Recuperação do Decúbito em Queda Livre (RDQL).

Procedimentos:

a) Desaparecimento da Preensão Palmar (PP) – utilizando-se um bastonete metálico, com aproximadamente, 5 cm de comprimento por 1 mm de diâmetro, fez-se uma leve percussão na palma da pata dianteira esquerda ou direita de cada animal. Em resposta, ocorreu a flexão rápida dos artelhos. Com o desenvolvimento do recém-nascido, ocorre o desaparecimento dessa resposta;

b) Recuperação Postural de Decúbito (RPD) – colocou-se o animal em decúbito dorsal sobre uma superfície plana e lisa. Em resposta, observou-se o retorno ao decúbito ventral. A resposta foi considerada positiva quando o animal assumia o decúbito ventral, apoiado sobre as quatro patas;

c) Resposta de Colocação Espacial Desencadeada pelas Vibrissas (CPV) – o filhote foi suspenso pela cauda, de tal forma que suas vibrissas tocavam levemente a borda de uma superfície plana. Em resposta, o animal tende a colocar as duas patas anteriores sobre a mesa e realizar movimentos de marcha, associados com extensão de tronco;

d) Aversão ao Precipício (AP) – o animal foi colocado sobre uma superfície plana e alta (mesa), com as patas dianteiras na extremidade da mesa, de maneira que ele detectava o precipício. Em resposta, o animal tende a se deslocar para um dos lados e caminhar em sentido contrário à borda, caracterizando a aversão ao precipício;

e) Geotaxia Negativa (GN) – o animal foi colocado no centro de uma rampa medindo 34 x 24 cm, revestida com papel antiderrapante (papel crepom), com inclinação aproximada de 45º, com a cabeça na parte mais baixa da rampa. Em resposta, o animal tende a girar o corpo, em um ângulo de 180º graus, posicionando a cabeça em sentido ascendente;

f) Resposta ao Susto (RS) – o animal foi submetido a um estímulo sonoro intenso e súbito, produzido pela percussão de um bastão de madeira sobre um recipiente metálico (6 cm de diâmetro x 1,5 cm de altura), a uma distância aproximada de 10 cm da cabeça. Em resposta, tende a haver uma retração das patas anteriores e posteriores, com imobilização rápida e involuntária do corpo do animal;

g) Recuperação do Decúbito em Queda Livre (RDQL) – o animal foi segurado pelas quatro patas, com o dorso voltado para baixo, a uma altura de 30 cm, e solto em queda livre sobre um leito de espuma sintética (30 x 12 cm). Em resposta, o animal tende a recuperar o decúbito durante a queda livre caindo na superfície apoiado sobre as quatro patas.

3.6.2 Indicadores de Maturação Somática

A partir do 1º dia pós-natal em diante os filhotes foram examinados diariamente, sempre no mesmo horário da avaliação da ontogenia reflexa, de modo a determinar o dia em que a maturação somática se completou. Para isso, foram avaliadas as seguintes características físicas (tomadas como indicadores de desenvolvimento somático).

Procedimentos:

a) Abertura do Pavilhão Auricular (APA) – normalmente, o animal nasce com o pavilhão auditivo dobrado; portanto, o pavilhão auricular aberto foi detectado no dia em que a dobra foi desfeita. Nesta avaliação, a maturação foi considerada positiva quando os dois pavilhões estiveram desdobrados;

b) Abertura do Conduto Auditivo (ACA) – Ao nascimento, o conduto auditivo encontra-se fechado. Considerou madura a ACA no dia em que o orifício auricular podia ser visualizado. Nesta avaliação, a maturação foi considerada positiva quando os condutos, direito e esquerdo, encontraram-se abertos;

c) Erupção dos Dentes Incisivos Superiores (EIS) – foi registrado o dia em que houve a erupção dos dentes incisivos superiores. Levou-se em consideração a resposta positiva quando ambos os incisivos estiveram expostos;

d) Erupção dos Dentes Incisivos Inferiores (EII) – foi registrado o dia em que houve a erupção dos dentes incisivos inferiores. Levou-se em consideração a resposta positiva quando ambos estiveram expostos;

e) Abertura dos Olhos (AOL) – no rato, os olhos encontram-se totalmente encobertos pelas pálpebras, durante alguns dias após o nascimento. A resposta foi considerada positiva quando os dois olhos estiveram abertos, com presença de movimento reflexo das pálpebras;

f) Aparecimento dos Pelos Epidérmicos (APE) – os ratos nascem sem pelos, o seu aparecimento foi confirmado quando detectada a presença da pelugem, para tal teste deslizou-se gentilmente uma haste plástica sobre a epiderme do animal;

g) Comprimento da Cauda (CC) – o animal foi colocado sobre uma régua milimetrada, sendo a cauda delicadamente mantida bem estendida, desde a base até a extremidade.

3.7 TESTES COMPORTAMENTAIS

Uma semana antes do final da adolescência (T45) e início da fase adulta (T90), foram avaliados o comportamento de ansiedade através do teste de campo aberto com a finalidade de verificar parâmetros de ambulação, comportamentos de levantar, tempo de autolimpeza e defecação (PELLOW; CHOPIN; FILE; BRILEY, 1985); Teste do Labirinto em Cruz Elevado (HANDLEY; MITHANI, 1984; PELLOW; FILE 1985) e Caixa Claro-escuro (COSTALL *et al*., 1989).

Quanto a avaliação da memória utilizou-se o Teste de Habituação do Campo Aberto (RACHETTI *et al*., (2013); Teste de Reconhecimento de Objetos, para avaliação da memória em curto e longo prazo (NAVA-MESA; LAMPREA; MÚNERA, 2013) e o Labirinto Aquático de Morris para avaliação da memória espacial dos roedores (VORHEES; WILLIAMS, 2006).

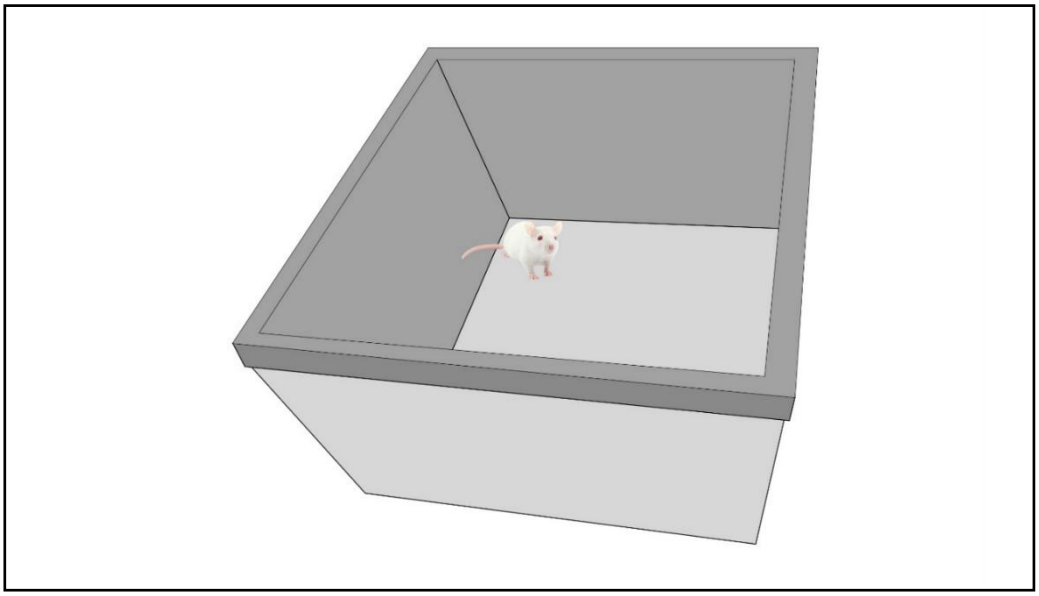
Os testes foram realizados sempre no período da manhã, no horário das 6:00 as 8:00 h. Todas as sessões de testes comportamentais foram filmadas com câmera de vídeos instalada no teto e os dados posteriormente associados como indicador dos efeitos da suplementação dietética materna.

3.7.1 AVALIAÇÃO DO COMPORTAMENTO DE ANSIEDADE

3.7.1.1 Teste do Campo Aberto

O campo aberto é um instrumento para testar comportamento de ansiedade e atividade exploratória, a fim de verificar os efeitos de ambientes não familiares sobre a emocionalidade em ratos (PRUT; BELZUNG, 2003). O teste foi realizado com a prole de ratas Wistar suplementadas no período de gestação e lactação. Cada animal foi inserido no centro do aparelho e observado durante 10 minutos. Foram avaliados parâmetros como: ambulação (número de cruzamentos dos segmentos pelo animal com as quatro patas), número de comportamentos de levantar (*rearing*), tempo de comportamentos de autolimpeza (*grooming*) e defecação (registrada por meio do número de bolos fecais) (RACHETTI *et al*., 2013). As sessões foram filmadas e, posteriormente, analisadas. O aparelho foi higienizado com uma solução de 10% de álcool após cada sessão de comportamento (Figura 6).

Figura 6 -Representação do teste do Campo Aberto.

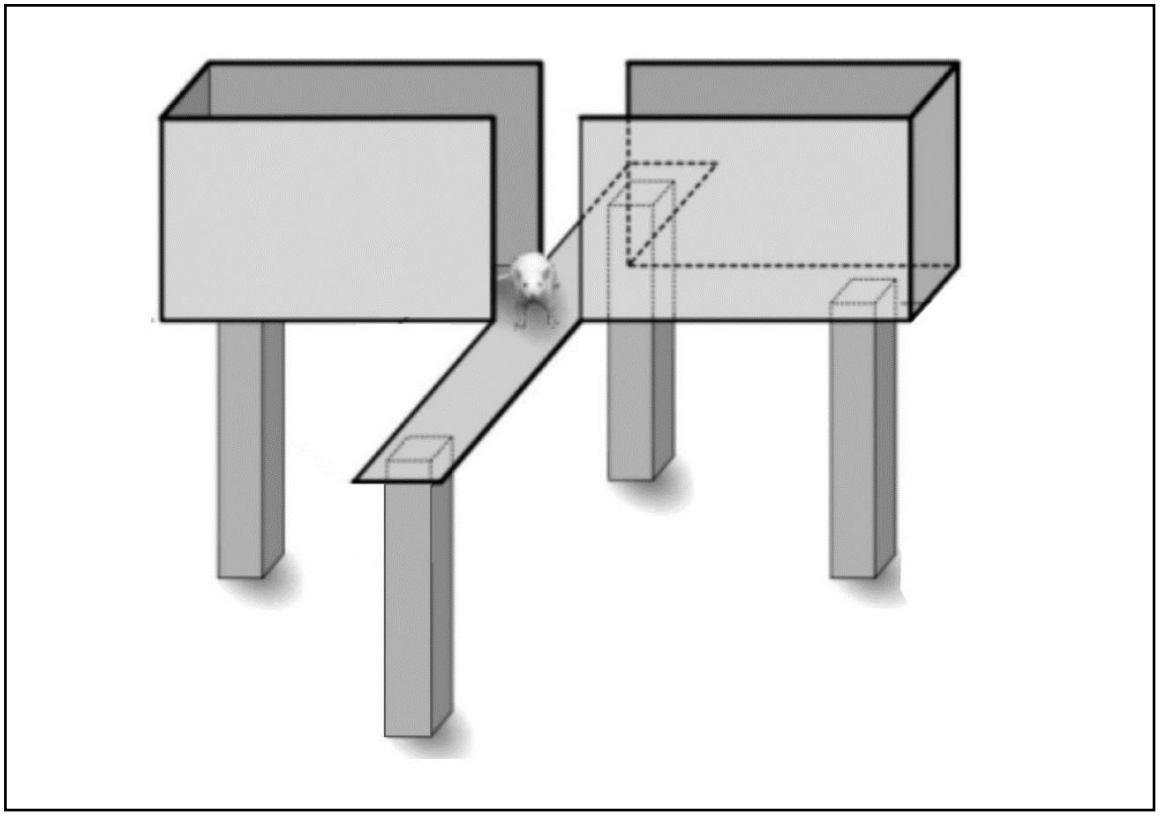


F**onte:** Próprio autor.

3.7.1.2 Teste do Labirinto em Cruz Elevado (LCE)

O LCE é comumente utilizado como modelo não condicionado de avaliação do comportamento tipo ansioso em roedores (FLINT, 2003; BRADLEY *et al*., 2007). O LCE constitui um aparato formado por dois braços fechados e dois braços abertos perpendiculares aos primeiros, uma área central, sendo este elevado do solo. O animal foi posto no centro do aparelho, sempre pelo mesmo pesquisador, com o focinho voltado para o braço fechado direito. Durante 5 minutos, analisou-se a frequência de entradas nos braços fechados e abertos, o tempo gasto em cada braço e no centro do aparelho. Além disso, também foi contabilizada a quantidade de mergulhos da cabeça do animal nos braços abertos. A cada animal testado, o labirinto foi higienizado com álcool a 10%. As sessões foram filmadas e, posteriormente, analisadas. O aparelho foi higienizado com uma solução de 10% de álcool após cada sessão de comportamento (Figura 7).

Figura 7 -Representação do teste do Labirinto em Cruz Elevado.

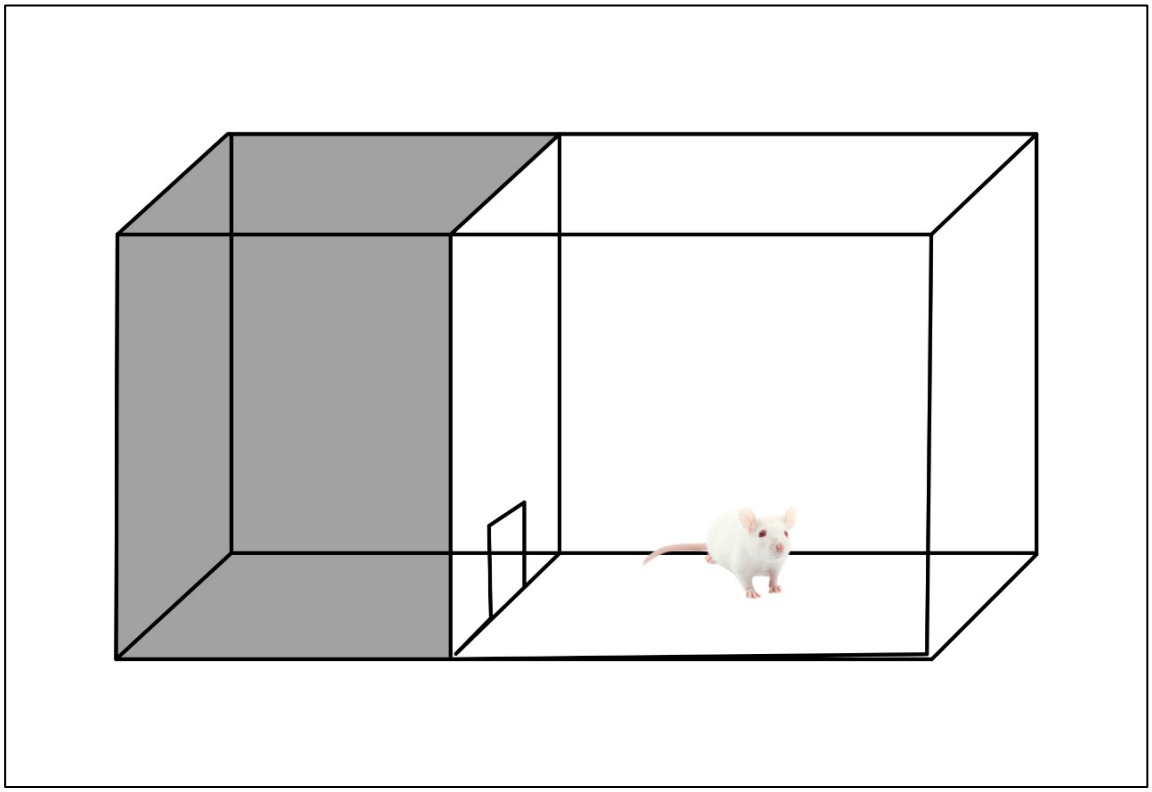


F**onte:** Próprio autor.

3.7.1.3 Teste da Caixa Claro-escuro

A caixa de transição claro-escuro mede a ansiedade incondicionada e comportamento exploratório em roedores (BOURIN; HASCOËT, 2003). O aparato feito de acrílico (45 x 27 x 27 cm) é dividido em dois compartimentos, sendo um escuro (18 x 27 cm) e outro claro (27 x 27 cm). Os animais foram colocados no centro do compartimento claro, com o focinho voltado para o compartimento escuro, sendo mantidos no parelho por 5 min para livre exploração. As sessões foram filmadas e, posteriormente, analisadas. Os comportamentos avaliados foram o tempo de permanência do animal em cada um dos compartimentos. Ao fim de cada sessão, o aparelho foi higienizado com uma solução de 10% de álcool, uma vez que a sujeira na caixa claro-escuro compromete o componente neofóbico associado ao aparato (Figura 8).

Figura 8 -Representação do teste da Caixa Claro-escuro.



F**onte:** Próprio autor.

3.7.2 AVALIAÇÃO DOS PARÂMETROS DE MEMÓRIA

3.7.2.1 Avaliação da memória usando o Teste de Habituação ao Campo Aberto

Para a avaliação da aprendizagem não associativa do animal, foi realizado o Teste de Habituação ao Campo Aberto com o objetivo de avaliar a capacidade de habituação do animal a longo prazo. Segundo Rachetti *et al*. (2013), a atividade locomotora do animal no campo aberto, está intimamente ligada à sua capacidade de aprendizagem, assim, a habituação do animal após exposição repetida ao campo aberto é considerado um indicativo de aprendizagem não associativa.

Para concretude do teste, utilizou-se a metodologia descrita por Rachetti *et al*. (2013). O animal foi exposto ao campo aberto em dois momentos. Primeiro, foi realizado o teste de habituação e após 7 (sete) dias repetiu-se o mesmo com a finalidade intuito de comparar a atividade locomotora dos animais para relacionar com a memória.

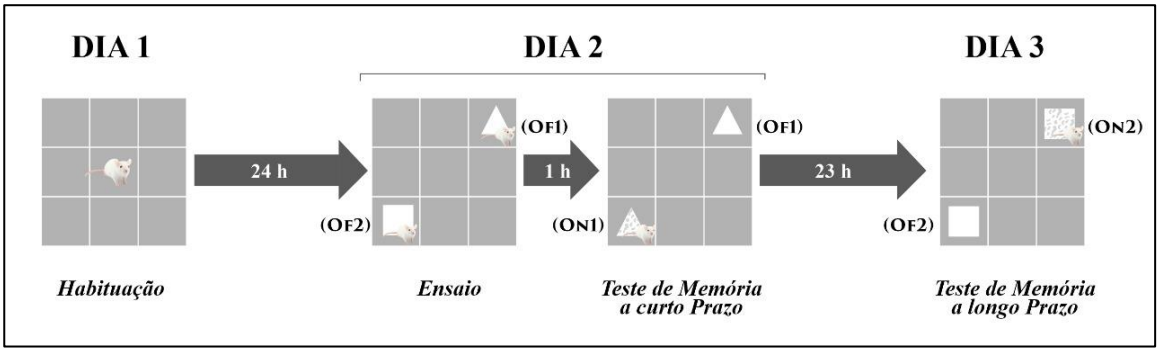
O parâmetro analisado na realização desse teste foi a quantidade de ambulações dos campos pelo animal, considerando a locomoção das quatro patas para o interior de cada campo. O tempo de observação foi de 10 minutos. O procedimento foi realizado sempre entre as 06:00 a 08:00h. No dia do experimento, os animais permaneceram em seus locais e só foram manipulados no momento de serem colocados no campo aberto (Figura 8) para que não tivesse mudança de ambiente ou alguma alteração no comportamento. Posteriormente, os vídeos foram analisados e as categorias comportamentais identificadas e registradas manualmente. A cada animal testado, o aparelho foi limpo, antes de iniciar e depois de concluído o teste, com solução de álcool a 10% e papel absorvente.

3.7.2.2 Avaliação da memória usando Teste de Reconhecimento de Objetos

Para avaliar a memória em curto e longo prazo foi utilizado o Teste de Reconhecimento de Objetos (TRO) (NAVA-MESA; LAMPREA; MÚNERA, 2013). O teste consiste em um campo aberto preto (60 x 60 x 60 cm), com seis linhas cruzadas formando 6 quadrantes de 20 x 20 cm, uniformemente iluminado e com objetos na cor preta, com formatos (pirâmides o prisma retangular) e texturas (liso ou áspero) diferentes.

O teste constituiu em 4 (quatro) ensaios de 10 minutos que aconteceu em 3 (três) etapas: 1) Dia 1 – habituação durante 10 minutos para minimizar o estresse da manipulação; 2) Dia 2 – realizado 24 h após o ensaio de habituação onde cada animal foi colocado no campo aberto contendo dois objetos (OF1 e OF2) de textura idêntica (lisa), mas com formatos diferentes (triângulo e prisma retangular) e localizados perto de dois cantos opostos escolhidos aleatoriamente. Para mensuração da memória à curto prazo, uma hora após, o animal foi colocado novamente para exploração de dois objetos (OF1 em sua localização original e um novo objeto – ON1), de forma idêntica, mas com diferente textura em relação ao OF1 e localizado no local onde OF2 tinham sido colocados durante o ensaio amostral; e 3) Dia 3 – realizado 24 h após o ensaio curta duração onde cada animal foi colocado no campo aberto para explorar dois objetos (OF2 em seu lugar original) e um novo objeto (ON2), de forma idêntica, mas com diferente textura (Figura 9). Para avaliação da memória à curto prazo foi observado o tempo gasto pelo animal para explorar o objeto novo de textura diferente (ON1) e para avaliação da memória à longo prazo foi observado o tempo gasto pelo animal para explorar o objeto novo de textura diferente (ON2), 24 h após a primeira exposição, no dia 2. As sessões foram filmadas com uma câmera de vídeo e a cada animal testado o aparelho foi limpo, antes de iniciar e depois de concluído o teste, com solução de álcool a 10%. O resultado da taxa de exploração foi calculado para cada animal e expresso pela razão TN/(TF + TN) (TN = tempo gasto explorando o objeto novo; TF = tempo gasto explorando o objeto familiar (D'AVILA *et al*., 2017; GUSTAVSSON *et al*., 2010).

Figura 9 – Representação do Teste de Reconhecimento de Objetos.



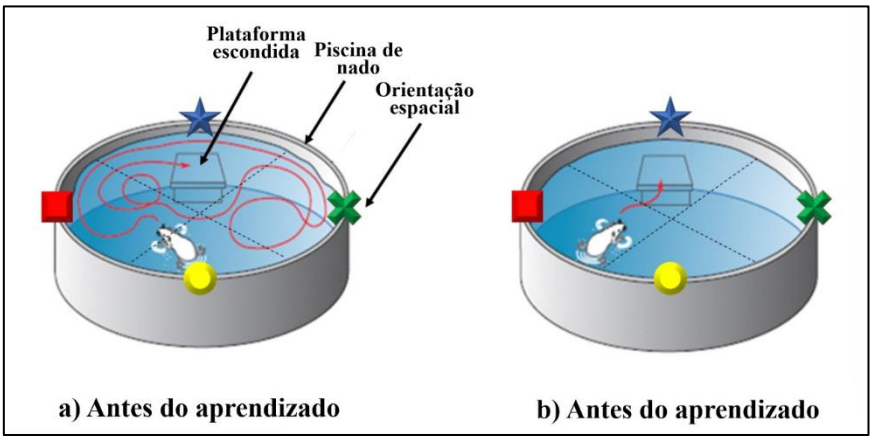
Fonte: Adaptado de Nava-Mesa; Lamprea; Múnera, (2013).

3.7.2.3 Avaliação da memória espacial utilizando o Labirinto aquático de Morris

O labirinto aquático de Morris foi utilizado para avaliação da memória espacial dos animais, ligada principalmente à região encefálica hipocampal, segundo Brandeis *et al*. (1989) e Sullivan (2010). O protocolo aplicado foi baseado em estudos anteriores de Delint-Ramírez *et al*. (2008) e Vorhees e Williams (2006), cuja duração correspondeu a quatro dias, sendo três deles de treino e o último dia de teste, no qual a plataforma foi retirada. Uma piscina redonda, fabricada com material plástico, com diâmetro de 1,60 m e 82 cm de altura, foi dividida em quatro quadrantes representados pelos pontos cardiais (norte, sul, leste e oeste), sendo a plataforma localizada na posição noroeste, cujas dimensões corresponderam a 10 cm x13 cm. A piscina foi cheia com água até a altura de 54 cm e a plataforma foi submersa a 1 cm.

Cada período de treinamento consistiu em quatro ensaios, com duração de 60 segundos cada, nos quais os animais eram aleatoriamente posicionados nos quadrantes. A ordem aleatória de início foi seguida para todos os animais. O objetivo dos treinos era que o animal encontrasse a plataforma e nela permanecesse por 10 segundos, caso não a encontrasse, o animal era gentilmente conduzido até a mesma. Após cada ensaio dos treinos, foi dado intervalo de 20 segundos, no qual o animal era acondicionado provisória e individualmente em uma caixa. Durante os dias de treinos, foi analisada a latência de escape. No quarto dia, o dia de treino, a plataforma foi retirada e os animais nadaram durante 60 segundos. Foram analisadas as variáveis: velocidade média, distância total percorrida, número de entradas no alvo (que seria a plataforma), tempo na zona da plataforma e latência para primeira entrada no alvo (Figura 10).

Figura 10 – Desenho experimental adaptado do Teste do Labirinto Aquático de Morris.



Fonte: Próprio autor.

3.8 ANÁLISE DA MICROBIOTA FECAL POR MEIO DE METAGENOMA AMPLICON rRNA 16S

3.8.1 SEQUENCIAMENTO DO GENE rRNA 16S

Amostras individuais de fezes dos animais foram coletadas diretamente em microtubos tipo Eppendorf aos 45 e 90 dias de vida e imediatamente armazenadas em freezer a -20°C. Seis amostras de cada grupo de tratamento foram usadas para sequenciamento de DNA do gene 16S rRNA. O DNA genômico foi extraído usando um kit comercial (PowerFecal Pro DNA, Qiagen, Hilden, Alemanha). A integridade do DNA foi avaliada por eletroforese em gel de agarose a 1% e quantificado por fluorometria (Qubit, ThermoFisher, Waltham, MA, EUA). As regiões V3-V4 do gene microbiano 16S rRNA foram amplificadas por PCR (95 °C por 3 min, seguido por 25 ciclos a 95 °C por 30 s, 55 °C por 30 s, 72 °C por 30 s e uma extensão final para 72 °C por 5 min) usando os iniciadores 341F: 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3' e 785R: 5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3'. A biblioteca de amplicons foi preparada usando o Nextera XT Index Kit Set A (Illumina Inc., San Diego, CA, EUA) e esferas magnéticas para limpeza e purificação (Agencourt AMPureXP, Beckman Coulter, Indianapolis, EUA). O sequenciamento do tipo paired-end foi realizado na plataforma Illumina MiSeq usando um kit V2 de 500 ciclos (2 × 250).

3.8.2. ANÁLISES DE BIOINFORMÁTICA

A pipelene de bioinformática utilizada foi segundo recomendações de Liu *et al.* (2021). As sequências Forward e Reverse desmultiplexadas brutas foram processadas na plataforma QIIME 2 v.20.8 30 Bolyen *et al*. (2019). Os *reads* com baixa qualidade foram removidos, tendo o score Q>20 como qualidade mínima por meio do programa DADA2 Callahan *et al*. (2016). As sequencias quiméricas e não classificadas foram removidas das análises assim como bases acima de 245 pb com baixa qualidade. As tabelas de Variantes de Sequência de Amplicon (ASV) geradas foram normalizadas em 3150 ASVs por amostra, usando DADA2. Para filogenia, as sequências foram rarefadas e alinhadas usando Silva 128 SEPP reference database disponível em https://docs.qiime2.org/2022.2/data-resources/ . A análise de diversidade alfa foi

avaliada pelos índices de Observed Features, Fisher, Simpson e Shannon por meio do QIIME 2 v.20.8 30 (BOLYEN *et al*., 2019). As classificações taxonômicas foram atribuídas pelo método Machine-learning baseada na base de dados Silva Databases com 99% de similaridade para as regiões V3-V4.

