an idea health journal
ISSN (online): 2797-0604

Volume 6 No 4, Page 760-773
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Effect of Docosahexaenoic Acid Supplementation on Spatial Working Memory and Cerebral Myelin Expression in Malnourished Wistar Rats

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Article Info:
Received: 22 May, 2026
Revised: 30 August, 2026
Accepted: 31 August, 2026
Available online: 16 Sept 2026

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How to Cite:
Widodo T & Susanti N. Effect of Docosahexaenoic Acid Supplementation on Spatial Working Memory and Cerebral Myelin Expression in Malnourished Wistar Rats. An Idea Health Journal. 2026; 6(04): 760-773. https://doi.org/10.53690/ihj.v6i04.834

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Abstract

Background: Malnutrition in early life can disrupt central nervous system development, including myelination and cognitive function. Docosahexaenoic acid (DHA), a long-chain omega-3 fatty acid, plays an important role in neuronal membrane integrity, neural development, and cognitive function. This study aimed to analyze the effects of different DHA doses on cerebral myelin profiles and spatial memory in malnourished Wistar rats.

Methods: This true experimental study involved 30 Wistar rats (Rattus norvegicus) randomly allocated into five groups (n=6): normal control (K−), malnourished control (K+), and malnourished groups receiving DHA at 10.8 mg/day (P1), 16.2 mg/day (P2), or 27 mg/day (P3) for 12 days. Spatial memory was assessed using the Y-maze test, and cerebral myelin profiles were examined using immunohistochemistry. Data were analyzed using One-Way ANOVA followed by appropriate post hoc tests.

Result: Body weight increased in all groups. Cerebral myelin profiles differed significantly between groups ($p=0.037$; $\eta p^2=0.325$), with a trend toward higher profiles at higher DHA doses. Spatial memory also differed significantly ($p=0.001$; $\eta p^2=0.499$). Games–Howell analysis showed lower spatial memory in malnourished controls than in normal controls ($p=0.002$), P2 ($p=0.024$), and P3 ($p<0.001$), whereas P1 did not differ significantly from malnourished controls.

Conclusion: DHA supplementation affected cerebral myelin profiles and spatial memory in malnourished Wistar rats, with more consistent spatial memory effects at 16.2 and 27 mg/day.

Keywords: Docosahexaenoic Acid, Malnutrition, Spatial Memory; Myelin; Y-Maze.

BACKGROUND

Stunting remains a major nutritional problem that impacts the quality of children's growth and development, particularly in early life. Stunting is a specific anthropometric indicator in children that describes impaired linear growth, characterized by length or height-for-age being below established growth standards. This condition stems from complex biological and environmental processes, including long-term exposure to nutritional deficiencies, recurrent infections, health conditions, and social and environmental factors. The impact of childhood growth disorders is not limited to physical growth; it can also affect nervous system development and cognitive function. Children with stunted linear growth are at risk of impaired brain development, delayed motor development, decreased learning ability, and impaired cognitive function, which can lead to health problems (1-3). consequences into later life. The first 1,000 days of life are a critical phase of brain development marked by neural network formation, synaptogenesis, myelination, and the maturation of interneuron connectivity. Disruptions in nutritional adequacy during this period can impact brain development and potentially lead to long-term changes in a child's structure, function, and neurocognitive development (4-6).

The terms malnutrition, growth restriction, chronic undernutrition, and stunted linear growth in childhood are distinct concepts and should not be used interchangeably. Malnutrition is a general term that encompasses deficiencies, excesses, or imbalances in energy and nutrient intake (1, 2). Malnutrition does not specifically refer to nutritional deficiencies or growth disorders. Malnutrition occurs when nutrient intake or availability is insufficient to meet the body's physiological needs. If this condition persists over a long period, it can be referred to as chronic malnutrition. It can potentially affect the growth and development of various organs, including the nervous system (4, 5).

Growth restriction refers to a condition in which an organism's growth process is hampered or occurs outside its biological growth potential. Growth restriction can result from various factors, including nutritional deficiencies, hormonal disorders, disease, infection, genetic disorders, metabolic disorders, and environmental exposures. Therefore, growth restriction is not synonymous with malnutrition, although nutritional deficiencies can cause or contribute to growth restriction. Conversely, malnutrition does not always result in the same degree of growth impairment because other biological and environmental factors also influence growth responses (1, 2).

Stunted linear growth in childhood, operationally assessed through anthropometric indicators of length or height for age, is a manifestation of growth disorders that has specific definitions and criteria in humans. Stunting cannot be understood simply as a synonym for chronic malnutrition or general growth restriction. Although long-term malnutrition is a significant factor associated with stunting, the condition is also influenced by infection, maternal health, quality of care, the environment, sanitation, and other social determinants.[1,2]Stunting in this study is used in a limited way to describe anthropometric conditions in humans and is not used as a term to describe conditions induced in experimental animals.

Experimental animal studies use models of malnutrition or growth restriction to study the biological consequences of nutritional deficiencies on growth, organ development, and nervous system function. These models are not intended to replicate all clinical, anthropometric, and multifactorial determinants of stunting in humans. Malnutrition-induced conditions in mice cannot be directly equated with stunting in human children because the definitions, measurement indicators, biological course, and environmental context differ. Therefore, this animal model serves as an experimental system to evaluate

the biological mechanisms of malnutrition with translational relevance to understanding growth and developmental disorders (4, 5, 7).

The relevance between animal models of malnutrition and human growth restriction should be understood through the underlying biological mechanisms, rather than through a direct association between diagnoses or conditions. These mechanisms include impaired nutrient availability, altered tissue growth, impaired brain development, altered myelin formation and maintenance, and altered cognitive function. Animal studies can provide information about the biological relationship between nutritional deficiencies and nervous system development. Still, their interpretation should be limited to the effects of interventions on experimentally induced malnutrition or growth restriction. These findings cannot be directly used as evidence that the same interventions are effective in preventing or addressing stunting in human children (4-6).

Docosahexaenoic acid (DHA) is a nutrient that plays a crucial role in nervous system development and function. DHA is a long-chain omega-3 fatty acid and an essential component of nerve cell membranes; it helps maintain membrane fluidity, synaptogenesis, dendrite formation, synaptic plasticity, and neurotransmitter function that supports learning and memory. Adequate DHA availability during development is necessary to support neural tissue formation and maturation (8-10). DHA deficiency can affect