3.8.3 INFERÊNCIA METABÓLICA

A predição funcional dos metagenomas foi realizada a partir de dados de rRNA 16S usando PICRUSt2 Langille *et al*. (2013). As tabelas de abundancias de ASVs gerados através do QIIME2 foram utilizadas como input para PICRSt2 FullPipile por meio do pluing q2-picrust2 do QIIME2 disponível em https://library.qiime2.org/plugins/q2-picrust2/13/. As abundâncias das vias metabólicas e KO foram mapeadas utilizando o banco de dados MetaCyc disponível em https://metacyc.org/.

3.9 EUTANÁSIA E COLETA DOS TECIDOS

Sob anestesia, a prole foi submetida a punção cardíaca. Em seguida, o cérebro foi coletado para análise de gorduras totais, composição de ácidos graxos, conteúdo de malonaldeído (MDA) níveis de glutationa peroxidase (GPx) e histologia do hipocampo e córtex. Após a coleta, os tecidos foram imediatamente armazenados em freezer sob temperatura de -20 ºC e/ou - 80 ºC até o momento das análises.

3.9.1 COLETA DO LEITE MATERNO E TECIDO CEREBRAL DA PROLE PARA A ANÁLISE DO CONTEÚDO LIPÍDICO E PERFIL DE ÁCIDOS GRAXOS

O leite materno foi obtido no 21º dia de lactação, após jejum de 8 horas. As mães foram anestesiadas com uma mistura de Cloridrato de Ketamina + Cloridrato de Xilasina (1 mg/kg de peso do animal), que correspondeu a uma dose de 50 mg/kg de peso de Cloridrato de Ketamina e 20 mg/kg de peso de Cloridrato de Xilasina, e o leite materno coletado por ordenha manual, de acordo com metodologia adaptada de Keen *et al*. (1981). As mães foram separadas de sua prole por um período de 1 h antes da ordenha e 5 minutos antes da mesma receberam injeção intraperitoneal de 0,30 mL de ocitocina. A ordenha

manual durou cerca de 20 a 30 minutos e o leite coletado foi acondicionado em um microtubo e, posteriormente, congelado em freezer a - 20ºC.

Na prole, a coleta do tecido cerebral ocorreu após anestesia e procedimento de punção cardíaca. O tecido foi removido por inteiro e após a coleta houve a separação dos hemisférios através de um corte longitudinal no sentido antero-posterior. O hemisfério esquerdo, utilizado para a análise do perfil de ácidos graxos, foi imediatamente pesado e colocado em uma superfície com gelo e, em seguida, mantido em ultra freezer a - 80 ºC até o momento das análise.

A extração dos lipídios tanto do leite materno quanto do tecido cerebral foi realizado pelo método de Folch, Less e Stanley (1957), seguido da transmetilação dos ácidos graxos de acordo com Hartman e Lago (1973) e posterior injeção e quantificação dos ácidos graxos em cromatografia gasosa, conforme descrito no tópico 3.3 (“McCANCE; WIDDOWSON’S, 2002).

3.9.2 DETERMINAÇÃO DO CONTEÚDO DE MALONDIALDEÍDO (MDA)

A determinação do conteúdo de MDA foi realizada pelo método descrito por Esterbauer e Cheeseman (1990). Após fragmentação do cérebro, uma tira do hemisfério direito do tecido de cada animal foi separada para esta análise, colocada em microtubo de 2,0 mL e levada ao congelamento a uma temperatura de - 80 °C. Para iniciar a determinação do MDA, as amostras do tecido foram descongeladas e, posteriormente, pesadas. Mediante o peso foi calculada a quantidade de tampão Tris HCl para cada amostra, utilizando-se a proporção de 1:5 (p/v). O tecido foi picado com uma tesoura por, aproximadamente, 15 segundos, em meio resfriado. O material fragmentado foi triturado e homogeneizado a frio, sob ação de um triturador (Ultra Stirrer modelo: Ultra 80), e a ele adicionado o tampão Tris HCl. O homogenato obtido foi centrifugado a 2500 *xg* por 10 min a 4 °C e 300 μL do sobrenadante foram transferidos para um microtubo, sendo adicionados 750 μL do reativo cromogênico e 225 μL de ácido clorídrico (HCl – 37%). Em seguida, o material foi colocado em banho-maria com agitação a 45 °C, durante 40 min e, posteriormente, levado à uma centrifugação a 2500 *xg* durante 5 min a 4 °C. 300 μL do sobrenadante foram transferidos para microplaca de 96 poços, em duplicata, e levado para leitor de microplacas (Polaris, Belo Horizonte, Minas Gerais, Brasil) a um comprimento de onda de 586 nm. O conteúdo de MDA foi calculado através de interpolação em curva padrão com o 1,1,3,3 – tetraetoxipropano, o qual foi hidrolisado

durante o passo de incubação com HCl a 45 °C, gerando o MDA. Os resultados foram expressos em nmoL/g tecido.

3.9.3 DETERMINAÇÃO DOS NÍVEIS DE GLUTATIONA TOTAL

O conteúdo total de glutationa do tecido cerebral da prole foi quantificado como descrito por Anderson (1985). As amostras do cérebro foram descongeladas, picadas, diluída para uma concentração de 1:20 (m/v) de ácido tricloroacético a 5% e homogeneizadas (Homogeneizador Ultra Stirrer, Modelo 80) em banho de gelo. Os homogenatos foram centrifugados a 10.000 G (15 min a 4ºC) e os sobrenadantes utilizados para quantificação do conteúdo total de glutationa. O conteúdo de glutationa total foi medido imediatamente em Leitor de microplacas Polaris® (Celer Biotecnologia S. A.), a 412 nm. Os resultados foram expressos como nmol/ g de tecido. Todos os reagentes foram adquiridos pela Sigma-Aldrich® (St Louis, MO, EUA).

3.9.4 AVALIAÇÃO HISTOLÓGICA DO TECIDO CEREBRAL DA PROLE

Os cérebros de 3 ratos por tratamento foram coletados, lavados em solução salina (0,9% NaCl) e fixados em formalina tamponada a 10%. O hipocampo e o córtex foram submetidos a procedimento histológico conforme técnica de rotina do Laboratório de Patologia (Departamento de Fisiologia e Patologia/CCS/UFPB) para obtenção de blocos dos quais foram cortados cortes semi-seriados de 4 μm. As lâminas foram hidratadas, coradas com hematoxilina-eosina (HE), desidratadas, diafanizadas em xilol e montadas com Entellan® para análise microscópica óptica (Motic BA 200, Olympus Optical Co, Filipinas). Para a análise morfométrica, foram utilizadas vinte imagens aleatórias de lâminas do tecidos do hipocampo e do córtex. Sob um microscópio de luz Axiolab (Zeiss) com resolução de 400 ×, vinte imagens de cada tecido foram retransmitidas para um sistema de análise de imagem (Kontron Elektronik image analyser; Carl Zeiss, Alemanha—KS300 software). A leitura das lâminas foi realizada aleatoriamente por dois patologistas. Uma objetiva de 10 × e uma fotomicrografia de 40 × dos tecidos foram usadas para obter as imagens.

3.10 ANÁLISES ESTATÍSTICAS

Os resultados foram expressos em média ± desvio padrão (DP) ou erro padrão da média (EPM). O teste de normalidade Shapiro-Wilk foi aplicado e analisados pelo ANOVA One Way, seguido de teste de Tukey, para comparação entre os grupos. Porém, os resultados da ontogenia reflexa e deseonvolvimento somático foram expressos em valores de mediana do dia (Min-Máx) e analisados por Kruskal-Wallis seguido de Teste de Dunn's. Foi utilizado o programa estatístico GraphPad Prism e considerado o nível de significância para rejeição da hipótese nula de $p<0,05$.

Em relação a análise taxonômica, o teste não paramétrico de Kruskal-Wallis foi escolhido para comparações dos índices de diversidade alfa. As análises de abundância diferencial tanto de taxonômicas quanto metabólicas foram realizadas por meio do programa estatístico STAMP Parks *et al*. (2014). Para as análises de cluster e *heatmap* foi utilizado o método de Farthest Neighbor. Para as análises comparativas de múltiplos grupos foi aplicado o teste estatístico de análise de variância (ANOVA), tendo Post-hoc test como Games-Howel e effect size: Eto-squared aplicados. Como correção foi aplicado teste múltiplo de Benjamini-Hochberg.

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4 RESULTADOS

Os resultados obtidos nesta tese estão apresentados em forma de dois Artigos Científicos originais que foram submetidos a periódicos de alto fator de impacto.

ARTIGO I: Suplementação materna da amêndoa de baru (*Dipteryx alata* Vog.) modula a microbiota fecal, acelera o neurodesenvolvimento e melhora a memória da prole de ratas Wistar
(Submetido ao periódico: Scientific Reports. Fator de impacto: 5.51 - Qualis A1)

ARTIGO II: Maternal consumption of baru almond (*Dipteryx alata* Vog.) induces anxiolytic-like behavior, reduces oxidative stress in the brain and preserves cell bodies in the offspring's cortex at different stages of life.
(Submetido ao periódico: Journal of Ethnopharmacology. Fator de impacto: 4.36 - Qualis A2)

ARTIGO I

MATERNAL SUPPLEMENTATION WITH BARU ALMOND (*Dipteryx alata* Vog.) MODULATES FECAL MICROBIOTA, ACCELERATES NEURODEVELOPMENT, AND IMPROVES MEMORY IN THE OFFSPRING OF RATS.

(**Submetido ao periódico:** Scientific Reports. **Fator de impacto:** 5.51 - **Qualis**: A1**).**

Maternal supplementation with baru almond (*Dipteryx alata* Vog.) modulates fecal microbiota, accelerates neurodevelopment, and improves memory in the offspring of rats.

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ABSTRACT

We tested the hypothesis that PUFAs and phytocomplexes present in the Baru almond can modulate the intestinal microbiota, the brain fatty acid profile, and memory in the offspring of rats treated during the initial phases of life. *Wistar* rats were mated and randomized into three groups: Control (CG) - received distilled water by gavage; (Oil) – received 2,000 mg of baru oil/kg, and (Almond) - received 2,000 mg of baru almond. The offspring were submitted to evaluation of somatic development and reflex ontogenesis during the first 21 days of life. During adolescence (T45) and adulthood (T90) the Open Field Habituation (OF), Object Recognition (ORT), and Morris Water Maze (MWM) tests; evaluation of the fatty acid profile and brain histology; and analysis of the fecal microbiota were performed. The supplementation promoted an increase in PUFAs in both breast milk and in the brains of the adolescent and adult offspring, as well as in docosahexaenoic and arachidonic acid content. We observed acceleration of reflex ontogeny and somatic development in both the almond and oil groups, as well as better performance in the OF, ORT, and MWM tests in both life stages. Supplementation promoted a relative abundance of intestinal bacteria, producers of neuroprotective compounds.

Keywords: PUFAs; Phytocomplexes; Gut microbiome; Neurodevelopment; Cognition.

1. INTRODUCTION

The period of embryonic development is considered critical and can be influenced by many stimuli, including nutrition, which in turn, promotes significant changes in the maternal and fetal intestinal microbiome[1,2]. The intrauterine environment is a fundamental interface between mother and fetus, and early formation of a microbiota at this stage of life has been postulated[3]. After birth, breastfeeding becomes a key factor in molding the intestinal microbiota of the offspring[3].

Microbial diversity evolves rapidly, migrating from a relatively simple state to a more complex and stable state by the first thousand days of life[4]. Evidence indicates that essential nutrients, such as polyunsaturated fatty acids (PUFAs) and antioxidants compounds are important to a relative abundance of beneficial intestinal microorganisms, as well as to the production of metabolites which act on the central nervous system (CNS) [as reviewed in Jayapala; Lim, 2022 and Filosa; Di Meo; Crisp, 2018][5,6].

The underlying mechanisms by which PUFAs exert positive impacts on the CNS may well include their ability to modify neuronal membrane fluidity and signaling, supporting neurogenesis, and organization of neural networks, regulating processes involving neurotransmission, cell survival, and neuroinflammation[7–10]. Further, the gut microbiota is capable of producing a variety of neuroactive compounds through metabolism of dietary PUFAs, to impact the CNS through the gut-brain axis[11]. Antioxidants compounds are also able to cross the blood-brain barrier to bioaccumulate in the brain[12]. Some molecules cross the blood-brain barrier (BBB) intact, while others need to be biotransformed by intestinal bacteria[13]. In the CNS, these compounds reduce oxidative stress, and help maintain the molecular, cellular, structural, and functional integrity of the brain[14].

Among the many sources of PUFAs and phytocomplexes available for maternal supplementation, the baru almond (*Dipteryx alata* Vog.) stands out. The *Barueiro* fruit, belonging to the Leguminosae family, and native to the Brazilian Cerrado regions has aroused scientific interest (due to its lipid composition), as a source of monounsaturated and polyunsaturated fatty acids[15]. The almond also contains antioxidants compounds with neuroprotective action (gallic acid, ferulic acid, catechins, epicatechin, tannins, phytic acid, and tocopherols)[16,17].

Previous studies have reported that if offered during early life, PUFAs and antioxidants compounds can modulate neurodevelopment[18,19]. However, the literature still lacks studies regarding maternal supplementation and its effects on offspring. We hypothesized that maternal supplementation with baru oil during the critical period of embryonic development would induce

an abundantly beneficial microbiota, adequate maturation of the CNS, and better cognition and memory in the offspring.

The present research aimed to evaluate the impact of maternal supplementation with baru oil and almonds on somatic and reflex development, brain fatty acid profile, intestinal microbiota, and memory in the offspring of rats treated during the initial phase of life.

2 MATERIAL AND METHODS

2.1 Baru Almond

The baru almond used in the experiment belonged to the species *Dipteryx alata* Vog., from the city of Goiânia/GO, Brazil: latitude -16º40`43``S, longitude -49º15`14``W, at an altitude of 749 m. Baru almond was used to extract the oil which was placed in amber glass and stored at -20°C. The almonds were also crushed and mixed with distilled water to be administered immediately to the animals in the form of a solution by gavage. The fatty acid profile of baru almond and oil was analyzed according to the methodology described by Folch et al., (1957)[21] and Hartman and Lago., (1973)[23]. To analyze the phenolic compounds, Liu et al., (2002)[24] was used; for antioxidant activity, the FRAP methodology described by Benzie and Strain, (1999)[25], with modifications proposed by Pulido et al., (2000)[26], and also the ABTS methodology described by Surveswaran et al., (2007)[27] were used. The results of these analyses are described in Table 1.

Table 1 – Fatty acid composition, phenolic compounds, and antioxidant activities in baru oil and almonds.

		BARU OIL (%)	BARU ALMOND (%)
Acids Fat		**100g^{-1} lipid**	
SATURATED			
Palmitic acid	C16:0	7.03	9.44
Stearic acid	C18:0	4.91	7.46
Arachidic acid	C20:0	1.07	0.40
Behenic acid	C22:0	3.02	6.34
Lignoceric acid	C24:0	4.02	9.24
Cerotic acid	C26:00	0.24	0.79
∑ SFA		**20.29**	**33.67**
MONOUNSATURATED			
Oleic acid	C18:1ω9	47.54	30.73
Gondoic acid	C20:1ω9	-	4.37
Gadoleic	C20:1	2.39	1.05
Erucic acid	C22:1ω9	-	0.57
∑ MUFA		**49.93**	**36.72**
POLYUNSATURATED			
Linoleic acid	C18:2ω6	29.05	27.52
α-linolenic acid	C18:3ω-3	4.02	3.37
Eicosadienoic acid	C20:2ω6	-	0.12
∑ PUFA		**33.07**	**31.01**
PHENOLIC COMPOUNDS			
Total phenolics (mg GAE/100g)		39.43 (±2.940)	41.31 (±1.224)
Total flavonoids (mg CE/100g)		2.12 (±0.001)	3.25 (±0.001)
ANTIOXIDANT ACTIVIES			
FRAP (µmol TE/g)		0.044 (±0.001)	0.094 (±0.003)
ABTS (µmol TE/g)		0.90 (±0.011)	2.50 (±0.000)

GAE: Gallic acid equivalent; EC: Catechin equivalent; FRAP: Ferric reducing activity; ABTS: radical-scavenging capacity; TEAC: Trolox equivalent; SD: Standard deviation

2.2 Animals and Experimental Groups

All of the experimental methods were previously approved by the Ethics Committee for Animal Use - CEUA of UFCG - Certification No. 104-2017, in compliance with the standards established by the National Council for the Control of Animal Experimentation (CONCEA, Brazil), under Law No. 11,794 /2008 (Arouca Law), and with the guidelines for *in vivo* experiments with animals of the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) 2.0[28]. The baru almond used in the supplementation was registered in SisGen; protocol No. A071A68 (see attachment). Twenty-four female Wistar strains, from the Experimental Nutrition Laboratory of the Federal University of Campina Grande - LANEX/UFCG, aged 90 days and weighing 250 ±50 g were used to obtain offspring. Females were mated at the rate of two females for each male. After confirmation of pregnancy, the animals were housed in individual polypropylene cages (60 cm long, 50 cm wide, and 22 cm high), and kept under standard laboratory conditions (temperature 22 ±1°C, humidity 55 ±5%, light/dark cycle of 12/12 hours - artificial light from 6:00 to 18:00), in three groups: Control - received distilled water; Oil - supplemented with 2,000 mg of baru oil/kg of animal weight; and Almond - supplemented with 2000 mg of baru almond solution/kg of animal weight. Gavage was administered from the 7th day of pregnancy through the 21st day of lactation. The mothers were offered standard feed (Presence Purina®, São Paulo, Brazil) and water *ad libitum*. After weaning, the offspring also received standard feed until adulthood. The dosages offered to the animals were based on studies described by Reis et al., (2018)[29], which evaluated the hepatoprotective and antioxidant activity of baru almond oil (*Dipteryx alata* Vog.) at 1g/kg in a clinical assessment of lipo-toxicity and dyslipidemia, as well as a study by Siqueira et al., (2012)[30], which evaluated the impact of consuming the AIN-93M diet with 10% baru almonds for prevention of iron-induced oxidative stress in rats.

2.3 Experimental Design

The animals were submitted to somatic maturation and reflex ontogeny tests from the first day of lactation to the 21st postnatal day. For quantification of fatty acids and brain histology, tissue was removed at times (T45) and (T90). The associative learning test using the open field (OF) and the object recognition test (ORT) also took place at times (T45) and (T90). The Morris Water Maze test (MWM) was performed with the animals only at time (T90), as indicated in figure 1.

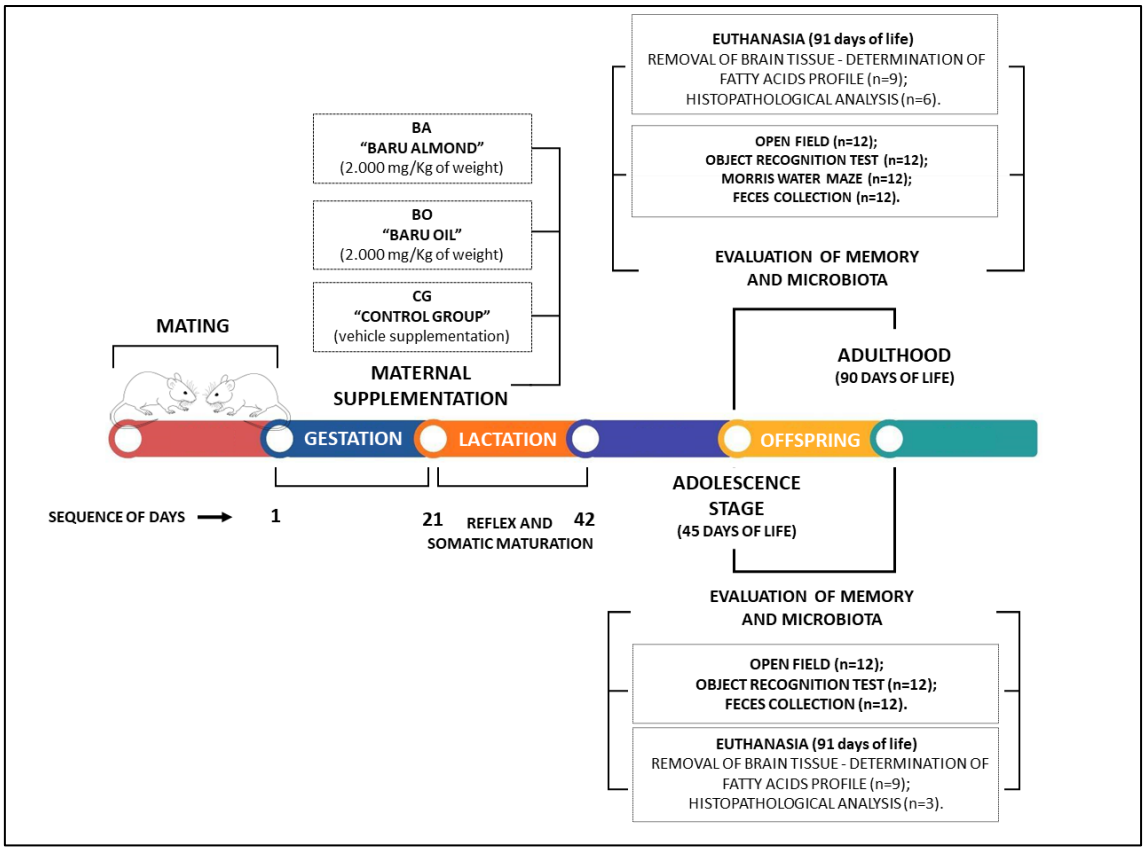


Figure 1. Experimental protocol. Sequence of experimental days conducted with the offspring of Wistar rats treated during pregnancy and lactation. Feces collection was performed to evaluate the relative abundance and functional metabolic prediction of microorganisms. (n) = number of animals used for tests.

2.4 Somatic maturation and reflex ontogeny

Somatic maturation and reflex ontogeny responses were checked daily from the 1st to the 21st day after birth, between 8 am and 9 am, and were considered consolidated when the expected response repeated for three consecutive days, the first day being considered the day of consolidation. The reflexes studied followed the experimental model established by Smart and Dobbing, (1971)[31], as described in Table 3. The maximum observation time considered was 10 seconds, timed manually using a Kenko KK-2808 digital timer.

The somatic maturation indicators evaluated were: Aural Pinna Opening (APO), Auditory Conduit Opening (ACO), Eye Opening (EO), Eruption of Upper Incisor Teeth (EUIT) and Lower Teeth (EIIT), Appearance of Epidermal Hair (AEH), and Tail Length (TL) (Smart and Dobbing, 1971)[31].

Table 2 – Description of the reflex test.

REFLEX	STIMULUS	RESPONSE
Palmar grasp (PG)	Light percussion on the palm of the right foreleg	Quick bending of ankles.
Righting (RR)	The rat is placed in supine position on a surface.	Return to the prone position with all paws in 10s.
Cliff-avoidance (CA)	The rat is placed on a flat and high surface (table), with legs towards the extremity.	Moves to one side and walks in the opposite direction to the edge
Vibrissa-placing (VP)	The animal is suspended by the tail and its vibrissae lightly touch the edge of a flat surface.	Both front legs are placed on the table, performing march movements.
Negative-geotaxis (GN)	The rat is placed at the center of an inclined ramp with head facing downwards	Body spin at an angle of 180 °, positioning head upwards.
Auditory-startle response (AS)	Intense and sudden sound stimulus	Retraction of anterior and posterior legs, with rapid and involuntary body immobilization
Free-fall righting (FFR)	Held by the four legs, at a height of 30 cm, it is released in free fall on a synthetic foam bed.	Position recovery during freefall on the surface supported on four paws.

2.5 Behavioral tests

2.5.1 Assessment of non-associative learning

Open Field Habituation Test During adolescent phase and adulthood the animals were submitted to the Open Field Habituation test and the Object Recognition Test (ORT). Each animal was exposed to the open field twice, in the first stage, the habituation test was performed; and after 7 (seven) days, the same test was repeated in order to compare the locomotor activity of the animals for evaluation of non-associative learning[32]. The parameter analyzed through this test is the amount of explorative interactions taken by the animal to the field, considering the locomotion of the four legs toward the interior of each field. The test observation time was 10 min. The procedure was performed between 06:00 and 08:00 a.m., on each test day, and the sessions were filmed with a video camera. For each animal tested, the apparatus was cleaned before starting, and after completion of the test with a 10% alcohol solution.

2.5.2 Object recognition test (ORT)

To evaluate the short and long term memory, the Object Recognition Task (ORT) was used. The test was performed in the open field apparatus (60 × 60 × 60 cm), colored black, with six lines crossing forming 6–20 × 20 cm quadrants, uniformly lit, and with black color objects, with different shapes (rectangular or pyramid), and textures (smooth or rough)[33].

The test consisted of 4 (four) 10 min trials, taking place in 3 (three) steps: (1) Day 1–habituation for 10 min to minimize manipulation stress; (2) Day 2–performed 24 h after the habituation test, where each animal was placed in the open field containing two objects (FO1 and FO2) with identical textures (smooth), but with different forms (triangle and prismatic rectangle), located in two randomly chosen opposite corners. On the same day, yet 1 h later, the animal was placed in the open field again to explore two objects (FO1 in its original location, and a new object–NO1, identical to FO1 but with a different texture, and located in the place where FO2 had been placed during the habituation test; and (3) Day 3–was performed 24 h after the short duration test; each animal was placed in the open field to explore two objects (FO2 in its original place) and a new object (NO2) being identical to FO2 but with different texture.

2.5.3 Morris' Water Maze

The Morris water maze was used to assess the animals' spatial memory, and is linked to the hippocampal brain region [34,35]. The protocol applied was based on previous studies [36,37]. The duration was four days, three of which were for training and the last day (in which the platform was removed) was for testing. A round pool, made of plastic, with a diameter of 1.60 m and 82 cm in height, was divided into four quadrants represented by the cardinal points (north, south, east, and west), with the 10cm x 13cm platform located in the northwest position. The pool was filled with water to a height of 54 cm and the platform was submerged (1 cm).

Each training period consisted of four trials, each lasting 60 seconds, in which the animals were randomly positioned in the quadrants. A random start order was followed for all animals. The objective of the training was for the animal to find the platform and remain on it for 10 seconds, if it did not find it, the animal was gently led to it. After each training trial, a 20-second interval was given, in which the animal was individually provided a waiting box.

During the training days, escape latency was analyzed. On the fourth day, the training day, the platform was removed and the animals swam for 60 seconds. The variables analyzed were: average speed, total distance traveled, number of entries into the target (which would be the platform), time in the platform zone, and latency to the first entry into the target.

2.6 Analysis of the intestinal microbiota using the 16S amplicon rRNA metagenome

2.6.1 Sequencing of 16S rRNA gene

Individual stool samples from the animals were collected at 45 and 90 days of age and immediately stored at -20°C. Six samples from each group were used for DNA sequencing of the 16S rRNA gene. Genomic DNA was extracted using a commercial kit (PowerFecal Pro DNA, Qiagen, Hilden, Germany). DNA integrity was assessed by 1% agarose gel electrophoresis and quantified by fluorometry (Qubit, ThermoFisher, Waltham, MA, USA). The V3-V4 regions of the microbial 16S rRNA gene were amplified by PCR (95°C for 3 min, followed by 25 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final extension to 72°C for 5 min) using primers 341F: 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3', and 785R: 5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3'. The amplicon library was prepared using the Nextera XT

Index Kit Set A (Illumina Inc., San Diego, CA, USA) and magnetic beads for cleaning and purification (Agencourt AMPureXP, Beckman Coulter, Indianapolis, USA). Paired-end sequencing was performed on the Illumina MiSeq platform using a 500 cycle V2 kit (2 × 250).

2.6.2. Bioinformatics analysis

The bioinformatics pipelene was used according to the recommendations of LIU *et al.*, (2021)[39]. The raw demultiplexed Forward and Reverse sequences were processed in the QIIME 2 v.20.8 platform 30[40]. Low -quality *reads* were removed, with a Q>20 minimum score for quality using the DADA2 program[41]. Chimeric and unsorted sequences were removed from the analysis as well as low quality bases above 245 bp. The generated Amplicon Sequence Variant (ASV) tables were normalized to 3150 ASVs per sample using DADA2. For phylogeny, the sequences were rarefied and aligned using the Silva 128 SEPP reference database available at https://docs.qiime2.org/2022.2/data-resources/. The alpha diversity analysis was evaluated using: Observed Features, Fisher, Simpson, and Shannon indices with QIIME 2 v.20.8 30[40]. Taxonomic classifications were assigned by the Machine-learning method based on the Silva Databases with 99% similarity for the V3-V4 regions.

2.6.3 Metabolic inference

Functional prediction of metagenomes was performed from 16S rRNA data using PICRUSt2 [42]. The abundance tables of ASVs generated through QIIME2 were used as input for PICRSt2 FullPipile through the q2-picrust2 plugin of QIIME2 available at https://library.qiime2.org/plugins/q2-picrust2/13/. Metabolic pathway and KO abundances were mapped using the MetaCyc database available at https://metacyc.org/.

2.7 Obtaining breast milk, offspring brain tissue, and performing fatty acid profile analysis

Breast milk was collected according to an adapted methodology described by Keen et al. (1981)[43]. Mothers were separated from their respective children on the 21st day of lactation, one hour before the procedure. The animals were anesthetized intramuscularly with xylazine hydrochloride (20 mg/kg) and ketamine hydrochloride (50 mg/kg), and intraperitoneally injected with 3 IU of

oxytocin. Nipple massage was performed to stimulate milk ejection. Milking was done manually and the milk was housed in an Eppendorf and frozen for further analysis.

On the 41st and 91st days of life, brain tissue was removed from the offspring after decapitation. Tissue was stored at -20°C until the day of analysis (n = 6). The fatty acid profile of the brains was determined according to the methodology described by Folch et al., (1957)[21], and Hartman and Lago, (1973)[22], with subsequent identification by gas chromatography (Varian 430GC).

2.7.1 Analysis of fatty acid composition in breast milk and offspring brain tissue

2.7.1.1 Lipidic Extraction

Sample were weighed (2 g of each) in a beaker and added to 30 ml of chloroform:methanol mixture (2:1). After this addition, the content was transferred to a deep glass container with the side covered with aluminum foil and stirred for 2 min with the help of grinder. The triturate was filtered through qualitative filter paper into a 100 ml graduated cylinder with a polished mouth. Next, the vessel walls were washed with an additional 10 mL of chloroform:methanol which was also filtered with the previous volume. The volume of the filtered extract of the graduated cylinder was recorded with the graduated cylinder closed. Twenty percentage of the final volume of the filtered extract was added to 1.5% sodium sulfate. The mixture was stirred with the graduated cylinder closed and given time for the phases to separate. It was observed that the upper phase was 40% and the bottom 60% of the total volume. The volume of the lower phase was recorded and then the upper phase was discarded by suction with a graduated pipette. For lipid quantification, an extracted aliquot of 5 mL (lower phase) was separated with a volumetric pipette and transferred to a previously weighed beaker. This beaker was placed in an oven at 105°C so the solvent mixture could evaporate, being careful that the fat would not be degraded by heat. After cooling in a desiccator, the beaker was weighed and the fat residue weight was obtained from the difference [20].

2.7.1.2 Transesterification of Fatty Acids

In the sample treatment, methylation of fatty acids present in the lipid extract was carried out following the methodology described by Hartman and Lago (1973)[22]. An aliquot of the lipid extract was taken, calculated for each sample according to the fat content found in the lipid measurement, and performed according to the (Folch et al., 1957) [20], adding 1 ml of internal

standard (C19:0) and a saponification (KOH) solution. This solution was subsequently brought to heating under reflux for 4 min. Esterification solution was added immediately after, returning the solution to heating under reflux for 3 more minutes. Next, the sample was allowed to cool before subsequent washings with ether, hexane and distilled water, finally obtaining an extract (with the methyl esters and solvents), which was conditioned into a properly identified amber glass until complete drying of the solvents. After drying, a suspension in 1 ml of hexane was made and packaged into a vial for further chromatographic analysis. The aliquots of saponification and esterification solutions were determined according to the methodology described by Hartman and Lago (1973) [22].

2.7.1.3 Gas Chromatography Analysis

A gas chromatograph (VARIAN 430-GC, California, EUA), coupled to a capillary column of fused silica (CP WAX 52 CB, VARIAN, California, EUA) with dimensions of 60 m × 0.25 mm and 0.25 mm film thickness was used with helium as carrier gas (Flow rate of 1 ml/min). The initial oven temperature was 100°C programmed to reach 240°C, increasing 2.5°C per minute for 30 min, totaling 86 min. The injector temperature was maintained at 250°C and the detector at 260°C. 1.0 µl aliquots of esterified extract were injected in a Split/Splitless injector. The chromatograms were recorded using Galaxie Chromatography Data System software. The fatty acids results were quantified by integration the areas of the methyl esters and are expressed in percentage by area.

2.8 Histological assessment of offspring brain tissue

The brains from 3 rats per treatment group were collected, washed in saline (0.9% NaCl) and fixed in 10% buffered formalin. The hippocampus was submitted to histological procedures in accordance with the routine techniques of the Pathology Laboratory (Department of Physiology and Pathology/CCS/UFPB, to obtain blocks, from which 4 µm semi-serial sections were cut. The slices were hydrated, stained with hematoxylin-eosin (HE), dehydrated, cleared in xylene, and mounted with Entellan® for microscopic optical analysis (Motic BA 200, Olympus Optical Co, Philippines). For morphometric analysis, twenty random images of the hippocampal tissue slides were used. Under an Axiolab light microscope (Zeiss) with a resolution of 400×, the twenty images were relayed to an image analysis system (Kontron Elektronik image analyzer; Carl Zeiss,

Germany—KS300 software). The slides were read randomly by two pathologists. To obtain the images, a 10× objective and a 40× photomicrograph of the tissues were used.

2.9 Statistical Analysis

Results concerning reflex ontogeny and somatic development were expressed as median values for each day (Min-Max), and using Kruskal-Wallis analysis of variance followed by Dunn's test ($p < 0.05$). The remaining results were expressed as mean ± SEM and analyzed by ANOVA, and followed by Tukey's test ($p < 0.05$).

For taxonomic analysis, the non-parametric Kruskal-Wallis test was used to compare alpha diversity indices. Using the STAMP statistical program, both taxonomic and metabolic differential abundance analyses were performed [44]. For cluster and *heatmap analysis*, the Farthest Neighbor method was used. For comparative analysis of multiple groups, ANOVA statistical tests were applied, with the Games-Howel Post-hoc test and effect size: (ETO)-squared applied. As a correction, multiple Benjamini-Hochberg testing was applied.

3 RESULTS

3.1 Fatty acid profile of breast milk and offspring brain tissue

3.1.1 Composition of fatty acids in breast milk

When evaluating the composition of saturated fatty acids in breast milk, it was observed that the highest concentration of these fatty acids was detected in the oil and almond groups (Table 3). In addition to total saturated fats, a reduction in capric and benic acids was verified in the milk of mothers supplemented with baru almond and oil, and an increase in myristic and palmitic acids compared to control mothers ($p < 0.05$). In relation to the control and almond groups, lower concentrations of lauric acid and higher concentrations of stearic acid were observed in the milk of mothers belonging to the oil group ($p < 0.05$).

Lower levels of total monounsaturated fatty acids were detected in the milk of the experimental mothers ($p< 0.05$). However, when analyzing the fatty acids in isolation, an increase in eicosenoic

acid and a reduction in tetradecenoic, palmitoleic, and oleic acids was observed in the breast milk of mothers in the oil and almond groups ($p < 0.05$). Pentadecenoic acid was observed only in the milk of mothers supplemented with baru almond.

For polyunsaturated fatty acids in the milk, in relation to the control group there was an increase of ≅ 105% in the total content of PUFAs in the oil supplemented mothers´ milk, with a ≅ 136% increase in the almond supplemented mothers´ milk ($p < 0.05$). There was a reduction in gamma linolenic and alpha linolenic acids and an increase in eicosatrienoic and docasahexaenoic in the milk of mothers supplemented with baru oil and almond as compared to the control group ($p < 0.05$). The animals supplemented with baru almond presented an increase in linoleic, eicosadienoic and dihomo gamma linoleic acids when compared to the control and oil groups ($p < 0.05$). The mothers in the oil group presented a reduction in eicosadienoic acid and an increase in arachidonic acid when compared to the mothers in the control group ($p < 0.05$); concentrations of docastetraenoic acid were found only in the milk of mothers in the oil group.

3.1.2 Composition of fatty acids in the brains of adolescent offspring

In adolescence, when we quantified the saturated fatty acids, we found an increase in miristic acid content and a reduction in stearic acid content in the offspring of rats treated with baru oil and almond in relation to the control offspring ($p < 0.05$). There was a reduction in palmitic acid content in the offspring belonging to the oil group when compared to the control and almond groups ($p < 0.05$); and concentrations of caprylic and behenic acid were identified only in the oil group offspring.

For monounsaturates, lower levels of palmitoleic and oleic acids, and an increase in the concentration of pentadecenoic acid was observed in the brain of the animals in the oil and almond groups ($p < 0.05$); Eicosenoic acid concentrations were identified only in the brains of the almond group animals.

The total polyunsaturated content in the brain of the animals in the almond group was ≅ 128% higher than in the control group, and ≅ 105% higher in relation to the oil group. In the brain tissue of the experimental offspring, docosahexaenoic and docosatetraenoic levels presented increases relative to the control offspring ($p < 0.05$). There was a decrease in the concentration of arachidonic acid in the animals of the oil group, and an increase in its concentration in the animals of the almond group ($p < 0.05$).

3.1.3 Composition of fatty acids in the brains of adult offspring

In the brain tissue of the adult offspring, the concentrations of saturated fatty acids were lower in the almond group than the oil and control groups ($p < 0.05$). Myristic acid was found in higher concentrations in the almond group animals ($p < 0.05$), and palmitic and stearic acid levels were reduced in the brain of the almond group animals, yet increased in the oil group when compared to the control group ($p < 0.05$).

For monounsaturates, we observed higher levels in the brains of the oil and almond offspring than in the control offspring ($p < 0.05$). Higher concentrations of tetradecenoic and oleic acid were also increased in the brains of animals in the experimental groups ($p < 0.05$). Eicosenoic acid was reduced in the brain tissue of the almond group when compared to both the oil and control groups ($p < 0.05$). Palmitoleic acid presented higher concentrations in the almond group than in the oil or control groups ($p < 0.05$).

Total polyunsaturated fatty acid levels were about ≅ 84% lower in the brain tissue of the oil group offspring thanalmond and control offspring ($p < 0.05$). However, eicosadienoic, eicosatrienoic, arachidonic, docosahexaenoic, and docosatetraenoic acids presented higher concentrations in the brains of animals in the experimental groups than in the controls ($p < 0.05$). Dihomo Gamma Linoleic acid (DGLA**),** presented lower levels (while linoleic acid presented higher levels) in the brains of the almond group, when compared to the oil and control groups ($p < 0.05$).

Table 3 – Composition of fatty acids in breast milk and brain tissue of offspring treated during pregnancy and lactation with oil and baru almonds.

FATTY ACIDS		BREAST MILK			BRAIN - 45th DAY OF LIFE			BRAIN - 90th DAY OF LIFE		
	NO. OF CARBON ATOM	CONTROL	OIL	ALMOND	CONTROL	OIL	ALMOND	CONTROL	OIL	ALMOND
SATURATED										
Capric	**C10:0**	3.31±0.01a	0.25±0.02b	1.08±0.01c	-	0.07±0.01	-	-	-	-
Lauric	**C12:0**	5.23±0.01a	4.83±0.02b	6.06±0.01c	-		-	-	-	-
Myristic	**C14:0**	3.96±0.01a	5.44±0.02b	7.01±0.01c	0.22±0.01a	0.28±0.02b	0.28±0.01b	0.05±0.02a	-	0.11±0.03b
Palmitic	**C16:0**	18.56±0.03a	19.03±0.01b	21.14±0.01c	22.36±0.02a	20.58±0.01b	22.01±0.02c	23.19±0.02a	24.82±0.03b	21.25±0.03c
Stearic	**C18:0**	3.61±0.02a	7.05±0.01b	2.88±0.02c	25.04±0.02a	21.68±0.02b	22.82±0.01c	24.44±0.03a	26.79±0.03b	22.95±0.03c
Arachidic	**C20:0**	-	-	-	0.35±0.01	-	-	-	-	-
Behenic	**C22:0**	0.75±0.03a	0.23±0.01b	-	-	0.12±0.02	-	-	-	-
	TOTAL	35.42	36.83	38.17	47.62	42.73	45.11	47.68	51.61	44.31
MONOUNSATURATED										
Tetradecenoic	**C14:1**	0.09±0.01a	0.05±0.01a	-	-	-	-	-	1.84±0.02a	2.25±0.03b
Pentadecenoic	**C15:1**	-	-	0.87±0.03	1.20±0.02a	2.06±0.02b	2.40±0.02c	2.17±0.02	-	-
Palmitoleic	**C16:1**	1.96±0.02a	0.86±0.01b	0.59±0.01c	0.56±0.01a	0.47±0.01b	0.43±0.01c	0.33±0.01a	0.34±0.01b	0.43±0.02c
Oleic	**C18:1n9**	25.00±0.03a	19.62±0.02b	19.39±0.01c	20.41±0.03a	17.47±0.03b	17.47±0.03b	9.19±0.04a	17.12±0.02b	18.69±0.02c
Eicosenoic	**C20:1n9**	0.25±0.02a	0.54±0.01b	0.58±0.01c	-	-	0.64±0.01	1.84±0.02a	1.92±0.02b	0.82±0.01c
	TOTAL	28.03	21.07	21.43	22.17	20.00	20.94	13.53	21.22	22.19
POLYUNSATURATED										
Linoleic	**C18:2n6**	26.09±0.03a	22.93±0.03b	27.52±0.02c	0.27±0.02a	0.27±0.01a	0.55±0.02b	16.2±0.01a	0.15±0.01b	0.32±0.01c
Gamma Linolenic	**C18:3n6**	0.12±0.01a	0.07±0.01b	0.02±0.01c	0.17±0.01	-	-	-	-	-

Alpha Linolenic	**C18:3n3**	0.77±0.02a	0.11±0.01b	0.05±0.01c	-	-	-	-	-	-
Eicosadienoic	**C20:2n6**	0.64±0.01a	0.26±0.02b	1.18±0.03c	1.25±0.02a	1.43±0.02b	1.26±0.02c	3.22±0.02a	3.44±0.02b	4.27±0.02c
Dihomo Gamma Linoleic	**C20:3n6**	0.11±0.02a	0.11±0.01a	0.56±0.01b	9.14±0.03a	-	10.20±0.03b	0.48±0.01a	0.48±0.01a	0.44±0.01b
Eicosatrienoic	**C20:3n3**	0.61±0.03a	0.77±0.02b	9.41±0.03c	-	11.24±0.03	-	8.41±0.02a	10.25±0.03b	12.20±0.03c
Arachidonic	**C20:4**	0.65±0.01a	2.87±0.01b	-	7.18±0.02a	4.67±0.02b	9.36±0.02c	1.21±0.02a	5.45±0.02b	4.65±0.02c
DocasaHexaenoic	**C22:6n3**	0.08±0.02a	2.51±0.02b	1.01±0.02c	6.68±0.03a	8.07±0.02b	9.93±0.02c	1.87±0.03a	4.81±0.03b	5.77±0.02c
Docasatetraenoic	**C22:4n6**	-	0.93±0.04	-	1.62±0.02a	1.98±0.01b	2.48±0.02c	-	2.05±0.01a	2.66±0.02b
	TOTAL	29.07	30.56	39.75	26.31	27.66	33.78	31.39	26.63	30.31
AGP/AGS		0.82	0.83	1.04	0.55	0.64	0.74	0.65	0.51	0.68
N6		26.96	24.30	29.28	12.45	3.68	14.49	19.9	6.12	7.69
N3		1.46	3.39	10.47	6.68	19.31	9.93	10.28	15.06	17.97
N9		25.25	20.16	19.97	20.41	17.47	18.11	11.03	19.04	19.51
N6/N3		18.46	2.32	2.79	1.86	0.19	1.55	1.93	0.40	0.42

3.2 Somatic maturation and reflex ontogeny

For somatic indicators, the newborns belonging to the baru oil and almond groups presented anticipations in opening of the eyes, eruption of the upper and lower incisors, and in appearance of epidermal hair compared to offspring belonging to the control group ($p < 0.05$). For the other parameters, no statistical difference was observed ($p > 0.05$), as shown in Table 4.

Table 4 – Somatic development of newborn rats whose mothers were supplemented with baru and almond oil during pregnancy and lactation.

Physical Characteristics	**GROUPS**		
	CONTROL	**OIL**	**ALMOND**
Ear unfolding	3 (2-4)	2 (2-3)	2 (2-3)
Auditory conduit opening	13 (12-15)	12 (12-13)	12 (12-13)
Eye opening	14 (14-15)	10 (10-12)*	10 (10-11)*
Eruption of superior incisors	10 (10-11)	7 (7-9)*	8 (8-9)*
Eruption of inferior incisors	7 (5-7)	3 (3-4)*	4 (4-5)*
Epidermic hair appearance	6 (5-6)	3 (3-4)*	3 (3-4)*

Data were expressed as mean values of the day (Min-Max) and analyzed by Kruskal-Wallis analysis of variance followed by Dunn's test ($p < 0.05$). (*) compared to control group; (#) compared to the baru oil group. CONTROL (Control Group - n = 12), OIL (Baru Oil Group - n = 12), ALMOND (Baru Almond Group - n = 12).

As to the reflex ontogeny parameters, the offspring of mothers treated with the baru almond and oil presented loss of Palmar Grasp and anticipation of Free-Fall Righting when compared to control offspring ($p < 0.05$). As for the Righting Reflex, we observed anticipation in animals whose mothers were treated with baru almond ($p < 0.05$). For the Negative Geotaxis reflex parameter, only the animals belonging to the oil group presented anticipation in relation to the other groups ($p < 0.05$). For the Vibrissa Placing, Cliff Avoidance and Auditory Startle parameters, there was no statistically significant difference between the groups ($p < 0.05$).

Tabela 5 – Reflex ontogenesis of neonatal rats whose mothers were supplemented with baru and almond oil during pregnancy and lactation.

Reflexes	**GROUPS**		
	CONTROL	**OIL**	**ALMOND**
Palmar Grasp [a]	10 (7-12)	8 (6-9)*	8 (6-10)*
Righting Reflex[b]	5 (3-8)	4 (2-7)	3 (2-6)*
Vibrissa Placing[b]	9 (5-13)	9 (7-13)	10 (7-12)
Cliff Avoidance[b]	10 (6-14)	10 (4-15)	10 (7-18)
Negative Geotaxis[b]	20 (19-20)	17 (15-20)*	19 (17-20)
Auditory Startle[b]	12 (12-13)	12 (12-14)	12 (12-13)
Free-Fall Righting[b]	11 (8-13)	9 (6-12)*	9 (5-13)*

Data were expressed as mean values of the day (Min-Max) and analyzed by Kruskal-Wallis analysis of variance followed by Dunn's test ($p < 0.05$). (*) compared to control group; ($^{\#}$) compared to the baru oil group. Considering: [a] = day of response disappearance and b = day of response appearance. CONTROL (Control Group - n = 12), OIL (Baru Oil Group - n = 12), ALMOND (Baru Almond Group - n = 12).

3.3 Fecal Microbiota

3.3.1 Taxonomic composition of the fecal microbiota

Through Principal coordinates analysis (PCoA) a significant effect of treatments on the fecal microbiota of the adolescent baru oil and almond animals was observed in relation to the control group (**Fig.2A)**. In the adult phase, similarity was observed between the samples from the three treatment groups, indicating a long term reduction in the effect of baru oil and almond (**Fig.2B)**.

As for richness and uniformity of the microbial communities, significant differences were observed between the almond and control treatments in the adolescent offspring; as well as in the adult offspring, this was shown evidenced in the Simpson and Shannon diversity indices (**Supplementary Fig. S1)**.

Figure 2 - Similarity Analysis (Taxonomy)

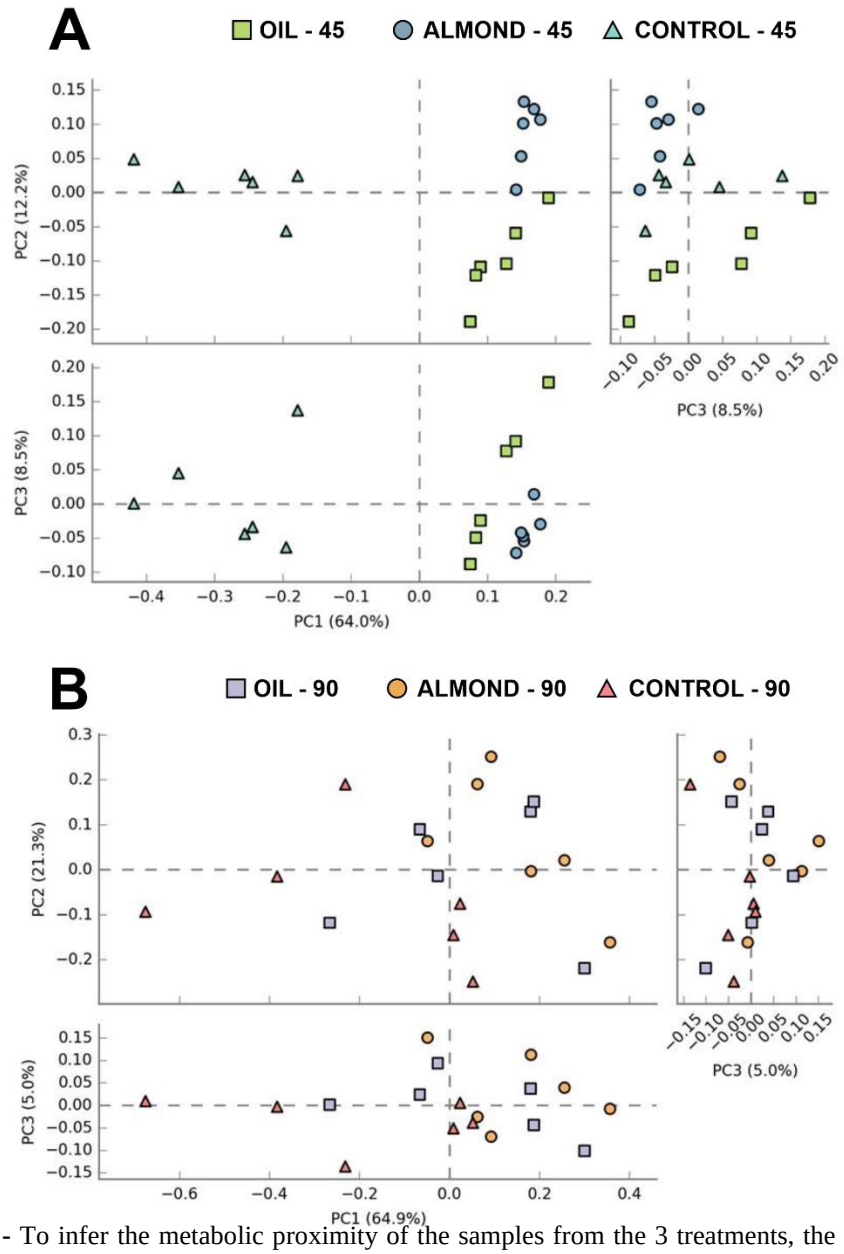
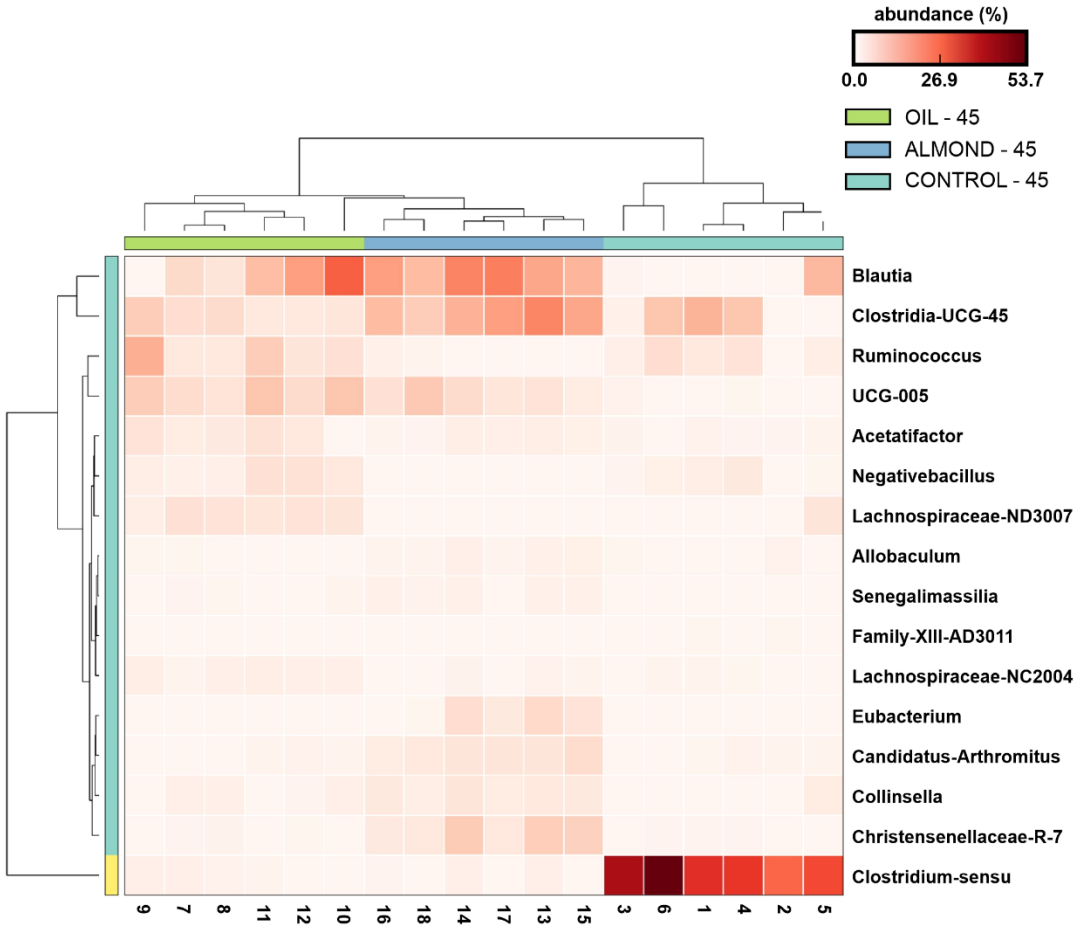


Figure 2 - To infer the metabolic proximity of the samples from the 3 treatments, the PCoA graph was used, which demonstrates similarity between the samples from the communities of the 3 treatments only at 90 days of life (Figure B).

For *Relative Abundance*, when evaluating the *Heatmap chart* using cluster analysis, we found a significant reduction (almost 50%) in the clostridium-sensu taxon during adolescence in the feces of both almond and oil animals. The cluster, composed by the taxa *Eubacterium, Candidates-Arthromitus, Collinsella and Christensenellaceae-R-7* was about 29% more abundant in the feces of animals in the almond group. The taxa *Blautia, Clostridia-UCG-014* were more abundant in the almond and oil treatments compared to the control group. The cluster composed of

Ruminococcus, UCG-005, Acetatifactor, Negativibacillus, and Lachnospiraceae-ND3007 was about 10% more abundant in the oil treatment group than in the almond or control groups (**Fig.3)**. There were no significant differences regarding the relative abundance in the fecal microbiota of the animals in the adult phase (data not shown).

Figure 3 - Analysis of multiple groups (Taxonomy)



The figure compares the abundances of the genera present in the 3 treatments through the *Heatmap graph*, using cluster analysis. The vertical dendrogram shows the clustering between treatment samples. The horizontal dendrogram shows the clusters formed between bacterial genera in each treatment that present statistical difference. The clusters are highlighted in red.

3.3.2 Functional prediction of the fecal microbiota

The functional similarity analysis revealed a significant effect of both oil and almond treatments on the abundance of metabolic pathways produced by microorganisms during adolescence (**Fig.4A)**. In adulthood, similarity was observed between the metabolic pathways, indicating a reduction in the effects of long-term treatment (**Fig.4B)**.

Figure 4 - Similarity Analysis (Functional Prediction)

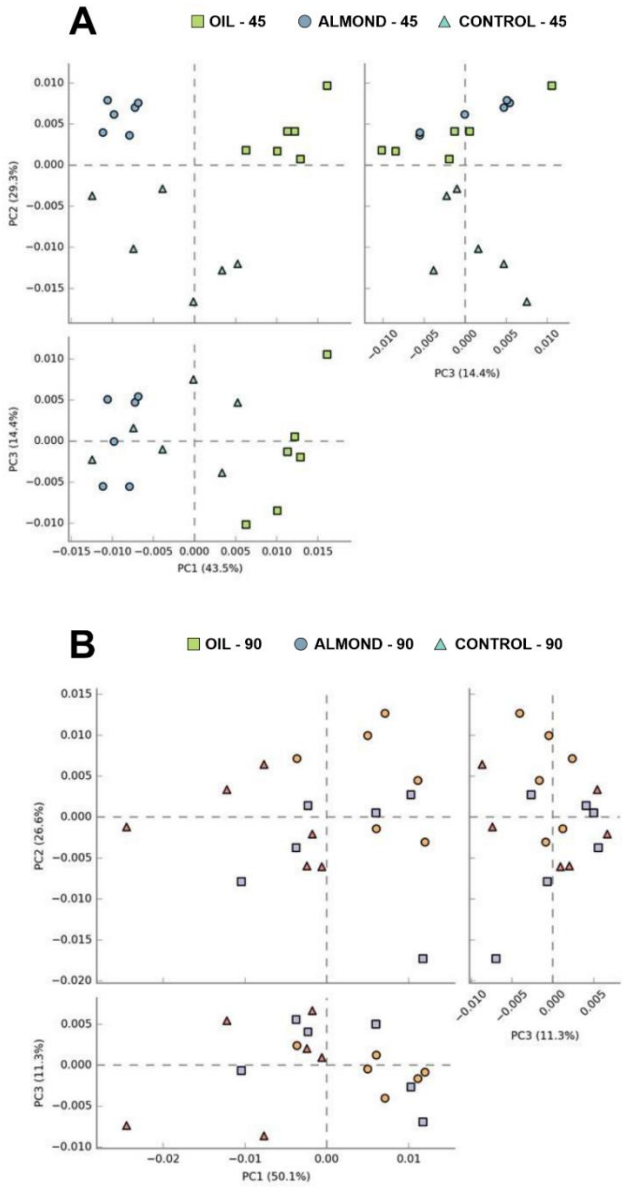
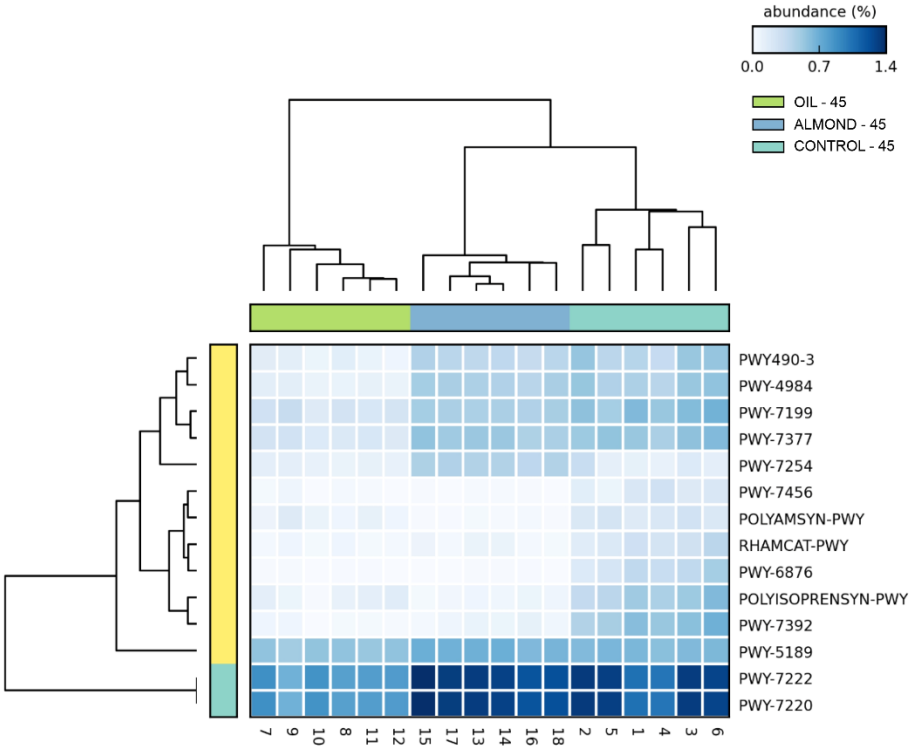


Figure 4- Similarity analysis. Figure (A), to infer the metabolic proximity of the samples in the 3 treatments, the PCoA graph reveals similarity between the samples from the 3 treatments in the adolescent phase. Figure (B), to infer the metabolic proximity of the samples in the 3 treatments, the PCoA graph reveals similarity between the samples from the 3 treatments in the adult phase.

As for the abundance of metabolic pathways using *Heatmap,* we verified a significant reduction in the biosynthesis pathways of tetrapyrrole II (PWY-5189); guanosine deoxyribonucleotide II (PWY-7222); adenosine deoxyribonucleotide II (PWY-7220); and tricarboxylic acid cycle (PWY-7254), in the adolescent animals treated with oil as compared to either the almond or control groups.

The oil and almond offspring (adolescents) differed statistically from the control group; presenting reduced abundance of plant mannan degradation pathways (PWY-7456), polyamine I biosynthesis (POLYAMSYN-PWY), rhamnose catabolism (RHAMCAT-PWY), isopropanol (PWY-6876) biosynthesis, polyisoprenoid biosynthesis (POLYISOPRENSYN-PWY), and taxadiene biosynthesis (PWY-7392). The nitrate assimilation pathways (PWY490-3), urea cycle (PWY-4984), pyrimidine deoxyribonucleosides (PWY-7199) rescue, and biosynthesis of Cob(I)yrinic acid a,c-diamide I (PWY-7377) were reduced in the oil group when compared to either the almond or control groups. There were no significant differences regarding the abundance of metabolic pathways in the fecal microbiota of animals in the adult phase (data not shown).

Figure 5 - Multiple group analysis (Functional prediction)

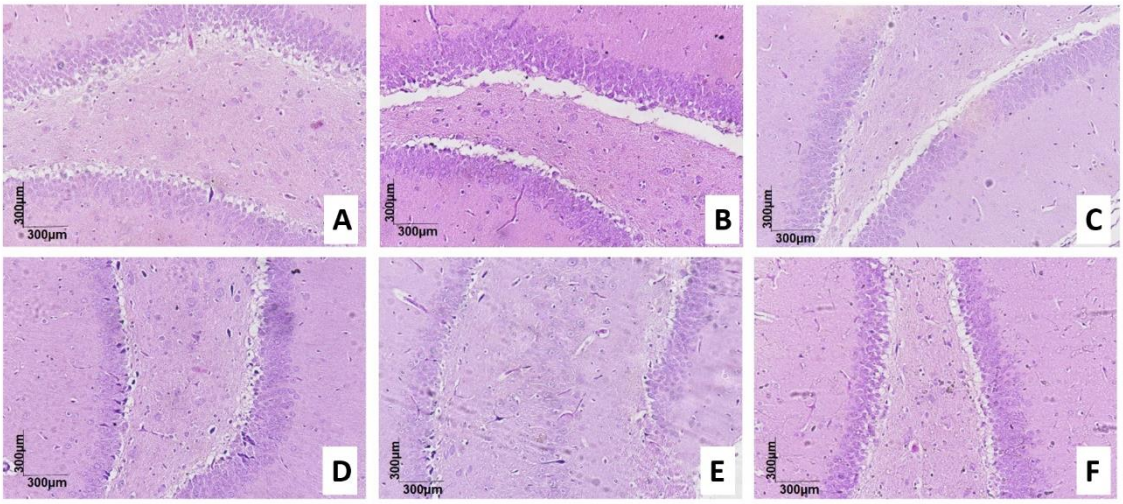


The figure compares the abundances of metabolic pathways present in the 3 treatments using the *Heatmap graph,* and cluster analysis. The vertical dendrogram presents clustering between treatment samples. The horizontal dendrogram presents the clusters formed between the metabolic pathways in each treatment which presented a statistical difference.

3.4 Histopathological assessment of the hippocampus

When analyzing the central nervous system stained histological sections with hematoxylin and eosin, we observed preservation of neurons in the dentate gyrus and in other hippocampal regions (CA1 – CA4), in the adolescent control offspring (A), as well as in all other experimental conditions, Baru oil (B) and Baru almond (C). The same results were verified in the adult control offspring (D), Baru oil (E) and Baru almond (F), as seen in Figure 6.

Figure 6 – Histopathological analysis of the hippocampus of the offspring of *Wistar* rats (treated with baru oil and almond).



Representative histological section of the hippocampus, respectively (A - C) - T45 Control, Baru Oil and Almond animals. (D - E) - T90 Control, Baru Oil and Almond animals.

3.5 Assessment of non-associative learning during adolescence

Habituation to the open field is related to non-associative learning being the decrease in the response that results from repetitive stimulation (BORGES et al., 2014) (REF). In figure 7A, it is possible to observe that the animals belonging to the oil group spent less locomotion time (second exposure) in the open field (99.33 ±4.61) as compared to the first (133.13 ±8.72) ($p < 0.05$). As for the number of crossings, it was found that all groups presented a reduction in this parameter in the second exposure: Control (98.00 ±9.57); (55.33 ±5.95), Oil (116.92 ±8.07); (60.38 ±6.82) and Almond (124.92 ±8.06); (59.89 ±5.51) **Fig. 7B** ($p<0.05$). For the number of entries into the inner zone of the apparatus, there was no statistical difference between the groups. However, with regard to the time spent in the inner zone, it was observed that the animals belonging to the oil

and almond groups remained longer in the internal zone when exposed to the apparatus for the second time; respectively (55.00 ±5.89 and 64.88 ±4.70); (60.89 ±3.33 and 77.13 ±5.59), (**Fig. 7D,** $p<0.05$).

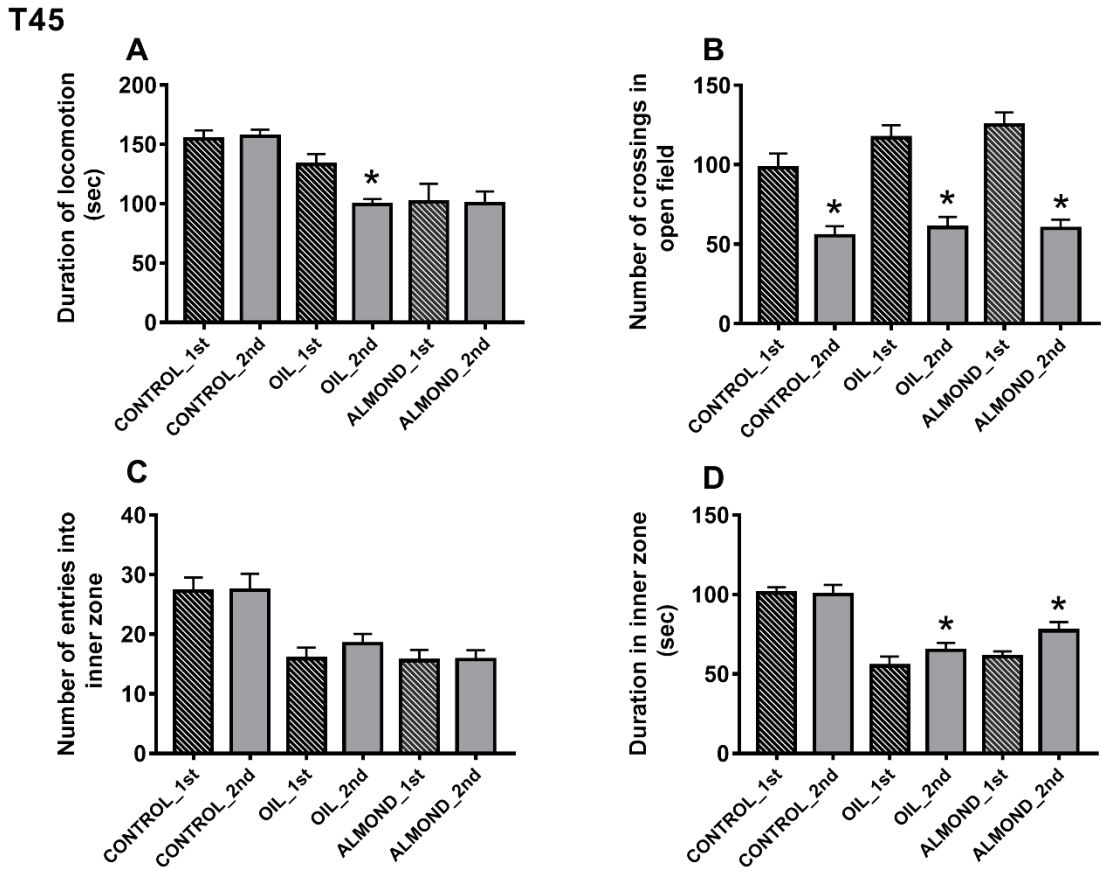


Figure 7. Habituation test performed at 45 days of age with the offspring of rats treated during pregnancy and lactation with baru oil and almond (2000 mg/kg of weight). Values are expressed as mean and standard deviation (One way ANOVA, Holm-Sidak); 1st exposure at 45 days of life, and 2nd exposure at 52 days of life; CONTROL (n = 12), OIL (n = 12), ALMOND (n = 12); ∗ versus first exposure in the same group. (A) Duration of locomotion: time spent by the animal moving in the open field. (B) Number of crossings in open field: ambulation was evaluated by the total number of segments, and counted when the animal inserted all four legs into the segments. (C) Number of entries into the inner zone: quantified when the animal placed all four legs inside each inner zone of the open field. (D) Duration in the inner zone - time spent by the animal in the inner zone of the open field.

3.6 Assessment of non-associative learning during adulthood

At 90 days of life (adult phase), a decrease in the ambulation time of oil (97.86 ± 6.72); (86.00 ±6.58) and almond (77.57 ±4.86); (57.63 ±3.34) groups was observed when exposed to the apparatus for the second time **Fig. 8A,** ($p < 0.05$). When the number of crossings was evaluated, it was verified that there was a reduction of this parameter in the animals of the control

(114.50 ±9.47); (53.55 ±5.91) and almond (76.07 ±7.36); (59.33 ±5.68) groups in relation to the second exposure **Fig. 8B,** ($p < 0.05$). When analyzing the number of entries and time spent in the inner zone, it was observed that the animals of the almond group presented longer times during the second exposure as compared to the first (20.31 ±1.64 and 22.67 ±2.74); (65.75 ±2.25 and 79.37 ±3.81), respectively **Fig. 8C and 8D,** ($p<0.05$).

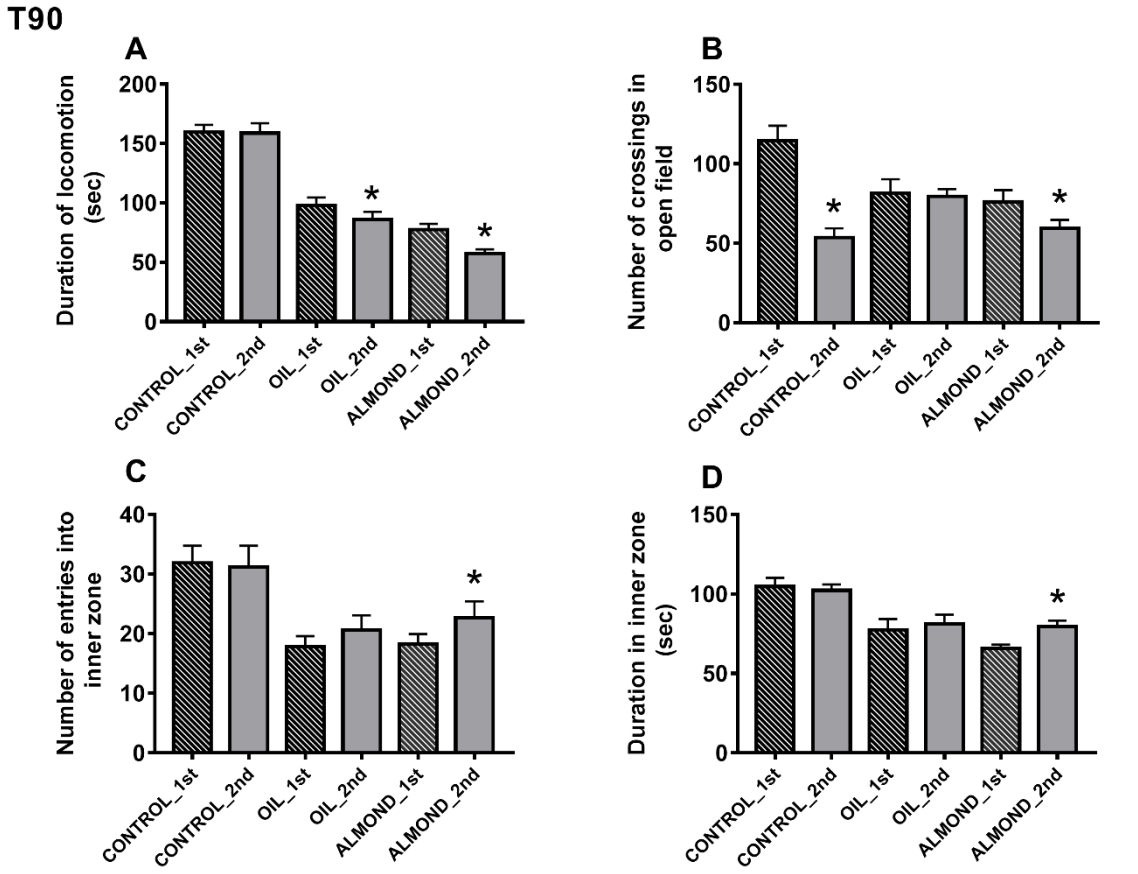


Figure 8 - Habituation test performed at 90 days of age of the offspring of rats treated during pregnancy and lactation with baru oil and almond (2000 mg/kg of weight). Values are expressed as mean and standard deviation (One way ANOVA, Holm-Sidak); 1st exposure at 45 days of life, and 2nd exposure at 52 days of life; CONTROL (n = 12), OIL (n = 12), ALMOND (n = 12); ∗ versus first exposure in the same group. (A) Duration of locomotion: time spent by the animal moving in the open field. (B) Number of open field crossings: ambulation was evaluated by the total number of segments covered, and counted when the animal inserted all four legs into the segments. (C) Number of entries in the inner zone was quantified when the animal placed all four legs inside each inner zone of the open field. (D) Duration in the inner zone - time spent by the animal in the inner zone of the open field.

3.7 Object Recognition: adolescence and adulthood test

In the adolescence phase, it was found that the animals of the experimental groups presented higher respective rates of new object exploration in both the short (0.72 ±0.04; 0.67 ±0.05) and

long term (0.56 ±0.05; 0.53 ±0.04) when compared to the control group (0.50 ±0.05; 0.47 ±0.04,) **Fig. 9A, 4 B (**p <0.05). In the adult phase regarding the short-term new object exploration rate **Fig. 9C, (**p<0.05) there was a statistically significant difference only for the animals belonging to the almond group (0.68 ±0.03) in relation to the controls (0.58 ±0.09). However, when analyzing the long-term exploration rate, a significant difference between the animals of the almond group (0.69 ±0.02) as compared to the oil (0.48 ±0.06) and control (0.47±0.06) groups was observed **(Fig. 9D,** p<0.05).

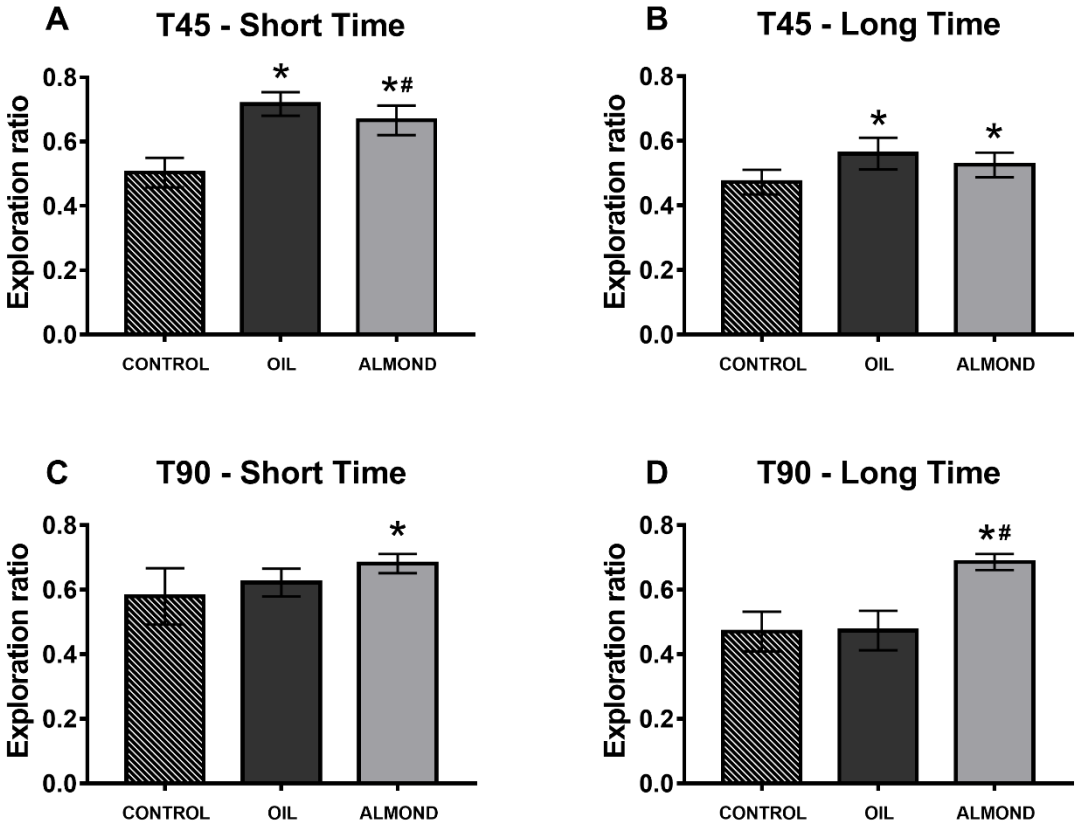


Figure 9 - Assessment of short-term and long-term memory using the object recognition test in adolescent and adult offspring of rats treated during pregnancy and lactation with baru oil and almond (2000 mg/kg body weight). Values are expressed as mean and standard deviation (One way ANOVA, Tukey p<0.05). (A) short-term object exploitation rate. (B) long-term object exploitation rate. CONTROL (n = 12), OIL (n = 12), ALMOND (n = 12); *Versus CONTROL group; # Versus the OIL group.

3.8 Morris water maze

To investigate the effects of baru oil and almond supplementation on hippocampus-dependent spatial memory, the escape latency time, time spent in the platform quadrant, and number of platform quadrant crossings were measured using the Morris aquatic maze test. In this task, the

animals are conditioned to learn to locate the platform submerged in water using signs located around the pool[45]. **Figure 5A** shows that animals from all groups presented a reduction in the latency time to finding the platform during training periods, the parameter is an indicator of learning in relation to the task. On the test day, the significant effect of supplementation was observed on the escape latency for animals in entering the platform quadrant, both oil and almond offspring completed the task in less time when compared to control offspring - respectively (4.91 ± 1.38); (8.82 ±2.09) and (5.82 ±1.33), **Fig. 5B,** ($p < 0.05$). The same behavior was verified when analyzing the number of crossings in the platform quadrant parameter, Controls 6.50 ±1.56; baru oil 2.00 ±0.85 and almond 2.83 ±0.83 **Fig. 5C,** ($p < 0.05$).

Statistical analysis also revealed a significant difference for the time spent by the animals in the platform quadrant, where the animals belonging to the oil (7.55 ±1.57) and almond (9.36 ±0.81) groups presented higher values than the control group (6.20 ±1.03) ($p<0.05$). The post hoc test revealed the even better performance of the almond offspring compared to the oil offspring (**Fig. 5D,** $p<0.05$).

For swimming speed, no statistical difference was observed, suggesting that supplementation did not favor or delay physical parameters, but was able to directly influence cognitive functions (data not shown).

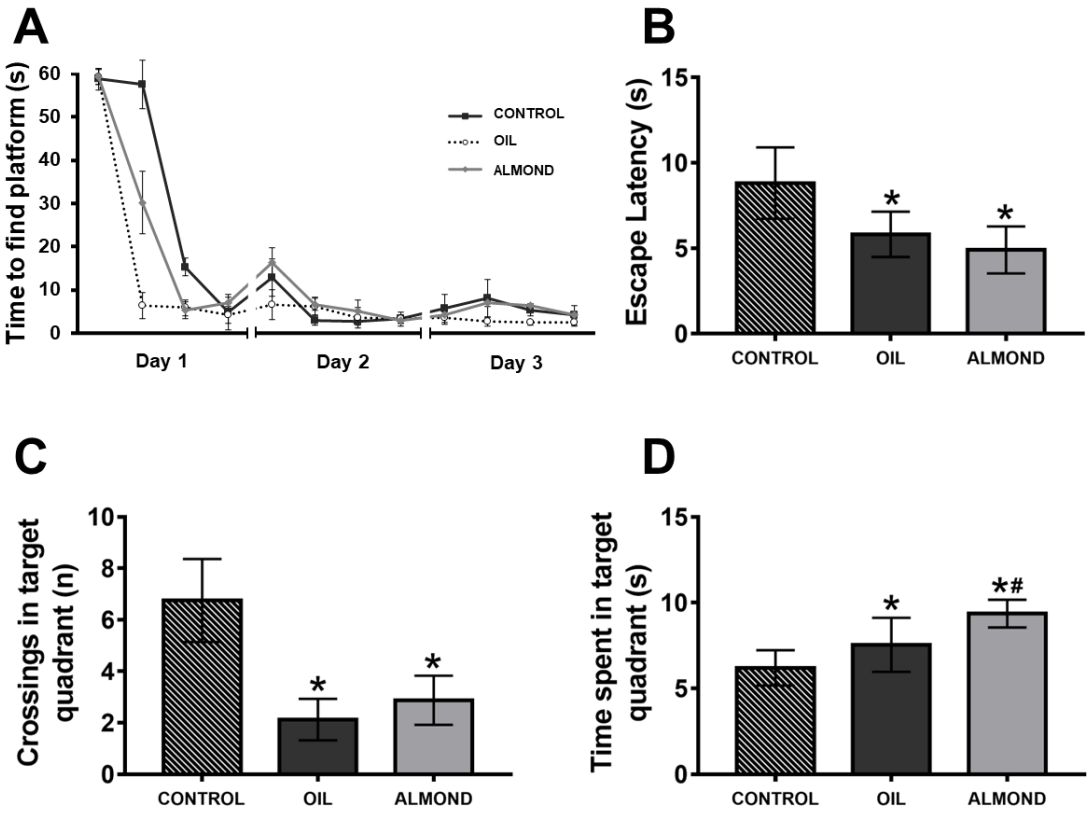


Figure 10 - Effects of treatment with baru oil and almond (2000 mg/kg body weight) on the performance of T90 offspring treated during pregnancy and lactation. (A) Training: Latency(s) spent by the animals to

find the submerged platform during training. (B) Test: Latency (s) spent by the animals to reach the quadrant where the platform was submerged. (C) Test: Crossing performed by the animals in the quadrant where the platform was submerged. (D) Test: Time spent by the animals in the quadrant where the platform was submerged. Values are expressed as mean and standard deviation (One way ANOVA, *Tukey p <0.05)*. CONTROL (n = 12), OIL (n = 12), ALMOND (n = 12); *Versus CONTROL; # Versus OIL.

4. DISCUSSION

We demonstrated for the first time, that maternal supplementation with baru oil and almond is able to increase PUFA concentrations in offspring brain tissue, accelerating neurodevelopment and improving the performance of these animals when object recognition and spatial memory tests are performed, whether in adolescence (T-45) or adulthood (T-90).

The results seem to be related to an increase in the relative abundance of intestinal bacteria in the oil and almond animals, which is inversely related to the production of neuroinflammatory metabolites which cause neural injury, neurodegeneration, and cognitive impairment. Although several studies have demonstrated the negative effects of excessive maternal consumption of lipids on the maternal and fetal microbiota [46,47], our findings suggest that supplementation of PUFAs together with the phytocomplexes present in baru, being administered during pregnancy and lactation, induces formation of a beneficial microbiota that supports adequate physical and cognitive development in the offspring.

The maternal treatments with baru oil and almonds also promoted acceleration of several somatic parameters in the offspring. Essential fatty acids are recognized for promoting somatic growth[48]. Both baru oil and almond present significant amounts of oleic and palmitoleic acids, in addition to n-6 and n-3 fatty acids in their composition, which have been correlated with physical development in the progeny[49]. Similar results have been found in the offspring of rats treated with avocado oil and pulp during the critical period of development[50]. Further, recent data suggest that PUFA supplementation modulates the gut microbiota, promoting production of metabolites that impact host postnatal growth kinetics[51,52].

Taxonomic analysis allowed verification that stool samples from adolescent rats supplemented with baru oil and almonds consistently presented higher relative abundances of *Blautia, Ruminococcus, Lachnospiraceae-ND3007, Collinsella* and *christensenellaceae r-7 taxa* than the control group. Previous research has demonstrated a relationship between these taxa and somatotropic axis activity via regulation of IGF-1, and production of growth hormone[53,4]. The hypothesis of a relationship between microbiota modulation resulting from the action of PUFAs, and physical growth in rodents was confirmed. However, further experimental studies are needed

to elucidate which metabolic pathways (used by these microorganisms) are involved in regulating IGF-1 and GH.

An important aspect to be considered is that PUFAs are deposited in offspring tissue through placental transport (intrauterine period) and postnatal breastfeeding[54,55]. During these phases, essential fatty acids are essential to proper physical and brain development[56]. DHA (docosahexaenoic acid) and arachidonic acid (ARA) are involved in neurogenesis, fluidity, and signaling activities in neuronal membranes[57,58,59]. Scarcity during this period compromises important brain regions such as the hippocampus, striatum, and visual cortex[60].

Sinclair, Guo and Abedin (2022) showed that alpha-linolenic acid supplementation during pregnancy increased levels of DHA in the offspring retina[61]. Greater incorporation of DHA into retinal phospholipids is associated with earlier eye opening and better visual acuity in guinea pigs[62]. In our study, we did not evaluate DHA content in the retina of the offspring, however, we did identify that the animals belonging to the oil and almond groups presented anticipated eye opening compared to the controls. Visual maturation results from DHA activity in retinal gene expression, photoreceptor membranes, and the neurotransmitters involved in signal transduction, rhodopsin activation, and rod and cone development[63].

Increased levels of ARA, EPA, and DHA in offspring brain tissue improve dendritic arborization, a gene expression that regulates neurogenesis and neurotransmission, contributing to proper formation and integrity of the vestibulospinal and sensorimotor systems[64–66]. In view of this, we decided to evaluate the breast milk and brain tissue fatty acid profiles in the rat offspring at different stages of life. Our results revealed higher concentrations of DHA (C22: 6n3) and ARA (C20: 6n4) in the milk of rats supplemented with baru oil and almond, which implies higher concentrations of these constituents in the offspring's brain during adolescence and adulthood. According to Innis (2011) and Morgane et al. (2002), the deposition of fatty acids in the pup´s brain is impacted by pre- and postnatal maternal supply, with the last gestational week and lactation being the most important or intense phases[8,6]. This helps explain our higher DHA results found for the adolescent and adult offspring.

Studies demonstrate that CNS maturation is dependent on essential fatty acid supply, and especially requires adequate amounts of ARA and DHA[50,68,69]. CNS maturation is measured in early life through the reflex ontogeny[70,31]. When evaluating these parameters, we found in the offspring that supplementation accelerated the disappearance of Palmar Grasp, and anticipated righting reflex, negative geotaxis, and decubitus recovery in free fall. These results, together with the breast milk and offspring brain tissue lipid profile analyses, support the hypothesis that increased levels of DHA and ARA are essential for CNS maturation[71,72].

Bioactive components also act as neuroprotective agents[73]. In view of this, we decided to analyze phytocomplex concentrations in baru oil and almonds. The results revealed significant amounts of phenolic compounds and total flavonoids. Antioxidant activity was verified through ABTS, FRAP, and IC_{50} assays in both matrices, the values were higher for the baru almonds. Data from our laboratory showed that the phytocomplexes present in baru improve oxidative stress markers in rat brain tissue, protecting PUFAs against lipid peroxidation. The effects of phytocomplexes on the maturation of the nervous system have been extensively investigated[71,72].

Evidence was presented to suggest an interaction between phytocomplexes, microbiota and neuroprotective activity[6]. A meta-analysis demonstrated that polyphenol supplementation in the range of 396 mg/d to 593 mg/d was able to stimulate the abundance of health-promoting taxa and the reduction of pathogenic species[74]. Depletion of the *Blautia, Ruminoccoccus, Eubacterium, Collinsella, Christensenellaceae r-7,* and *Lachnospiraceae-nd3007* taxa has been frequently reported in rodents under conditions of dysbiosis[75,76], oxidative stress[77], systemic inflammation[78,79] and neuropathologies associated with cognitive decline[80–82]. A relative abundance of these bacteria, as observed in our study, induces increased production of short-chain fatty acids (SCFA), anti-inflammatory cytokines, BNDF, and neurotransmitters, impacting memory and learning in rodents. The reduction of the *clostridium sensu taxon* in our study is similar to previous findings[83,84] and suggests that baru almond and oil supplementation has the potential for remodeling a healthy microbiota, with consequent prevention of cognitive decline.

In fact, when we correlate the fecal microbiota data with the histopathological evaluation, it is inferred that the oil and almond treatments, in addition to increasing the relative abundance of beneficial microorganisms, contribute to preservation of the offspring brain tissue, without altering frontal cortex cell bodies in the offspring while conserving neurons in the dentate gyrus and of the hippocampal regions CA1 and CA4. These histological results were evident both in adolescence and adulthood.

In view of this, we decided to investigate the cognitive performance of rodents using the Open Field (OF) Habituation and Object Recognition (ORT) tests (to verify non-associative learning), and the *Morris Water Maze* to assess spatial memory. According to Prut and Belzung, (2003) [85], non-associative learning by habituation in rodents consists of decreasing response to stimuli that become familiar. The OF habituation model assesses the exploratory activity of animals, which, when repeatedly exposed to the apparatus, tend to present less ambulation, which is interpreted as a form of learning[32]. We observed that the oil and almond treatments caused a locomotion reduction in the offspring (T-45 and T-90), and an increase in the time spent in the internal area of the OF in the second exposure. These results indicate that the animals were able to recognize the environment as being familiar and expressed reduced interest in exploration. In this same

evaluation, we observed longer times spent by rodents in the central area of the device, signifying that the reduction in locomotion did not occur due to anxiogenic stimuli. When evaluating the performance of the offspring in the ORT, we verified that the T-45 oil and almond groups presented short and long term recognition memory facilitation. In the adult phase, the results were more pronounced in the almond group.

The activity of ARA and DHA in maintaining structural and functional neuronal membrane integrity may well be the mechanism that explains our results[86–88]. The incorporation of these fatty acids into membrane phospholipids regulates plasticity mechanisms, while increasing synaptogenesis, BDNF levels, and hippocampal volume. This results in better cognitive performance in rodents[89–91]. Interestingly, the levels of these fatty acids were found in lower concentrations in the brain tissue of the animals in the oil group in the adult phase, and in higher concentrations in the animals of the almond group in the same phase, which justifies the more pronounced cognitive performance in the latter group. It is important to note that the concentration of antioxidant compounds present in baru almond is approximately ≅ 213% higher than in the oil. High concentrations of antioxidants (in addition to protecting the status of PUFAs in brain tissue), contribute to modulation of acetylcholine (AChE) activity, a neurotransmitter crucial to learning[92,93,94].

The influence of PUFAS on NMDA (N-methyl D-Aspartate) receptor synthesis, long-term hippocampal potentiation (LTP), and glutamatergic functions has also been associated with good learning and memory performance in rodent tests[91,95–98]. According to Rasoolijazi et al., (2015), antioxidants reduce oxidative stress and increase the neuron density in area CA1 of the hippocampus, and consequently improve spatial memory scores in MWM tests in rodents[99].

Although our study revealed significant MWM test effects of the almond treatment on adult offspring, with a significant reduction in escape latency and an increase in the animals' permanence time in the platform quadrant, it was not possible to evaluate the effects of supplementation on the spatial memory of the adolescent offspring, which is a limitation of our study. Nevertheless, by associating the MWM results with the CA and ORT tests, we achieved sufficient depth to support the hypothesis that baru almond and oil supplementation potentially improve rodent cognition, with the baru almond being more effective in long term memory.

To investigate the relationship between cognition and gut microbiome functions, we predicted the potential metagenome profiles of the 16S rRNA gene community using PICRSt2. The nitrate assimilation (PWY490-3) pathway, acetate production (PWY-7254), and cob(I)yrinic acid a,c-diamide I biosynthesis was differentially lower in the microbiota of the baru oil offspring in the adolescent phase. The nitrate assimilation pathway is extremely important for nitric oxide (NO) production by intestinal bacteria[100]. NO is a highly diffusible, short-lived free radical gas that

permeates biomembranes with a wide range of physiological functions[101], such as modulation of cerebral blood flow, regulation of synaptogenesis, and neurotransmission in the central and peripheral nervous system. All of which are essential mechanisms in the process of memory and learning[102–104]. The baru oil treatment, reduced (in adolescence) PWY-7254 metabolic pathway abundance which is related to acetate production. Acetate is one of the main SCFAs metabolized by intestinal bacteria during fermentation of dietary fiber[105]. Zheng et al (2021) showed that depletion of acetate-producing bacteria in the intestinal microbiota of mice promotes cognitive impairment, a result of decreases in synaptophysin (SYP) in the hippocampus region. Although the mechanisms are not well understood, the authors suggested a correlation between reductions in acetate-producing microorganisms and the risks of long-term cognitive decline[106]. Another important reduction caused by the oil treatment was in the cob(I)yrinic acid a,c-diamide I biosynthesis pathway, this reduction implies less vitamin B12 synthesis in intestinal bacteria. Although several studies have reported on the importance of vitamin B12 for the development and maintenance of the neurological system[107], other gut-brain axis studies point to the limited capacity of intestinal microbes to contribute to endogenous host B12 levels[108]. However, enteric production of B12 is a critical cofactor used by other intestinal commensals to regulate SCFA production, essential for the modulation of neurotransmitters such as glutamate, glutamine, and GABA in the hypothalamus[109]. Reduction of these clusters in the oil treatment may explain the less effective memory test results for this group.

Considering dose range, fatty acid and phytochemical profile, and administration times our results concerning rodent memory are similar to studies evaluating lipid matrix supplementation with PUFAs and phytocomplexes[50,110,111]. Yet despite these results, supplementation with baru oil 2,000 mg/kg suppressed metabolic pathways important to the integrity of the CNS, and intestinal microorganisms. It is important to investigate the mechanisms involved in this suppression, as well as assessing how it might impact cognitive and learning processes. Despite the consistent results described in this study, parallel research with pregnant women and offspring is necessary.

5. CONCLUSION

Maternal supplementation with baru oil and almonds during the initial phase of life contributes to adequate development of the offspring's nervous system, modulating both the microbiota and fatty acid profile in the brain, and contributing to anticipated reflex and somatic development, and short-term and long-term memory improvements.

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ARTIGO II

MATERNAL CONSUMPTION OF BARU ALMOND (*Dipteryx alata* Vog.) INDUCES ANXIOLYTIC-LIKE BEHAVIOR, REDUCES OXIDATIVE STRESS IN THE BRAIN AND PRESERVES CELL BODIES IN THE OFFSPRING'S CORTEX AT DIFFERENT STAGES OF LIFE.

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Maternal supplementation with oil and Baru almond (*Dipteryx alata* Vog.) reduces anxious-like behavior, oxidative parameters in the brain, in addition to preserving cell bodies in the cortex of the offspring at different stages of life

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ABSTRACT

The present study was to evaluate the impact of maternal consumption of oil and baru almond on anxious-like behavior and oxidative stress in the brain of the offspring treated during pregnancy and lactation. The dams were divided into three groups and treated by gavage (n = 8 mothers/group): Control- received distilled water; Oil - treated with 2,000 mg of baru oil/kg of animal weight and Almonds - treated with 2,000 mg of baru almond/kg of animal weight, and when the offspring (n = 12 pups/group) reached 45 (adolescence -T45), and 90 days of life (adult - T90), they were submitted to behavioral tests using the Open Field (OF), Elevated Plus Maze (EPM) and Light-Dark Box (LDB), in addition to cortex histology and parameters of oxidative stress in the brain. One-way ANOVA was used for data analysis, considering $p < 0.05$. The animals in the oil and almond groups showed greater ambulation and rearing and reduced grooming behavior and number of fecal buns. In the LCE, they presented a greater number of entries and time spent in the open arms compared to the CG ($p < 0.05$), they translocated and walked more in the clear area of the LDB, in addition, they presented a higher concentration of glutathione and lower MDA in the brain tissue ($p < 0.05$). Maternal supplementation with oil and baru almond reduces anxious-like behavior, oxidative parameters in the brain, in addition to preserving cell bodies in the cortex of adolescent and adult offspring.

Keywords: PUFAs; Antioxidant compounds; Malondialdehyde; Anxiety; Pregnancy and lactation.

1. INTRODUCTION

Maternal nutrition and lifestyle influence offspring phenotype, development, and behavior [1–3]. During the critical period, (the phase that comprises the formation of the nervous system), the scarcity and/or excess of nutrients, as well as exposure to stressful factors, contributes to changes in neural and cognitive function and also to neuro-endocrinal aspects [4–6]. Intake of essential fatty acids (EFA) and neuroprotective phytocomplexes appears to protect offspring brain tissues during pregnancy, lactation, and other stages of life as well [7–10].

The dietary lipids which structurally compose the nervous system and contribute to both tissue construction and determination of body growth are transferred to the fetus via the placenta and breast milk [11–14]. Neuronal membranes are rich in polyunsaturated fatty acids (PUFAs), especially arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). Due to the presence of unsaturated double bonds in their chemical structures, these membranes are particularly vulnerable to oxidative stress [15–17].

Brain tissue is extremely sensitive to oxidative damage that occurs for a variety of reasons, these include: high rates of oxygen consumption (O_2), large amounts of peroxidizable polyunsaturated fatty acids, weak expression (and thus activity) of antioxidant enzymes, neurotransmitter (dopamine and serotonin) auto-oxidation, and the presence of iron which catalyzes generation of Reactive Oxygen Species (ROS) in varied brain regions such as the substantia nigra, basal ganglia, caudate nucleus, putamen, and globus pallidus [18–20]. The influence of oxidative damage in neuropsychiatric disorders has already been extensively evaluated in experimental studies in rat offspring [21–24].

Increased lipid peroxidation can induce changes in brain tissue; compromising neural plasticity, and membrane fluidity, and induce cell death [25–27]. The presence of bioactive compounds and essential fatty acids in edible oilseeds can help increase antioxidant enzymes, reduce lipid peroxidation markers, and prevent neurological damage [28–31].

Of the various sources of fatty acids and phytocomplexes that can be used as supplements, baru almonds (*Dipteryx alata* Vog.), produced by the barueiro tree in the Brazilian *cerrado* have aroused scientific interest due to their nutritional composition. Their lipid profile is predominantly composed of unsaturated fatty acids: monounsaturated oleic (ω-9), and polyunsaturated linoleic acids; (ω-6) and (ω-3) [32–33]. In addition to lipids, other

studies have reported the presence of several bioactive compounds with antioxidant activities, such as gallic, ferulic, ellagic, and *p*-coumaric acids [34–35].

Since the baru almond is a source of dietary lipids and phytocomplexes, the present study hypothesized that maternal supplementation with baru almonds might protect the offspring's nervous system against oxidative damage. Considering the scarcity of information in the literature on the effect of baru almond and oil consumption in different stages of life, we aimed to evaluate the impact of maternal supplementation with oil and baru almond on anxiety-like behavior, oxidative stress in brain tissue and on the protection of the cortex of adolescent and adult offspring.

2 MATERIAL AND METHODS

2.1 Baru Almond

The baru almond used in these experiments belongs to the species *Dipteryx alata* Vog. and taken from the city of Goiânia/GO, Brazil: latitude - 16º40`43``S, longitude - 49º15`14``W, and altitude - 749 m. Material from the almond was used for an oil extraction and stored in amber glass at -20 ±2°C, while the other form was offered to the animals in a solution, in which minutes before gavage, the almond was crushed and mixed with distilled water.

2.1.1 Analysis of Fatty Acid Composition in Baru Oil and Almond

The fatty acid profiles of the oil and almond were analyzed [36–37] (Table 1).

2.1.2 Lipidic Extraction

Sample were weighed (2 g of each) in a beaker and added to 30 ml of chloroform:methanol mixture (2:1). After this addition, the content was transferred to a deep glass container with the side covered with aluminum foil and stirred for 2 min with the help of grinder. The triturate was filtered through qualitative filter paper into a 100 ml graduated cylinder with a polished mouth. Next, the vessel walls were washed with an additional 10 mL of chloroform:methanol which was also filtered with the previous volume. The volume of the filtered extract of the graduated cylinder was recorded with

the graduated cylinder closed. Twenty percentage of the final volume of the filtered extract was added to 1.5% sodium sulfate. The mixture was stirred with the graduated cylinder closed and given time for the phases to separate. It was observed that the upper phase was 40% and the bottom 60% of the total volume. The volume of the lower phase was recorded and then the upper phase was discarded by suction with a graduated pipette. For lipid quantification, an extracted aliquot of 5 mL (lower phase) was separated with a volumetric pipette and transferred to a previously weighed beaker. This beaker was placed in an oven at 105°C so the solvent mixture could evaporate, being careful that the fat would not be degraded by heat. After cooling in a desiccator, the beaker was weighed and the fat residue weight was obtained from the difference [36].

2.1.3 Transesterification of Fatty Acids

In the sample treatment, methylation of fatty acids present in the lipid extract was carried out following the methodology described by Hartman and Lago (1973) [37]. An aliquot of the lipid extract was taken, calculated for each sample according to the fat conte2nt found in the lipid measurement, and performed according to the (Folch *et al*., 1957) [36], adding 1 ml of internal standard (C19:0) and a saponification (KOH) solution. This solution was subsequently brought to heating under reflux for 4 min. Esterification solution was added immediately after, returning the solution to heating under reflux for 3 more minutes. Next, the sample was allowed to cool before subsequent washings with ether, hexane and distilled water, finally obtaining an extract (with the methyl esters and solvents), which was conditioned into a properly identified amber glass until complete drying of the solvents. After drying, a suspension in 1 ml of hexane was made and packaged into a vial for further chromatographic analysis. The aliquots of saponification and esterification solutions were determined according to the methodology described by Hartman and Lago (1973) [37].

2.1.4 Gas Chromatography Analysis

A gas chromatograph (VARIAN 430-GC, California, EUA), coupled to a capillary column of fused silica (CP WAX 52 CB, VARIAN, California, EUA) with dimensions of 60 m × 0.25 mm and 0.25 mm film thickness was used with helium as carrier gas (Flow rate of 1 ml/min). The initial oven temperature was 100°C programmed to reach 240°C,

increasing 2.5°C per minute for 30 min, totaling 86 min. The injector temperature was maintained at 250°C and the detector at 260°C. 1.0 µl aliquots of esterified extract were injected in a Split/Splitless injector. The chromatograms were recorded using Galaxie Chromatography Data System software. The fatty acids results were quantified by integration the areas of the methyl esters and are expressed in percentage by area.

Table 1. Fatty acid composition of oil and baru almond.

		BARU OIL (%)	BARU ALMOND (%)
Acids Fat		**100g^{-1} lipid**	
SATURATED			
Palmitic acid	C16:0	7.03	9.44
Stearic acid	C18:0	4.91	7.46
Arachidic acid	C20:0	1.07	0.40
Behenic acid	C22:0	3.02	6.34
Lignoceric acid	C24:0	4.2	9.24
Cerotic acid	C26:00	0.24	0.79
∑ SFA		**20.47**	**33.67**
MONOUNSATURATED			
Oleic acid	C18:1ω9	48.09	30.73
Gondoic acid	C20:1ω9	-	4.37
Gadoleic	C20:1	2.39	1.05
Erucic acid	C22:1ω9	-	0.57
∑ MUFA		**50.48**	**36.72**
POLYUNSATURATED			
Linoleic acid	C18:2ω6	29.05	10.57
α-linolenic acid	C18:3ω-3	-	3.37
Eicosadienoic acid	C20:2ω6	-	0.12
∑ PUFA		**29.05**	**14.06**

2.2 Analysis of antioxidant compounds present in Baru Oil and Almonds

Baru oil and almond were analyzed to determine phenolic components, flavonoids, and total carotenoids. Their antioxidant capacity was also analyzed using the ABTS, FRAP and the IC_{50} methods.

2.2.1 Extraction

Baru almond constituents were extracted with an 80% methanol solution and evaluated for ABTS• removal capacity, ferric reducing activity (FRAP), flavonoids, and total phenolics. Crushed baru almond (1 g) was placed in a test tube and then 10 mL of solvent was added. The test tube was left at room temperature for 24 hours, and after filtration, the volume was completed to 10 mL with extraction solvent and stored at 18°C until analysis. All extractions were performed in triplicate.

2.2.2 Determination of the Total Phenolic Content

To measure the total phenolic compounds present in the sample, we used the methodology described by Liu *et al*., (2002) [38] with minor adaptations. The absorbance of the extract was compared with a standard gallic acid curve to estimate the concentration of phenolic compounds in the sample. Results were expressed in milligrams equivalent of gallic acid/100g of sample (mg EAG/100g).

2.2.3 Determination of total flavonoids

The total flavonoid content was measured using the colorimetric assay developed by Zhishen *et al*., (1999) [39]. To estimate the concentration of flavonoid contents in the sample, the extract's absorbance was compared with a catechin standard curve. The total flavonoid content was expressed in mg equivalent of catechin/100g of sample (mg EC/100g).

2.2.4 Antioxidant activity - FRAP method

The FRAP method was performed according to Benzie & Strain, (1999) [40], with modifications proposed by Liu *et al*., (2002) [38]. The FRAP solution was used as a reference reagent and the absorbance was read in nm. Results were expressed as µmol trolox equivalents per gram sample (µmol TE/g^{-1}).

2.2.5 Antioxidant activity - ABTS methods

The ABTS method was performed in accordance with the methodology described by Surveswaran *et al*., (2007) [41], with modifications. Results were expressed as μmol trolox equivalents per gram of sample (μmol TE/g^{-1}). Where A_0 is the absorbance of the control. The effective concentration presented 50% radical inhibition activity (IC_{50}), expressed in mg extract/mL, which was determined from the graph of the free radical scavenging activity (%) against the extract concentration.

The baru almond and oil, respectively, presented total phenolic contents of 41.31 and 39.43 mg GAE/100 g, and total flavonoids of 3.25 and 2.12 mg EC/100 g. For antioxidant activity, the baru almond and the oil presented respective FRAP values of: 0.044 and 0.094 μmol TE/g, ABTS values of 0.90 and 2.50 μmol TE/g, and IC_{50} values of 14.13 and 28.1 mg/ml.

2.3 Animals and Experimental Groups

All of the experimental methods were previously approved by the Ethics Committee for Animal Use - CEUA of UFCG - Certification No. 104-2017, in compliance with the standards established by the National Council for the Control of Animal Experimentation (CONCEA, Brazil), under Law No. 11,794 /2008 (Arouca Law), and with the guidelines for *in vivo* experiments with animals of the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) 2.0 [42]. The baru almond used in the supplementation was registered in SisGen; protocol No. A071A68 (see attachment). Twenty-four female Wistar strains, from the Experimental Nutrition Laboratory of the Federal University of Campina Grande - LANEX/UFCG, aged 90 days and weighing 250 ±50 g were used to obtain offspring. Females were mated at the rate of two females for each male. After confirmation of pregnancy, the animals were housed in individual polypropylene cages (60 cm long, 50 cm wide, and 22 cm high), and kept under standard laboratory conditions (temperature 22 ±1°C, humidity 55 ±5%, light/dark cycle of 12/12 hours - artificial light from 6:00 to 18:00), in three groups: Control (CG) - supplemented with distilled water; Baru oil (OB) - supplemented with 2,000 mg of baru oil/kg of animal weight; and Baru almonds (AB) - supplemented with 2000 mg of baru almond solution/kg of animal weight. Gavage was administered from the 7th day of pregnancy through the 21st day of lactation. The mothers were offered standard feed (Presence Purina®, São Paulo, Brazil)

and water *ad libitum*. After weaning, the offspring also received standard feed until adulthood. The dosages offered to the animals were based on studies described by Reis *et al*., (2018) [43], which evaluated the hepatoprotective and antioxidant activity of baru almond oil (*Dipteryx alata* Vog.) at 1g/kg in a clinical assessment of lipo-toxicity and dyslipidemia, as well as a study by Siqueira *et al*., (2012) [34], which evaluated the impact of consuming the AIN-93M diet with 10% baru almonds for prevention of iron-induced oxidative stress in rats.

2.4 Experimental Design

During pregnancy and lactation, supplementation was performed using gavage. After weaning, the animals were expected to reach adolescence (T45) and adulthood (T90) to perform the anxiety tests. At the end of the experiment, the animals were euthanized to remove brain tissue and evaluate glutathione and malondialdehyde content. The experimental protocol is detailed in Figure 1.

Figure 1. Experimental protocol performed with the offspring of *Wistar rats* treated during pregnancy and lactation.

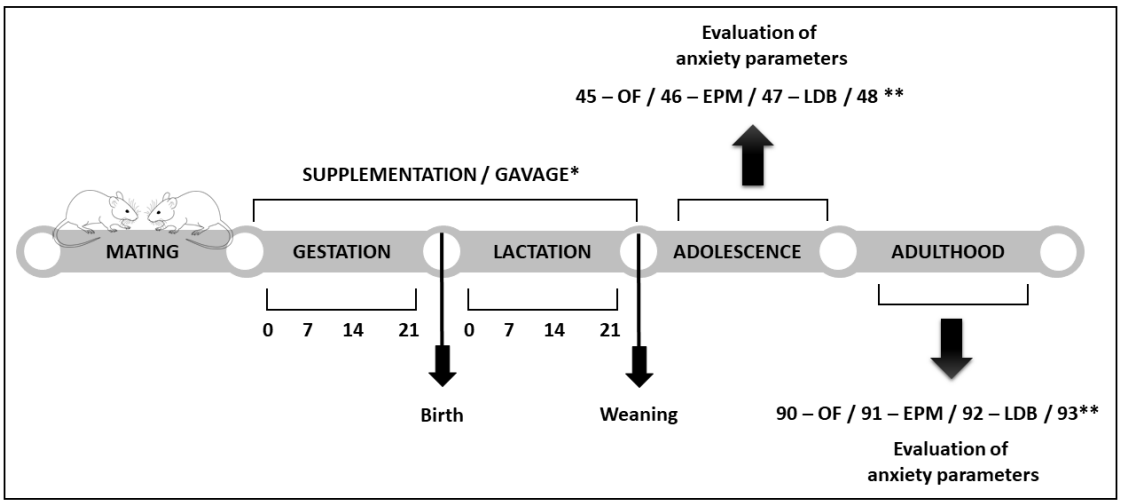

Figure 1. Experimental protocol. (*) Treatment period in which mothers received: distilled water (CONTROL) (n = 12); 2000 mg/kg of baru oil (OIL) weight (n = 12); and 2000 mg/kg of baru almonds weight (ALMOND) (n = 12). (**) Day of brain collection for analysis of glutathione, malondialdehyde, and cortex histology. OF = Open Field; EPM = Elevated Plus Maze; LDB = Light-Dark Box.

2.5 Behavioral tests

2.5.1 Open Field Testing (OF)

The open field is an instrument to test anxiety behavior and exploratory activity in order to verify the effects of unfamiliar environments on emotionality in rats [45]. The test was performed with Wistar rats in the post-lactation period. Each animal was inserted into the center of the apparatus and observed for 5 minutes. The test was performed in the dark, the light level was 0 lux. The following parameters were evaluated: ambulation (number of crosses of the segments by the animal with all four legs), number of rearing behaviors, time of self-cleaning (grooming) and defecation (recorded by number of defecation) [46]. The sessions were filmed and later analyzed using ANY-maze software. The apparatus was sanitized with a 10% alcohol solution after each behavioral session.

2.5.2 Elevated Plus Maze (EPM)

EPM is commonly used as an unconditioned model of anxiety in rodents [47,48]. The EPM is an apparatus formed by two closed arms and two open arms perpendicular to the first, and a central area which is raised from the ground. The animal was placed in the center of the device, always by the same researcher, with the muzzle facing the right closed arm. The frequency of entries in the closed and open arms, the time spent in each arm and in the center of the apparatus were analyzed during 5 minutes using ANY-maze software. In addition, the number of head dips in the open arms was also counted. Each animal was tested, and the maze was sanitized with a 10% alcohol solution. The entire test was performed at a luminosity level of 0 lux.

2.5.3 Dark Light Box Testing (DLB)

The light-dark transition box measures unconditioned anxiety and exploratory behavior in rodents [49]. The apparatus is made of acrylic glass (45 X 27 X 27cm) and is divided into two compartments, one dark (18 X 27cm) and one clear (27 X 27cm). The animals were placed in the center of the light compartment with the muzzle facing the dark compartment, and kept in the open for 5 min of free exploration. The sessions were filmed and later analyzed using ANY-maze software. The evaluated behaviors were the

permanence time of the animal in each of the compartments. At the end of each session the apparatus was sanitized with a 10% alcohol solution, as the dirt in the light-dark box compromised the neophobic component associated with the apparatus.

2.7 Determination of gluthatione content and malonyldialdehyde (MDA) levels

During adolescence (45 days of life) and adulthood (90 days of life), after 6 hours of fasting, the animals were anesthetized with Ketamine Hydrochloride and Xilasin (1 ml/kg body weight) and were sacrificed by cervical dislocation. Then, the brain tissue was removed to determine the content of glutathione and MDA. Total glutathione content was quantified by the recycling assay described by [50], and the results were expressed as nmol/g wet tissue. Samples that had been frozen with 1 mL of trichloroacetic acid were defrosted and minced with scissors for 15 s on an ice-cold plate. The resultant suspension was subsequently diluted to a final 1:20 (w/v) ratio and homogenised for 2 min with an automatic Potter homogenizer, and centrifuged at 2000 × g and 4 °C for 5 min. The supernatants were then centrifuged at 9000 × g and 4 °C for 5 min. The supernatants were assayed to determine total glutathione.

To assess lipid peroxidation, MDA production was measured in assay described by Esterbauer & Cheeseman (1990) [51]. Tissue (5 samples per group) homogenates (T Tris–HCl 20 mm, 1:5 p/v) were centrifuged at 2500 g at 48 °C for 15 min, then were added to a 750 ml solution (1-Methyl-2-phenylindole 10.3 mm in acetonitrile + 225 ml HCl 37%) and the mixture was placed in a water bath and heated to 4 °C for 40 min. Next, it was centrifuged at 2500 g at 4 °C for 5 min. Absorbance was measured at 586 nm (Genesys 10 s UV-VIS, Thermo Fisher Scientific, Loughborough, UK). The concentration of MDA was expressed as nmol of MDA per gram of brain tissue.

2.8 Histological evaluation of cortex

The cortex of 3 rats per treatment were collected, washed in saline solution (0.9% NaCl) and fixed in 10% buffered formalin. The major lobe was subjected to a histological procedure according to the routine technique at the Pathology Laboratory (Department of Physiology and Pathology/CCS/UFPB) for obtaining blocks from which semi-serial 4-μm sections were cut. The slides were hydrated, stained with haematoxylin–eosin (HE), dehydrated, diaphanised in xylol and mounted with Entellan® for optical microscopic

analysis (Motic BA 200, Olympus Optical Co, Philippines). For morphometric analysis, twenty random images from slides of cortex tissues were used. Under an Axiolab light microscope (Zeiss) with 400 × resolution, twenty images were relayed to an image analysis system (Kontron Elektronik image analyser; Carl Zeiss, Germany—KS300 software). Reading of slides was performed randomly by two pathologists. A 10 × objective and 40 × photomicrograph of cortex was used to obtain the images.

2.9 Statistical analysis

The results were expressed as mean ± SEM and analyzed by ANOVA followed by Tukey ($p < 0.05$). The program - GraphPad Prism 7.0 was used for data analysis.

3 RESULTS

3.1 Behavioral Tests

3.1.1 Assessment of anxious-like behavior using the Open Field – Adolescent phase (T45)

Submitting the adolescent offspring to the open field apparatus, it was possible to verify greater locomotor activity in the animals belonging to the experimental groups than in the control group animals [$F(2.23) = 82.24$, $p<0.0001$] (**Fig. 2A)**. *Post-hoc* analysis also revealed that the oil group animals presented greater locomotion than the almond group ($p < 0.01$ by the Tukey-Kramer test). For grooming behavior, there was a significant reduction observed for the oil and almond group animals as compared to the controls [$F(2, 22) = 8.605$, $p<0.0017$] (**Fig. 2B**). Regarding defecation, a reduction in the number of fecal boluses was observed for the oil and almond groups as compared to the control group [$F(2, 20) = 23.49$, $p <0.0001$] (**Fig. 2C)**. When analyzing the number of rearings, a significant difference between the experimental and control groups was also observed [$F(2, 25)=34.48$, $p<0.0001$] (**Fig. 2D**).

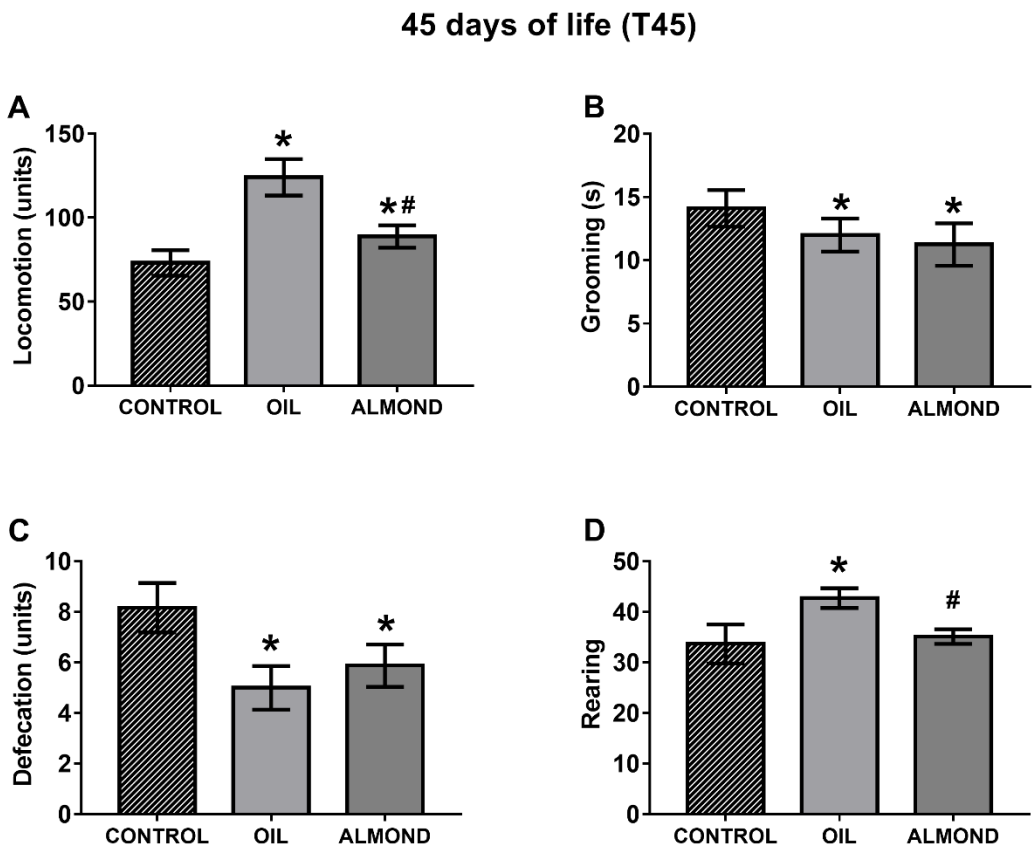

Figure 2. Open field test with adolescent offspring (T45) treated with baru oil (n = 12) and almonds (n = 12) during pregnancy and lactation (2000 mg/kg body weight). (A) locomotion, (B) grooming, (C) defecation, (D) rearing. Values expressed as mean and standard error (one-way ANOVA, Tukey-Kramer); *Versus CONTROL GROUP; # Versus OIL GROUP ($p < 0.05$).

3.1.2 Assessment of anxious-like behavior using the Open Field test - adult stage (T90)

When analyzing the behavior of the adult offspring in the open field apparatus, greater locomotor activity was observed in the animals belonging to the experimental groups than the control group [$F(2, 21) = 26.05$, $p<0.0001$] (**Fig. 3A)**. For grooming, a reduction was observed in the oil and almond group animals as compared to the control group [$F(2.21)=13.91$, $p<0.0001$] (**Fig. 3B)**. Further, there was a significant reduction in the number of fecal boluses in the almond group alone [$F(2.22)=4.765$, $p<0.0191$] (**Fig. 3C)**. For rearing behavior, a significant difference was seen between the animals in the almond group, as compared to the control and oil groups [$F (2, 24) = 10.15$, $p <0.0006$] (**Fig. 3D)**.

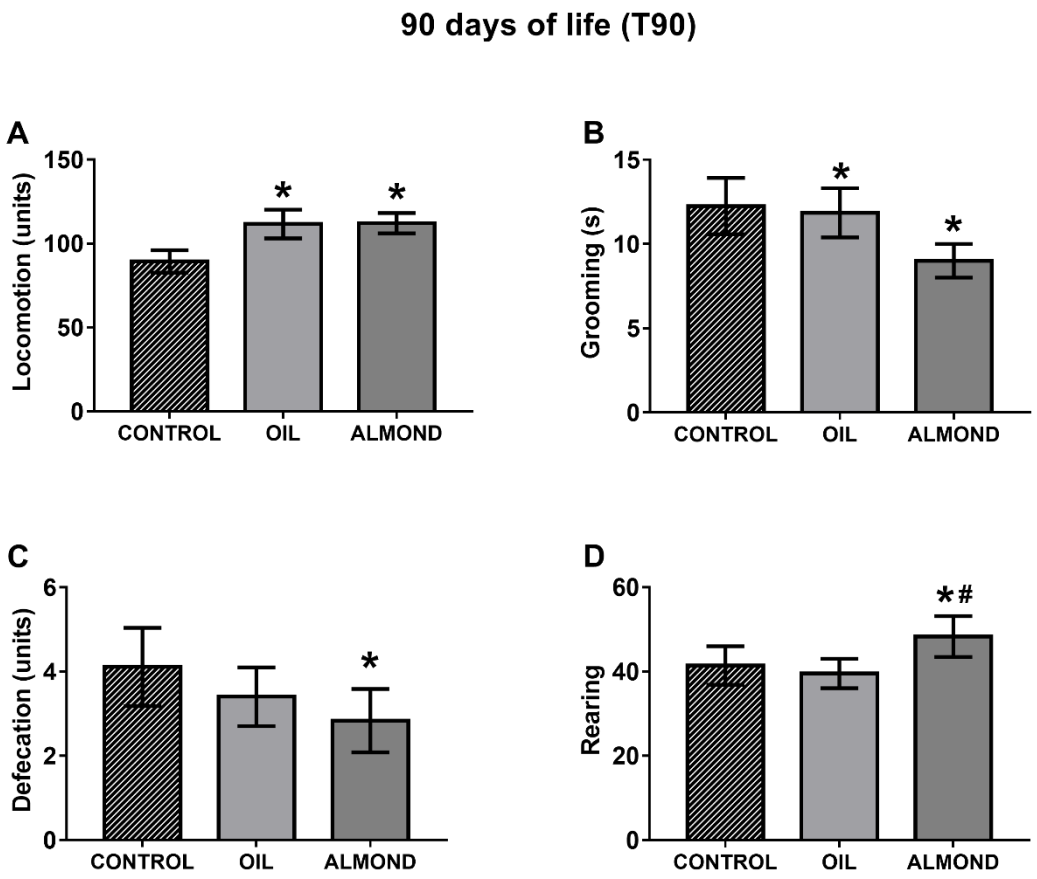

Figure 3. Open field test with adult offspring (T90) treated with baru oil (n = 12) and almonds (n = 12) during pregnancy and lactation (2000 mg/kg body weight). (A) locomotion, (B) grooming, (C) defecation, (D) rearing. Values expressed as mean and standard error (one-way ANOVA, Tukey-Kramer); *Versus CONTROL GROUP; # Versus OIL GROUP ($p < 0.05$).

3.1.3 Assessment of anxious-like behavior using the Elevated Plus Maze

The analyses indicated that baru almond supplementation augmented the number of entries and time spent in the open arms by the adolescent offspring treated during pregnancy and lactation [$F(2, 26) = 6.213$, $p < 0.0062$] (**Fig. 4A)**; [$F(2.27) = 5.769$, $p<0.0082$] (**Fig. 4B)**. When evaluating the time spent in the central area of the apparatus, it was found that the animals in the oil and almond groups remained longer than those belonging to the control group [$F(2, 24) = 147.1$, $p < 0.0001$] (**Fig. 4C)**. The *post-hoc* analysis also showed that the animals in the almond group remained longer in the central area than those of the oil group ($p < 0.01$ - Tukey-Kramer). In the adult phase, we observed a statistical difference only for the number of open arm entries, where the animals in the almond group presented a greater number of entries than the animals in the oil and control groups [$F(2, 26) = 14.64$, $p<0.0001$] (**Fig. 4A)**.

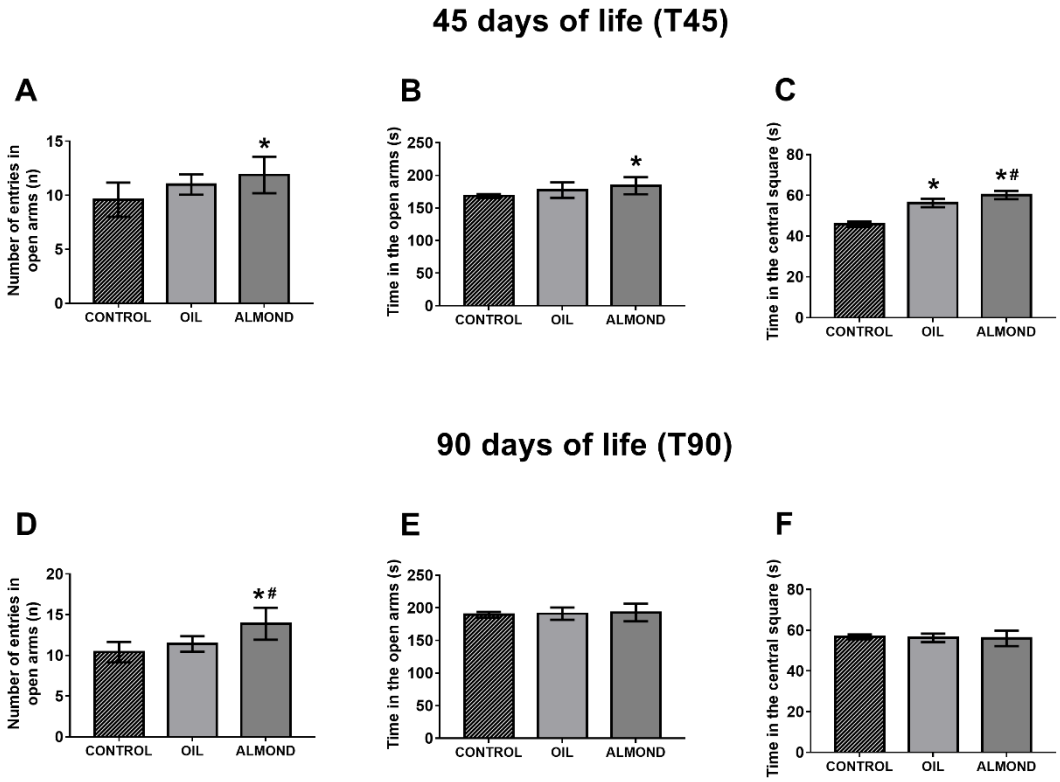

Figure 4. Elevated Plus Maze Test performed with adolescent (T45) and adult (T90) offspring treated with baru oil (n = 12) and almond (n = 12) during pregnancy and lactation (2000 mg/kg of weight). (A) number of entries in the open arms, (B) time in the open arms, (C) time in the central area. Values expressed as mean and standard error (one-way ANOVA, Tukey-Kramer); *Versus CONTROL GROUP; # Versus OIL GROUP ($p < 0.05$).

3.1.4 Assessment of anxious-like behavior using the light-dark box – adolescence phase (T45)

When verifying the number of transitions in the light and dark box test, it was observed that the animals belonging to the oil group presented significant differences in relation to the other groups, with a greater number of transitions [$F(2, 28) = 3.657$, $p < 0.0388$] (**Fig. 5A)**. Both experimental groups presented more time spent in the lighted compartment of the box as compared to the animals of the control group [$F(2, 24) = 123.1$, $p < 0.0001$] (**Fig. 5B)**. Post-hoc analysis revealed that animals in the almond group remained longer in the lighted compartment when compared to those of the oil group ($p < 0.01$ - Tukey-Kramer). For time spent in the dark compartment of the box, we found a significant difference only for animals in the almond group [$F(2, 20) = 4.007$, $p < 0.0344$] (**Fig. 5C)**.

For movements performed in the lighted compartment of the box, both the oil and almond group animals presented significant differences from the animals in the control group [F (2, 27) = 73.21, p <0.0001] (**Fig.5D)**.

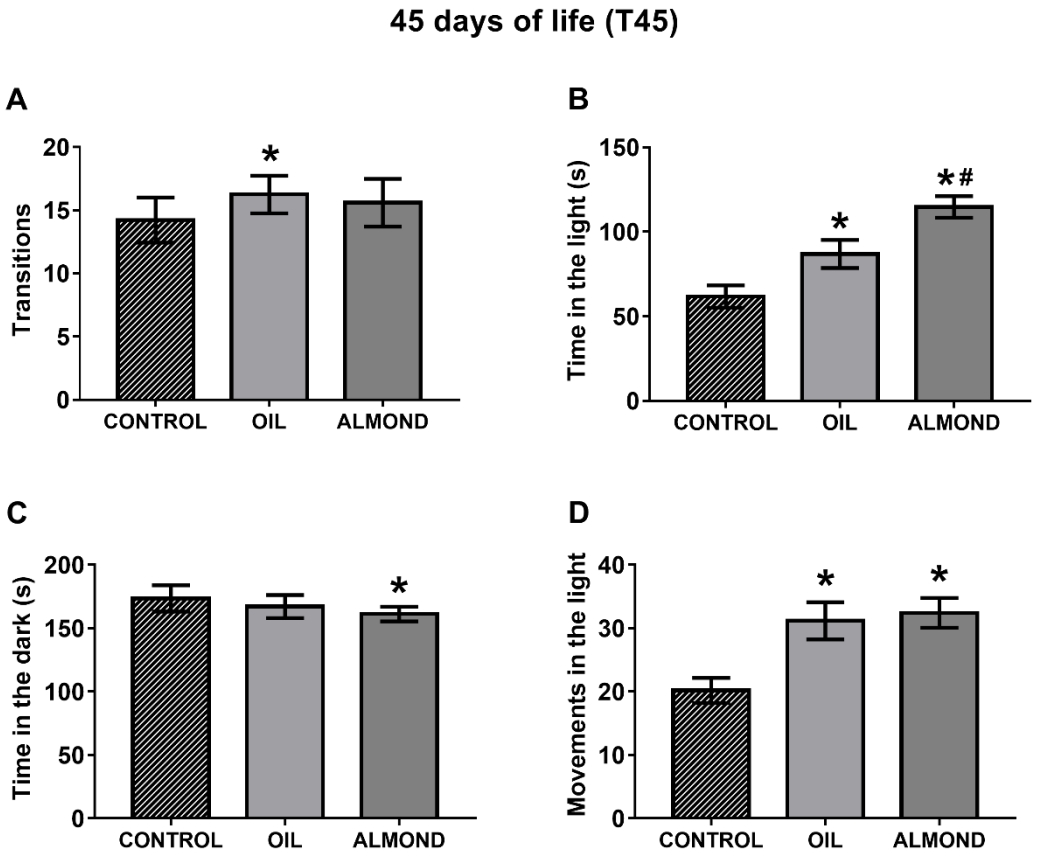

Figure 5. Light-dark box test for adolescent offspring (T45) treated with baru oil (n = 12) and almonds (n = 12) during pregnancy and lactation (2000 mg/kg body weight). (A) number of transitions; (B) time in the lighted compartment, (C) time in the dark compartment, (D) movements within the lighted compartment. Values expressed as mean and standard error (one-way ANOVA, Tukey-Kramer); *Versus CONTROL GROUP; # Versus OIL GROUP (p <0.05).

3.1.5 Assessment of anxious-like behavior using the light-dark box - adult stage (T90)

When evaluating the animals at 90 days of life, it was found that the animals belonging to the experimental groups presented more movement than the animals in the control group [F (2, 25) = 17.57, p <0.0001] (**Fig. 6A)**. As for the time spent in the lighted compartment, both experimental groups presented longer times than the control group [F (2, 27) = 31.24, p < 0.0001] (**Fig. 6B)**. Post-hoc analysis (Tukey-Kramer) also revealed that the animals in the almond group remained longer in the lighted compartment than

those of the oil group ($p < 0.01$). For time spent in the dark compartment, the almond group presented shorter times than the other groups [$F(2, 23) = 7.678$, $p < 0.0028$] (**Fig. 6C)**.

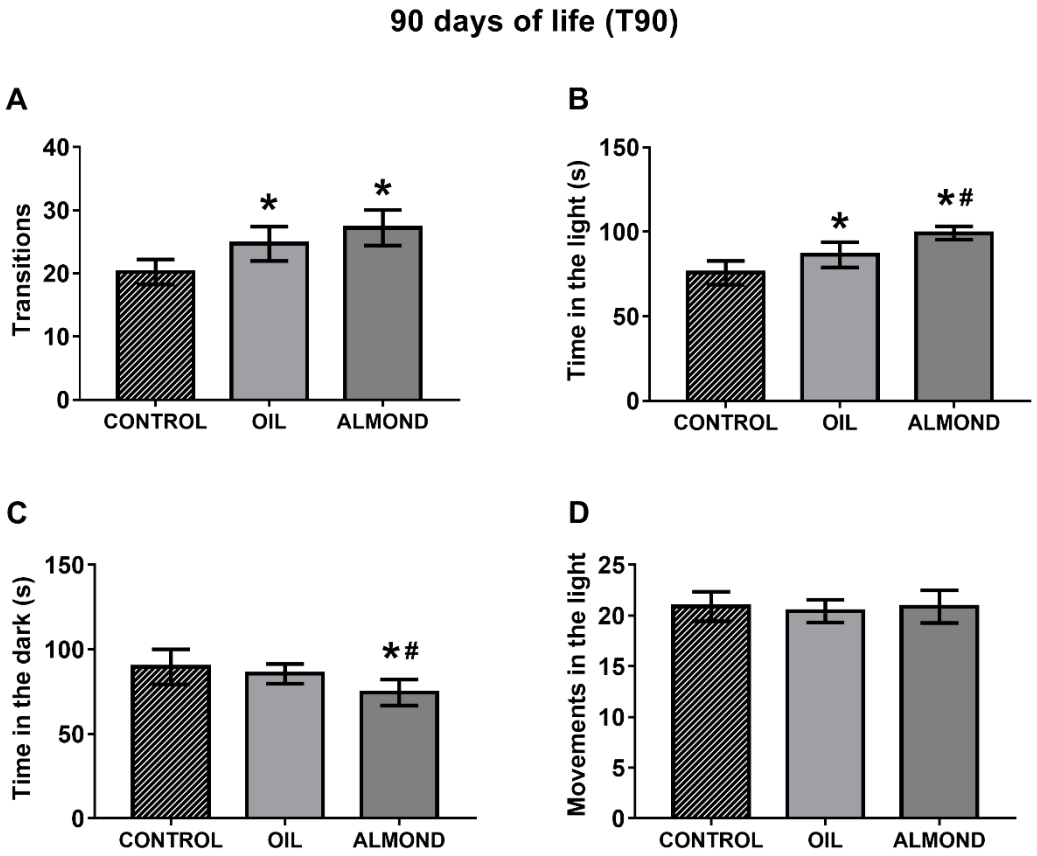

Figure 6. Light-dark box test with adult offspring (T90) treated with baru oil (n = 12) and almonds (n = 12) during pregnancy and lactation (2000 mg/kg body weight). (A) number of transitions; (B) time in the lighted compartment, (C) time in the dark compartment, (D) movement within the lighted compartment. Values expressed as mean and standard error (one-way ANOVA, Tukey-Kramer); *Versus CONTROL GROUP; # Versus OIL GROUP ($p < 0.05$).

3.2 Determination of total glutathione and MDA levels

When evaluating total glutathione levels in the brain tissue of the offspring, it was observed that the adolescence phase (T45) animals belonging to the oil and almond groups presented higher concentrations than the control group [$F(2, 15) = 320.3$, $p<0.0001$] (**Fig. 7A)**. However, in the adult phase (T90), only animals in the almond group continued to present higher concentrations [$F(2, 17) = 19.64$, $p < 0.0001$] (**Fig. 7B)**.

In the brains of the adolescent offspring, there was a reduction in MDA content in the oil and almond groups as compared to the control [F (2, 29) = 15.48, p < 0.0001; p<0.0008] (**Fig. 7C**). In the adult phase (T90) there was no statistical difference.

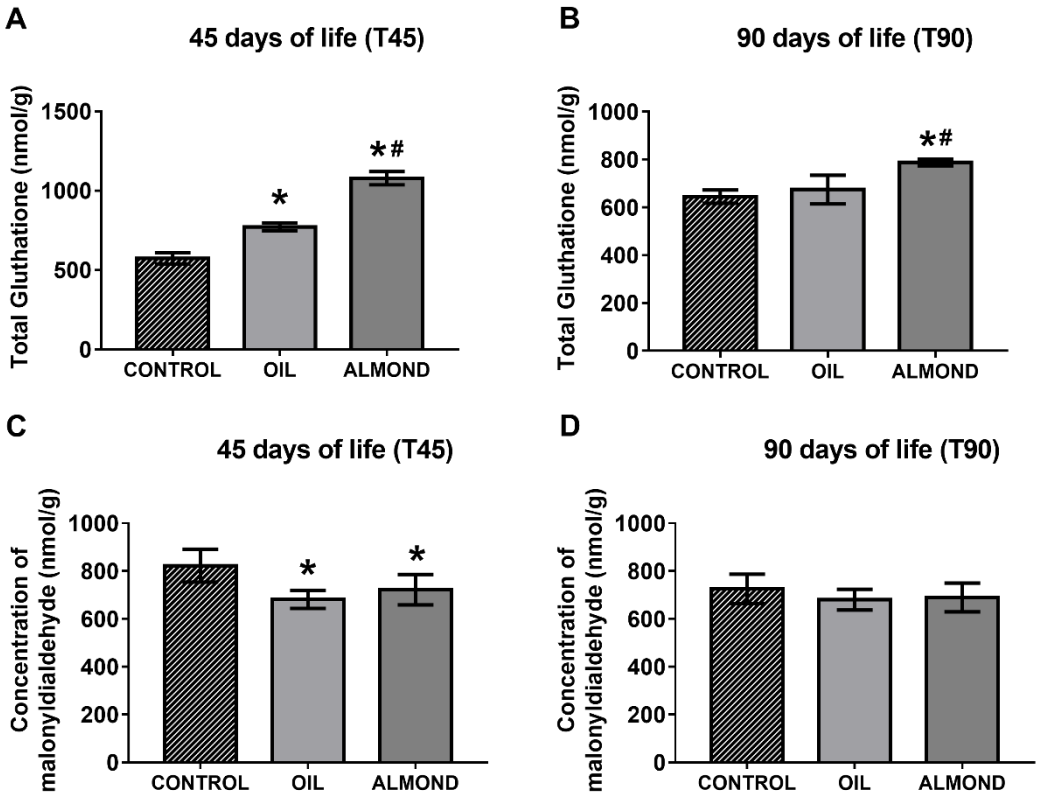

Figure 7. Evaluation of total glutathione and malondialdehyde concentrations in the brain of adolescent (T45) and adult (T90) offspring treated with baru oil (n = 12) and almonds (n = 12) during pregnancy and lactation (2000 mg/ kg of weight). Values expressed as mean and standard deviation (one-way ANOVA, Tukey-Kramer); *Versus CONTROL GROUP; $^{\#}$ Versus OIL GROUP (p <0.05).

3.3 Histological evaluation of cortex

When analyzing the histological section of the central nervous system stained with hematoxylin and eosin, we observed preserved cell bodies in the frontal cortex, with no change in shape or content compatible with normality in the control adolescent offspring (A), as well as in the other experimental conditions, baru (B) and baru almond (C). These same findings were verified in the control adult offspring (D), baru oil (E) and baru almond (C), as shown in figure 8.

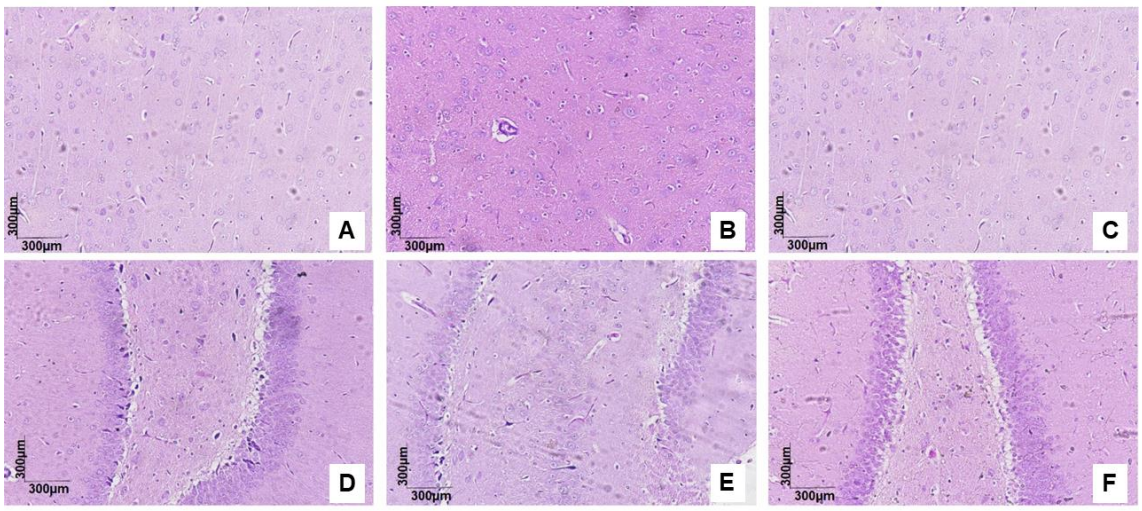

Figure 8. Representative histological section of the T45 frontal cortex (A – C) or T90 frontal cortex (D - E) of the animals under different experimental conditions. A and D control group; B and E Baru oil group; C and F Baru almond group.

4 DISCUSSION

In the present study, supplementation with baru oil and almonds (source of Polyunsaturated Fatty Acids – PUFAs, and phytocomplexes) during pregnancy and lactation positively influenced the offspring to promote: (1) the reduction of anxious-like behavior, (2) reduction oxidative stress in brain tissue and (3) preservation cell bodies in the cortex.

To evaluate the anxiety parameters, the Open Field (OF), Elevated Plus Maze (EPM), and the Light-Dark Box (LDB) model was used. Upon statistical analysis, it was observed that the animals belonging to the experimental groups, when exposed to the OF test in the adolescence (T45) and adult (T90) phases demonstrated greater locomotion activity and rearing behavior, with reductions in grooming and the number of fecal boluses as compared to the controls. The OF is a validated experimental model used to analyze locomotor activity in rodents [52]. According to Kraeuter and collaborators (2019) [53], Seibenhener and Wooten (2015) [52] and Hall (1934) [54], an increase in locomotor activity and rearing behavior, with reductions in the grooming parameter and the number of fecal boluses are indicative of anxiolytic-like behavior. Similar data were reported in a study by Frausto-González *et al*. (2021) evaluating the acute effects of Brazil nut extract supplementation in adult rats, revealing greater open field activity. According to the authors, the anxiolytic activity exerted by the extract is related to the ability of polyunsaturated fatty acids to interact with the $GABA_A$ receptor [55]. Other studies using

differing lipid matrices that are sources of PUFAs have also shown a reduction in grooming behavior and stools [56–58].

When we investigated the performance of the animals in our study in the EPM, we detected that the T-45 almond group presented higher number of entries and more time spent in the open arms. When the time spent in the central area of the EPM was evaluated, both the oil group and the almond group in adolescence (T-45) presented a more time than the control group. When only the experimental groups were analyzed, we verified greater permanence for animals in the almond group as compared to the oil group. In the adult phase (T-90), only the animals of the almond group presented a higher number of open arm entries. The anxiolytic effects observed in adolescence in the present study can be attributed to the ability of PUFAs to cross the placental and blood-brain barrier during pregnancy and lactation [13,59,60]. PUFAs are then deposited in offspring Nervous System (NS) structures, consequently increasing neuroplasticity, fluidity, and neuron survival [61–64]. Other studies point out the ability of PUFAs to regulate the concentration of Brain-Derived Neurotrophic Factor (BDNF) and glucocorticoid signaling, contributing to hypothalamic-pituitary-adrenal (HPA) axis regulation and modulating stress response, reducing anxious type behavior [65]. In the adult phase (T-90), the anxiolytic effects were persistent only in the animals of the almond group. We hypothesize that the presence of phytocomplexes present in the baru almond protects and preserves fatty acids deposited in the SN of the offspring against oxidative damage.

To further investigate the rodents' behavior, they were submitted to the Light-Dark Box (LDB) test. According to Hascoët *et al*., (2001) [49], this apparatus allows analyzing anxiety behavior using the time spent by the animal on both sides (light/dark) of the box. Research evaluating PUFAs and neuroprotective phytocomplexes has revealed the preference of tested rodents for the lighted side of the device, this effect is indicative of anxiolytic-like behavior [66–68].

Our data verified a significant increase in time spent on the lighted side of the box by both experimental groups in the different life stages (T-45 and T90) tested. Similar results have been evidenced in research evaluating the impact of maternal consumption of Conjugated Linoleic Acid (CLA), which is capable of protecting the offspring brain against oxidative damage, while producing anxiolytic effects during OF, EPM, and LDB tests [69]. In a study developed by Bernal-Morales *et al*., (2017) [70], it was shown that a mixture of fatty acids (lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic and elaidic acids) was able to modulate GABAergic neurotransmission, reducing anxiety-like behavior in

adolescent rats. Accordingly, such effects are related to the ability of certain fatty acids, including PUFAs, to modulate chloride ion channels, increasing GABAergic activity. Prévot & Sibille (2020) [71], demonstrated that a reduction of GABAergic activity implies cognitive dysfunction, it substantially affects the expression of somatostatin (SST +), which in turn alters excitatory signal encoding in cortical microcircuits, and results in depressive and anxiogenic behaviors.

Despite the benefits of PUFAs, they are highly oxidizable in brain tissue because of its extensive metabolic activity and iron concentration. Peroxidation of PUFAs leads to the formation of cytotoxic and genotoxic aldehydes, which possess a crucial pathogenic role in anxiety, depression, and neurodegenerative disease [72,73]. Some researchers have reported on the influence of bioactive compounds (present in foods) with neuroprotective antioxidant activity, that preserve PUFAs in brain tissue, and reduce anxious-like behavior in rodents [56,74,75].

When determining the contents in the baru almonds and oil, significant amounts of phenolic compounds and total flavonoids (phytocomplexes) were noted; the values were higher in the almonds. In the ABTS, FRAP, and IC_{50} assays, we found greater antioxidant activity in the almonds as compared to the oil. These findings may explain the expressive T-90 almond group results observed.

Gallic acid stands out among the phytocomplexes with anxiolytic activity. In addition to presenting antioxidant activity and restoring endogenous enzyme levels, such as superoxide dismutase, catalase, and glutathione peroxidase; gallic acid affects the GABAergic system, inhibiting the activity of gamma-acid transaminase, aminobutyric acid (GABA-T), modulating nitrergic mechanisms while reducing anxiety [76]. Although we did not measure gallic acid concentrations in our study, Lemos *et al*. (2012) [34], determined an average concentration of 170.9 mg of gallic acid per 100 g of baru almonds. Nabavi *et al*., (2012) [77] reports that 20 mg/kg of gallic acid is able to promote significant increases in levels of glutathione, catalase, and superoxide dismutase, reducing the oxidative stress induced by sodium fluoride in the rat brain. Yet it was shown that oxidative stress in brain lipid structures (lipid peroxidation) during the early stages of life contributes to neuronal loss in offspring, with consequent neural dysfunction in the short and long term [78,79].

We also sought to evaluate lipid peroxidation in offspring brain tissue. According to our results, it can be observed that maternal supplementation with both baru oil and almonds contributed significantly to malondialdehyde (MDA) concentration reductions in the T-

45 adolescent offspring. For glutathione, the data indicate significant concentration increases in both T-45 experimental groups. In the adult phase, we observed glutathione increases only in animals belonging to the almond group. The data corroborate França *et al*. (2021) [80], who observed a reduction in oxidative stress and anxiety-like behavior in dyslipidemic rats supplemented with a solution containing 1000 mg/kg of Macaíba seed (a source of lipids and phytocomplexes).

Increased MDA in brain tissue has been linked to neurodegenerative disease, anxiogenic behavior, and depression [81,82]. Elevated levels of MDA in the brain may result from the action of ROS on neuronal membranes, which are especially rich in arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) [83], and which due to their unsaturated bonds are particularly vulnerable to oxidative stress [84].

In contrast, increased levels of GSH in brain tissue contribute to neuronal cell protection. GSH plays a key role in antioxidant defense, maintaining redox homeostasis [85]. An increase in GSH levels in the brain attenuates ROS production, reduces the concentration of 8-Oxo-2'-deoxyguanosine (8-oxo-dG), and increases level of transcription factor 2-related erythroid nuclear factor 2 (Nrf2). The Nrf2 pathway (antioxidant response element) controls an array of endogenous cellular antioxidant systems, which are extremely important for ROS neutralization in the brain [86]. Additional studies have demonstrated the central importance of GSH in Nrf2-mediated neuroprotection [87–89].

When performing GSH analysis in the offspring of rats treated during pregnancy and lactation, significant values of the enzyme were verified during adolescence in the brain tissue of the animals belonging to the oil and almond groups (T-45). In the adult phase (T-90), there was an increase in GSH only in the animals from the baru almond group, thus suggesting that supplementation with the almonds promoted lasting effects, this possibly due to the greater phenolic and total flavonoid content in baru almonds as compared to baru oil. According to Batool *et al*. (2018) [90] and Mangge *et al*. (2014) [91], flavonoids present important activity against oxidative processes, attenuating the release of inflammatory cytokines (interleukin 1 β and tumor necrosis factor α - TNF - α), exposure to nitric oxide, and inhibiting activation of NADPH oxidase, while supporting regulation of oxidative process transcription factors.

Minich & Brown (2019) [92], reported that glutathione levels can be potentiated with intake of dietary nutrients; phenolics, flavonoids, and mono- and polyunsaturated lipids. This is in agreement with our study. Baru acts as a protective agent of the neural system in the developing offspring.

Lipid peroxidation of brain tissue appears to be involved in mechanisms underlying anxiety and other neurological disorders, and since the changes caused by oxidative damage can last throughout life, understanding the impact of the maternal diet on anxiety parameters in their offspring can help establish prevention and treatment protocols. The protective effects of baru on brain tissue are consistent and histological analysis shows that supplementation is able to promote the preservation of cell bodies in the cortex of adolescent and adult offspring. Despite these results, future translational research with pregnant women and offspring is encouraged. It is important to emphasize that according to Nair and Jacob (2016)[93], the dose of oil and baru almond used in the rodents of our experiment (2.000 mg/kg of weight) is equivalent to 340 mg/kg in humans.

CONCLUSION

Maternal supplementation with baru almonds during pregnancy and lactation is beneficial for the offspring's neural system development, protecting it against oxidative damage and reducing anxiety-like behaviors.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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5 CONSIDERAÇÕES FINAIS

A suplementação materna com óleo e amêndoa de baru mostrou potencial efeito para a modulação da microbiota fecal da prole, promovendo a abundância relativa de táxons produtores de compostos metabólicos neuroprotetores. Associado a estes efeitos, o aumento do conteúdo de ácidos graxos poliinsaturados no tecido cerebral da prole experimental, esteve correlacionada a um melhor desempenho destes animais frente aos testes de aprendizagem e memória, redução de estresse oxidativo no tecido cerebral, redução do comportamento tipo ansioso, bem como, maior preservação dos corpos celulares no hipocampo e córtex.

Os animais cujo as mães foram suplementadas com a amêndoa de baru apresentaram resultados mais pronunciados quando comparados a prole óleo, principalmente na fase adulta. Correlacionamos este resultado ao maior conteúdo e atividade antioxidante do baru, que foi capaz de proteger o tecido cerebral contra danos deletérios oriundos da ação de radicais livres e compostos pró-inflamatórios.

Diante destes resultados, sugerimos que a amendoa e o óleo de baru é uma alternativa interessante para garantir o suprimento adequado de PUFAs e antioxidantes durante a gestação e lactação, e assim, garantir o neurodesenvolvimento adequado. Porém, pesquisas translacionais devem ser realizadas avaliando a dose e tempo de administração utilizados em nossa pesquisa com seres humanos.

APÊNDICES

APÊNDICE A: Material suplementar artigo II: Metagenoma *amplicon* rDNA 16S Taxonomia

Análise de diversidade

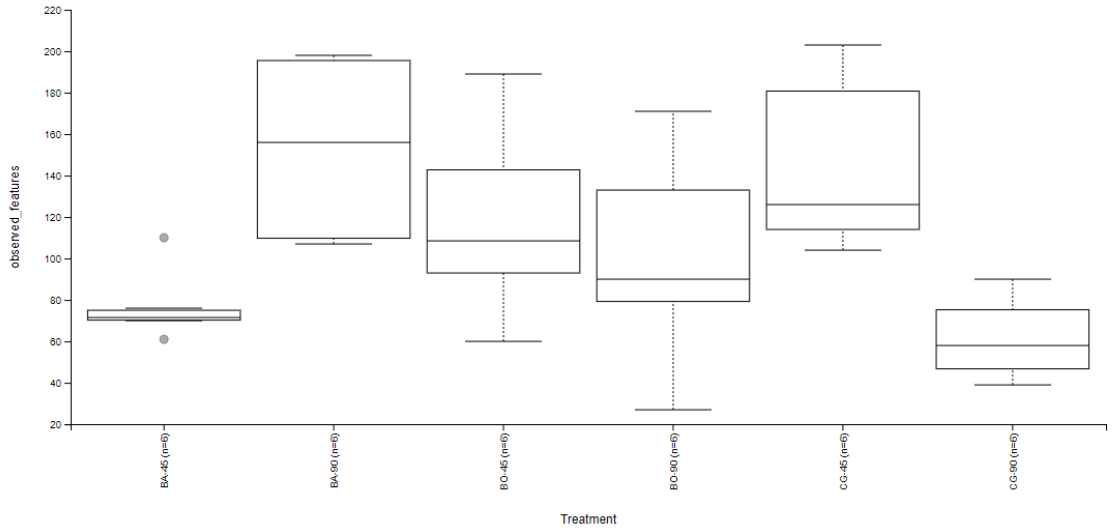


Figura 1 – Análise de diversidade alfa. O gráfico apresenta a diversidade alfa dos metagenomas em todos os tratamentos por meio do índice de Observed-features. O tratamento BA-45 apresentou diferença significativa com o controle CG-45, p-*value*= 0.006485. Assim como BA-90 comparado ao CG-90, *p-value* de 0.0038.

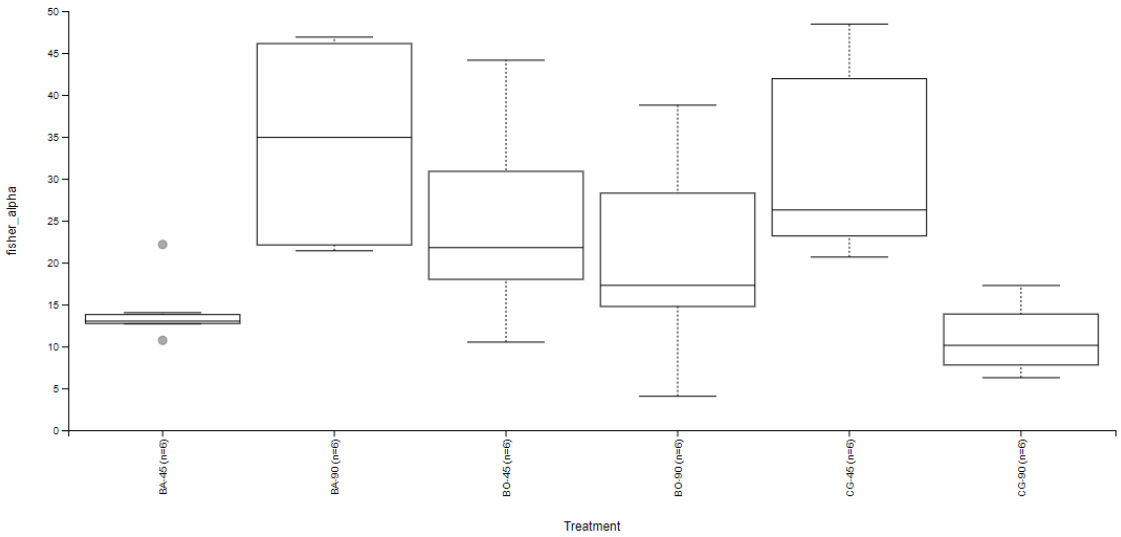


Figura 2 - Análise de diversidade alfa. O gráfico apresenta a diversidade alfa dos metagenomas em todos os tratamentos por meio do índice de Fisher. O tratamento BA-45 apresentou diferença significativa com o controle CG-45, p-*value*= 0.0064. Assim como BA-90 comparativamente ao CG-90, p-*value* de 0.0038.

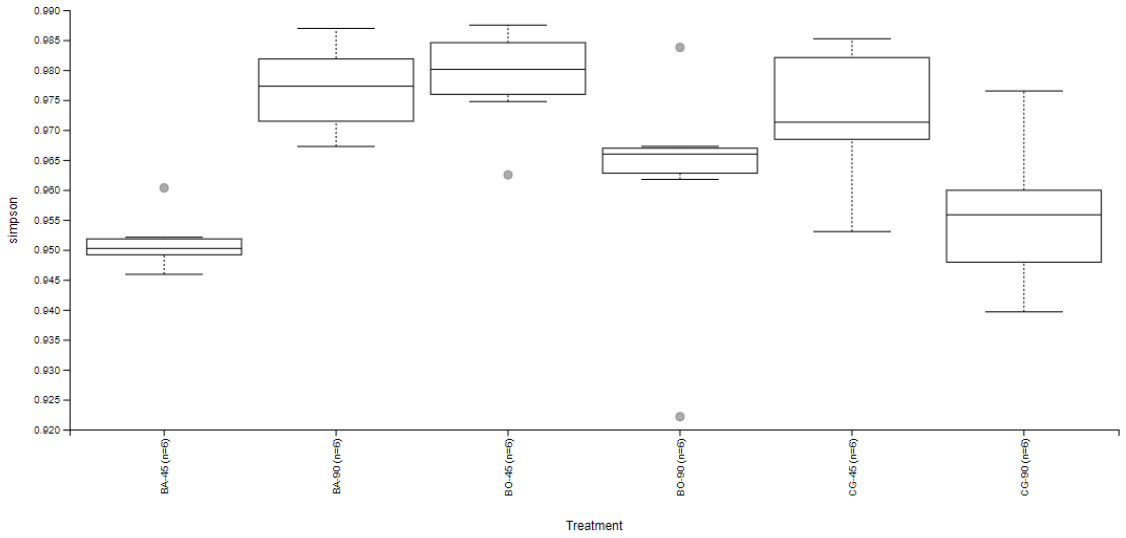


Figura 3 - Análise de diversidade alfa. O gráfico apresenta a diversidade alfa dos metagenomas em todos os tratamentos por meio do índice Simpson. O tratamento BA-45 apresentou diferença, comparativamente ao controle CG-45, *p-value* = 0.0064; assim como o tratamento BA-90, comparado ao controle CG-90, p *value* = 0.01630.

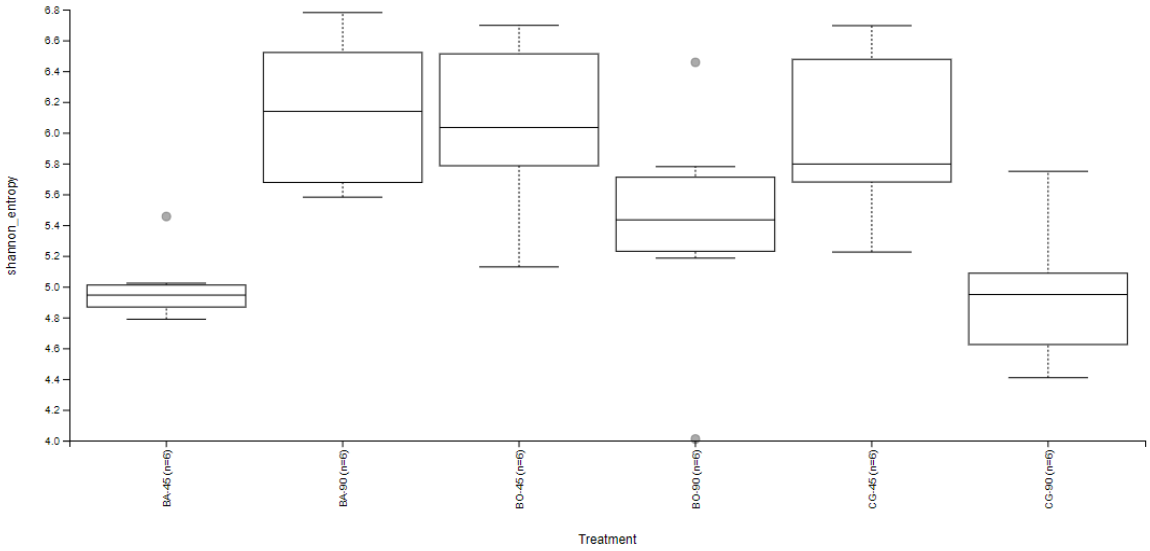


Figura 4 - Análise de diversidade alfa. O gráfico apresenta a diversidade alfa dos metagenomas em todos os tratamentos por meio do índice Shannon. O tratamento BA-45 apresentou diferença, comparativamente ao controle CG-45, p-*value* = 0.00648; assim como o tratamento BA-90, comparado ao controle CG-90, p *value* = 0.006485.

Análise de abundância diferencial

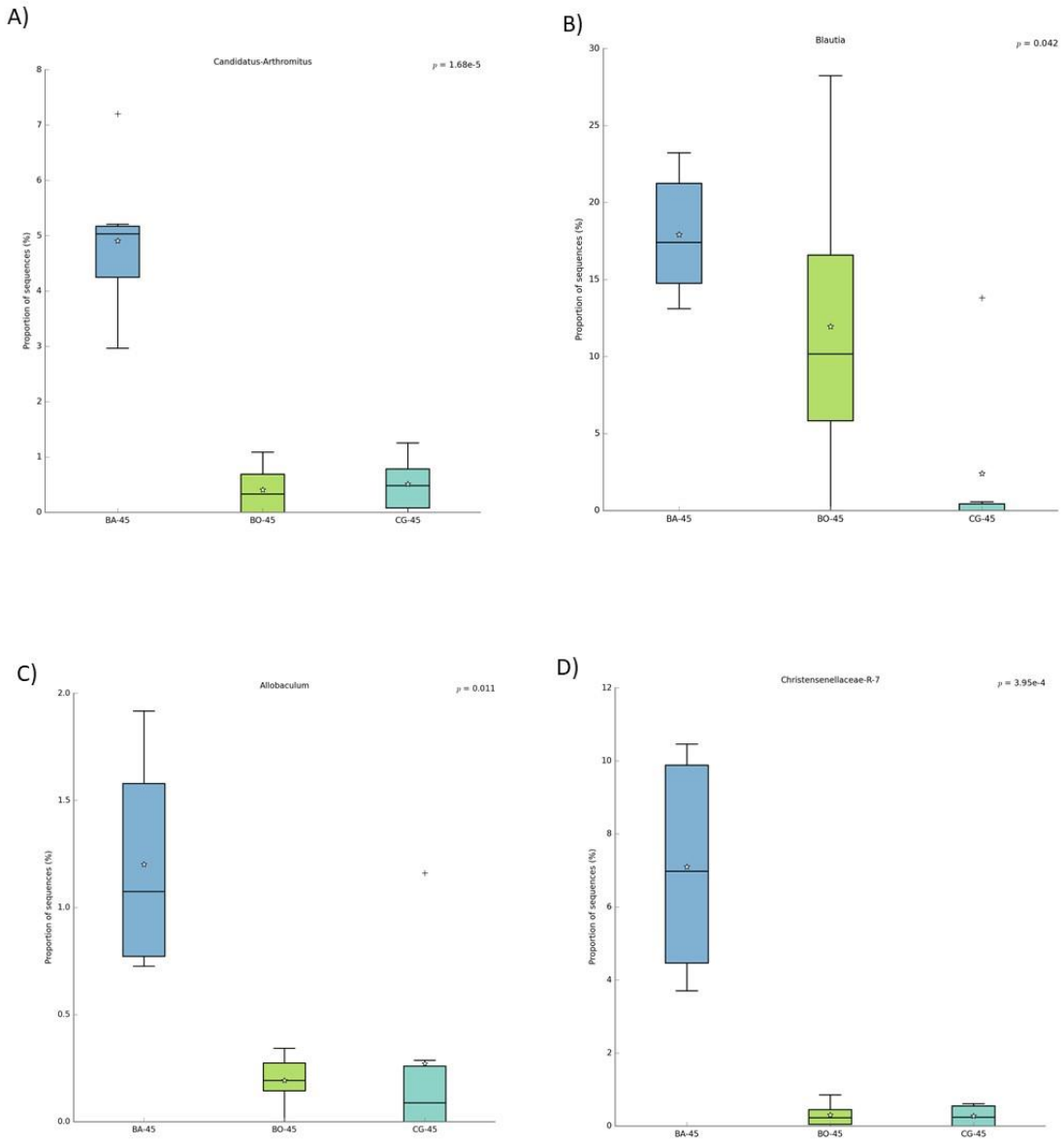


Figura 5 Análise de abundância diferencial

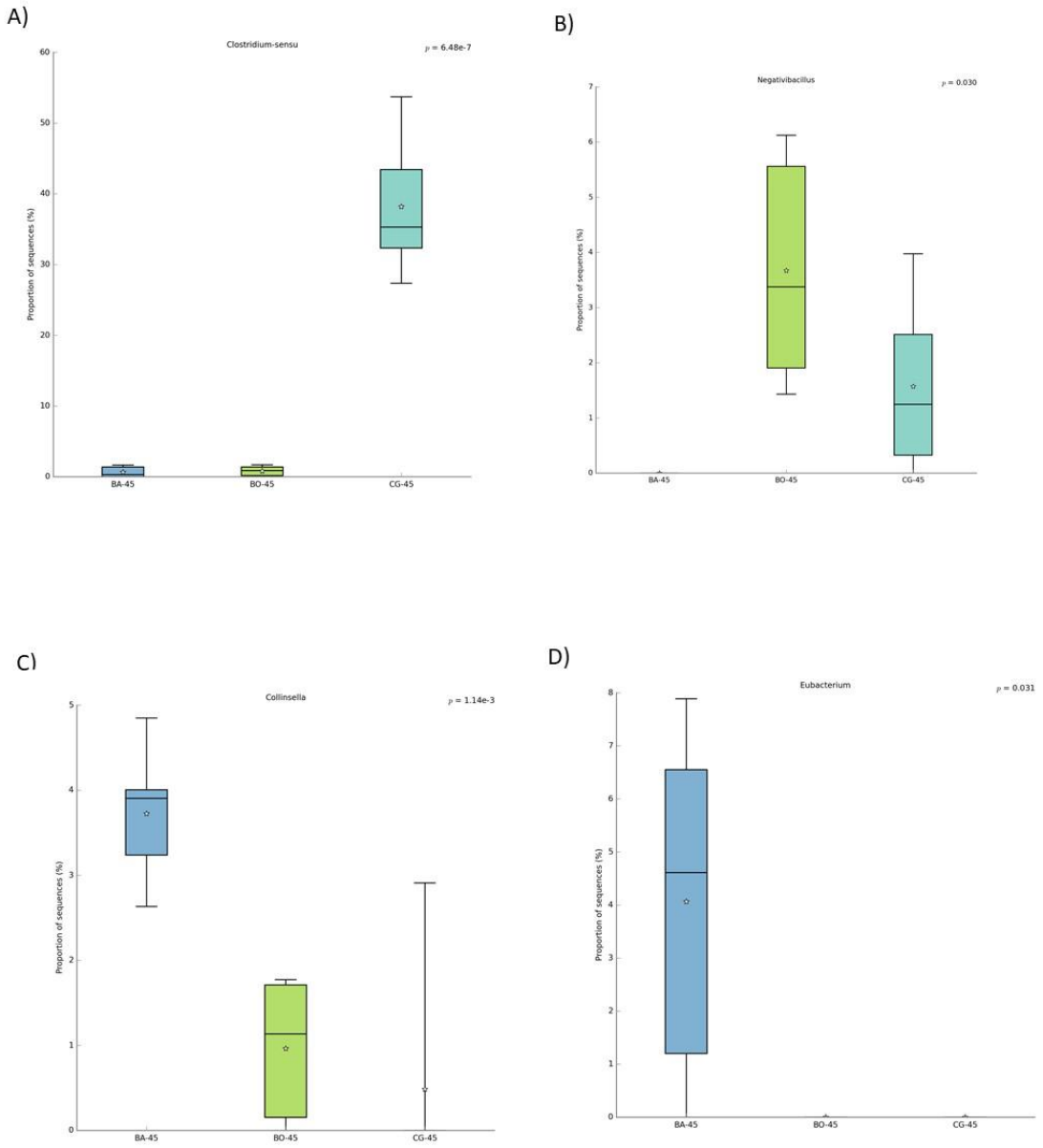


Figura 6 Análise de abundância diferencial

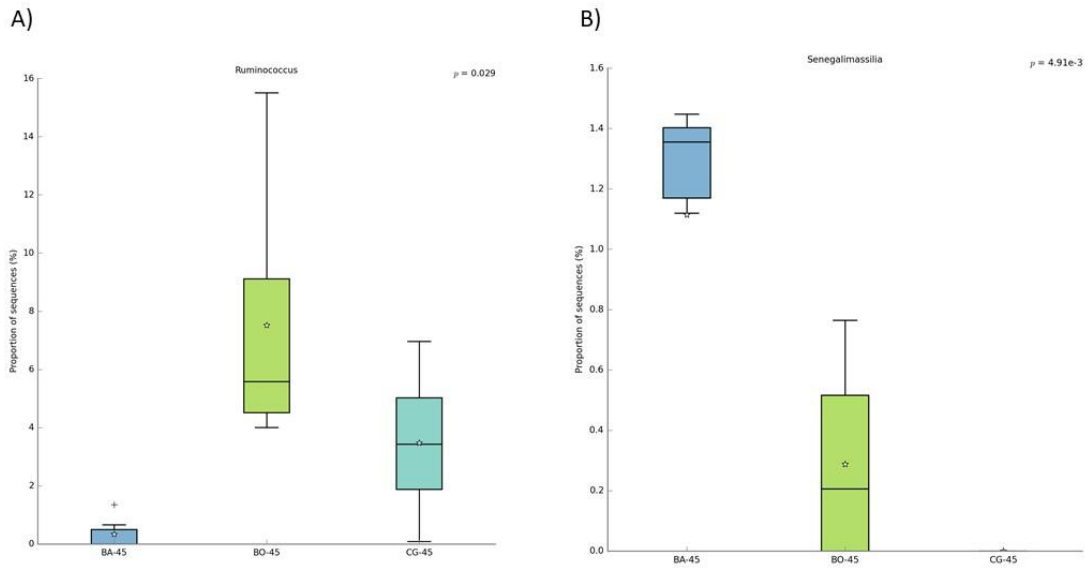


Figura 7 - Análise de abundância diferencial

APÊNDICE B: Material suplementar artigo II: Metagenoma *amplicon* rDNA 16S- predição funcional

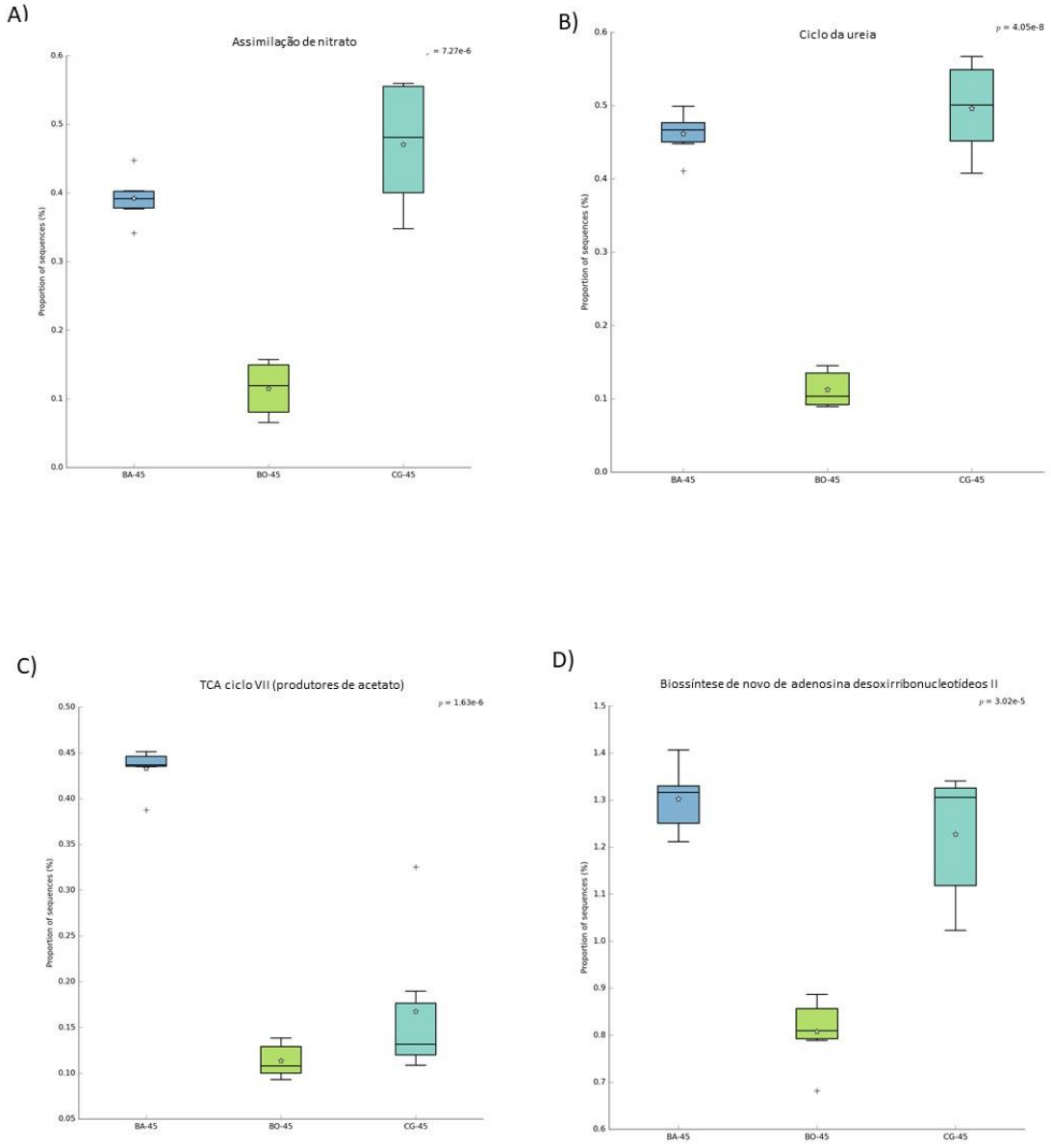


Figura 8- Abundância diferencial

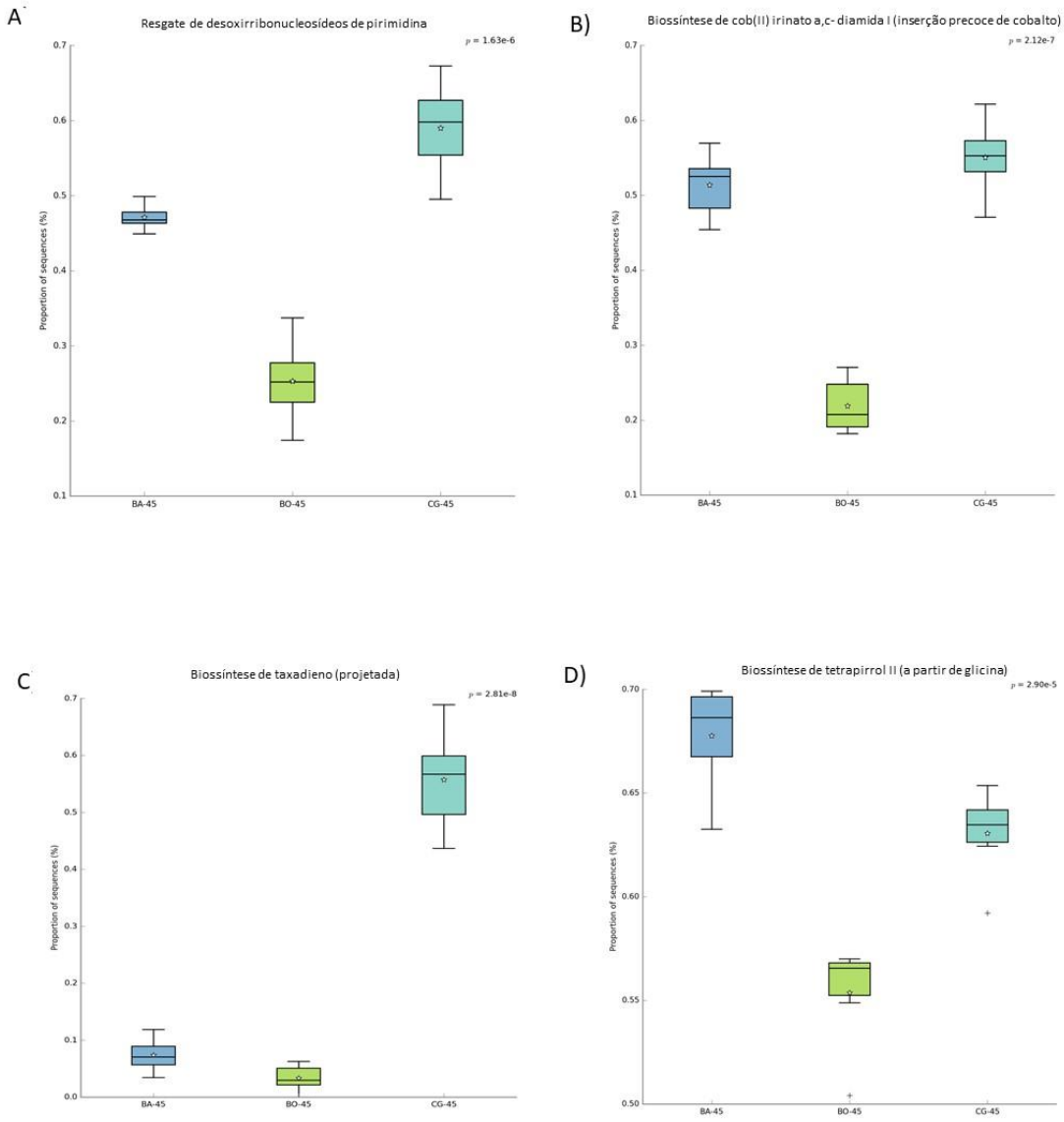


Figura 9 - Abundância diferencial

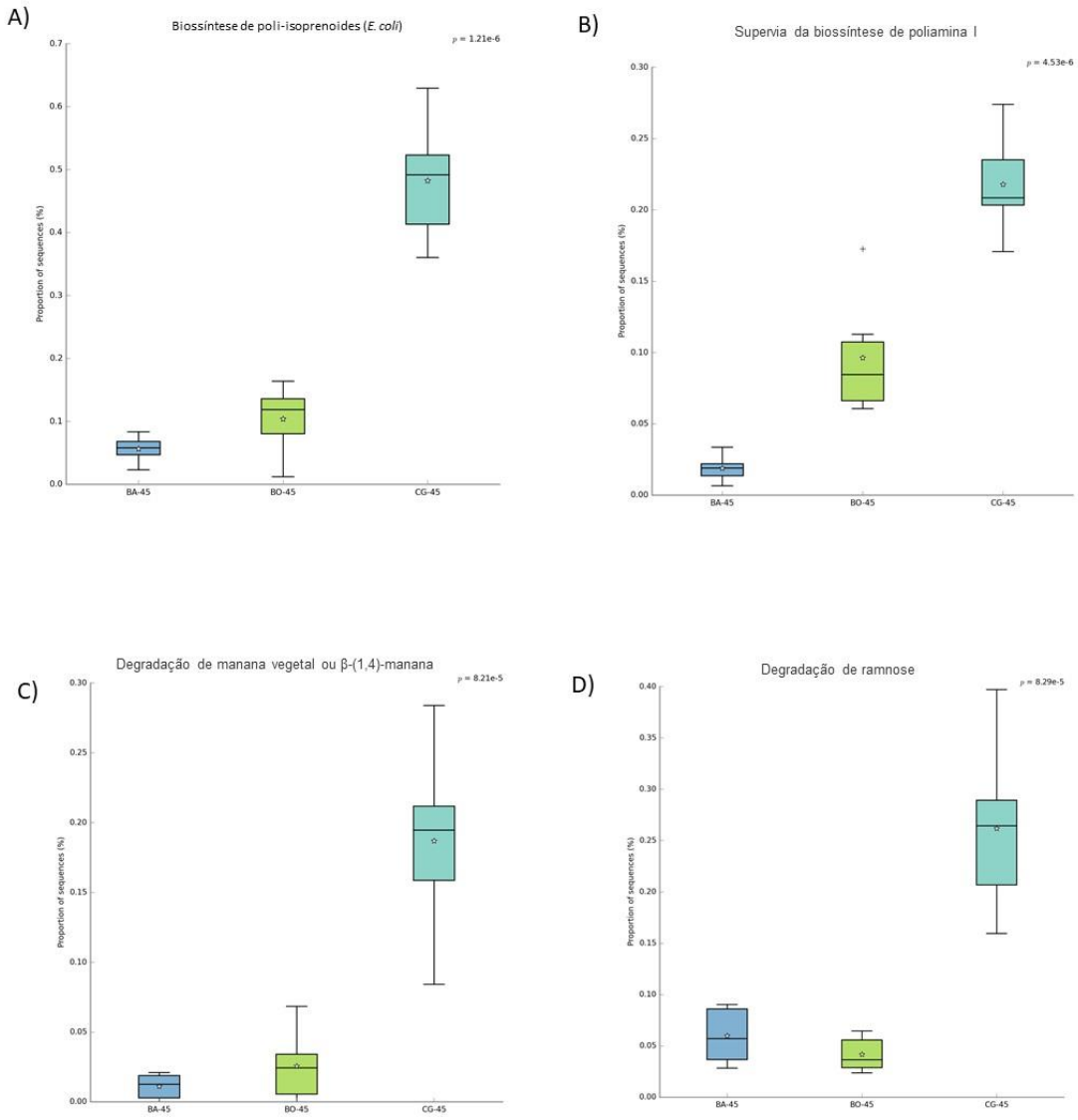


Figura 10 Abundância diferencial

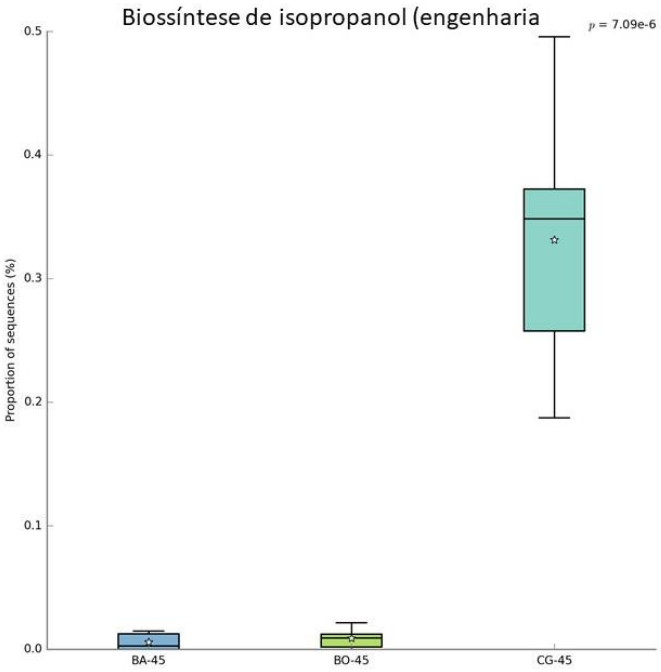
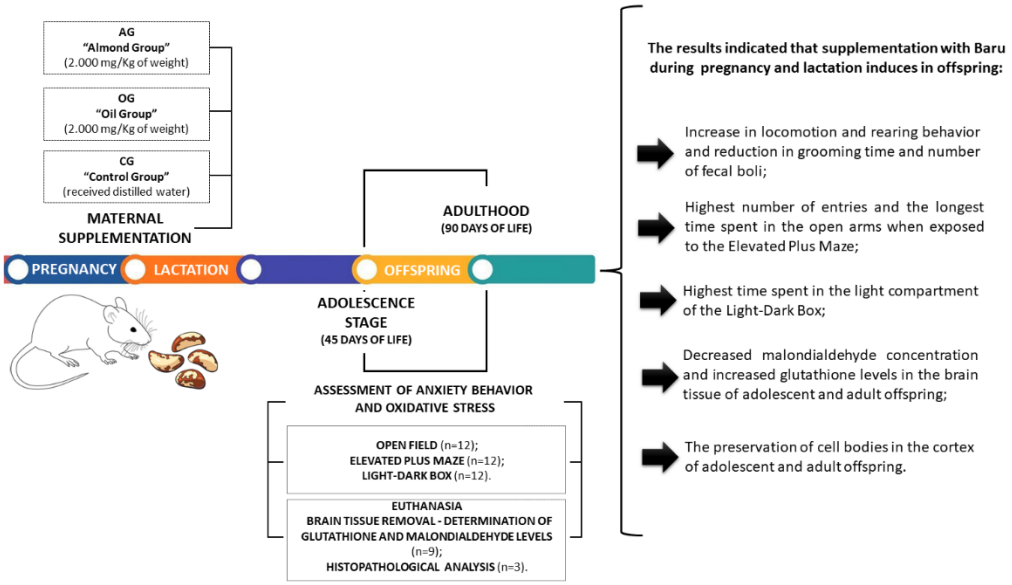
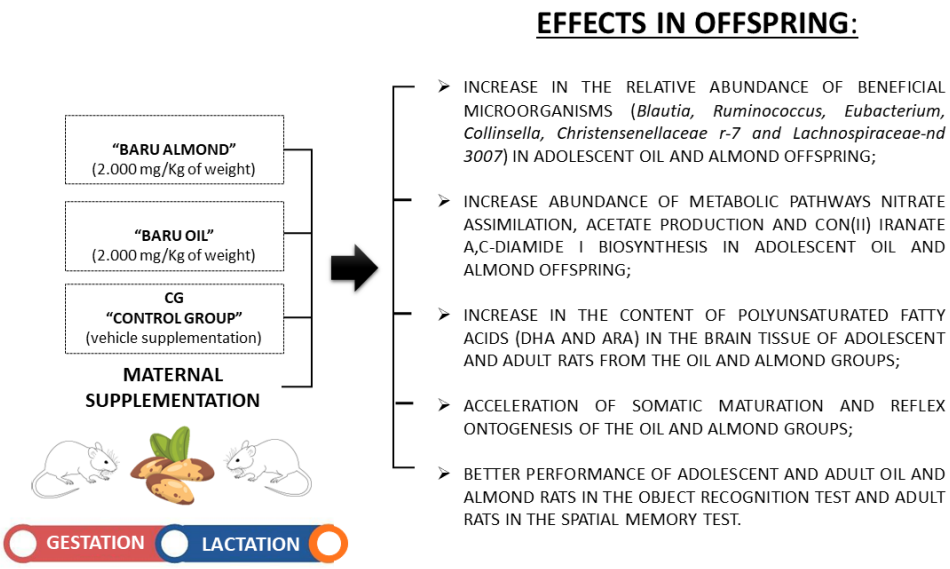


Figura 11- Abundância diferencial

APÊNDICE C: Graphical abstract artigo I



APÊNDICE D: Graphical abstract artigo II



ANEXOS

ANEXO A: Certidão de Aprovação do Comitê de Ética

UNIVERSIDADE FEDERAL DE CAMPINA GRANDE

CENTRO DE SAÚDE E TECNOLOGIA RURAL

Comitê de Ética em Pesquisa

DECLARAÇÃO

Declaro a quem possa interessar que **Profª Drª Juliana Késsia Barbosa Soares,** deu entrada via eletrônica em processo para apreciação de projeto de pesquisa, como coordenador deste *"IMPACTO DO CONSUMO DE BARU (Dipteryx alata Vog.) DURANTE A GESTAÇÃO E LACTAÇÃO SOBRE O DESENVOLVIMENTO FÍSICO, BIOQUÍMICO E COMPORTAMENTAL DE RATOS".* O referido projeto tem **Nº de protocolo CEP 104.2017.**

Patos, 11 de dezembro de 2017.

Atenciosamente.

Rosália Severo de Medeiros

Doutoranda em Qualidade Alimentar/FCT/UNL

Rosália Severo de Medeiros

Coordenadora do CEUA/CSTR/UFCG

ANEXO B: Certidão de Tradução do Artigo II.

Declaration of Document Editing - Corrections

David Peter Harding - U.S.A. - Native American Citizen

American English

Born in California, USA, **E-mail** kauaii@yahoo.com **Tel**: 055-083-99332-1042; **Skype**: kauaiilaura

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PUBLICATIONS AS AUTHOR - Royal Society of Chemistry - Analytical Methods; Great Britain, Trends in Analytical Chemistry - U.S.A., A.C.T.A., (The Netherlands), Talanta (Oxford).

Lattes: http://lattes.cnpq.br/6542007229560011 - **ORCID:** https://orcid.org/0000-0003-3604-6142

Research Gate: https://www.researchgate.net/scientific-contributions/59069231_David_P_Harding

DOCUMENT TITLE:

Maternal consumption of baru almond (*Dipteryx alata* Vog.) reduces anxiety-like behavior and oxidative stress in the brain of rat offspring in different stages of life

Date: 02 / 08 / 2022
Month / Day / Year

ANEXO C: Certidão de Submissão do Artigo II.

Journal of Ethnopharmacology

Maternal consumption of baru almond (Dipteryx alata Vog.) induces anxiolytic-like behavior, reduces oxidative stress in the brain and preserves cell bodies in the offspring's cortex at different stages of life

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Keywords:	PUFAs; Antioxidant compounds; Malondialdehyde; Anxiety; Pregnancy and lactation
Corresponding Author:	Larissa Dutra BRAZIL
First Author:	Diego Elias
Order of Authors:	Diego Elias
	Rita de Cássia Bidô
	Maciel Costa
	Marília Melo
	Ana Carolina Costa
	Larissa Dutra
	Vanessa Viera
	Daline Araújo
	Gerlane Guerra
	Juliana Soares
Abstract:	The present study was to evaluate the impact of maternal consumption of oil and baru almond on anxiety behavior and oxidative stress in the brain of the offspring treated during pregnancy and lactation. The mothers were divided into three groups and treated by gavage (n = 8 mothers/group): Control- received distilled water; Oil - treated with 2,000 mg of baru oil/kg of animal weight and Almonds - treated with 2,000 mg of baru almond/kg of animal weight, and when the offspring (n = 12 pups/group) reached 45 (adolescence -T45), and 90 days of life (adult - T90), they were submitted to behavioral tests using the Open Field (OF), Elevated Plus Maze (EPM) and Light-Dark Box (LDB), in addition to cortex histology and parameters of oxidative stress in the brain. One-way ANOVA was used for data analysis, considering $p < 0.05$. The animals in the oil and almond groups showed greater ambulation and rearing and reduced grooming and fecal buns behavior. In the LCE, they presented a greater number of entries and time spent in the open arms compared to the CG ($p < 0.05$), they translocated and walked more in the clear area of the LDB, in addition, they presented a higher concentration of glutathione and lower MDA in the brain tissue ($p < 0.05$). Maternal supplementation with oil and baru almonds induces anxiolytic-like behavior, reduces oxidative stress in the brain, and preserves cell bodies in the cortex of adolescent and adult offspring.
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	Rubem Guedes rguedes@ufpe.br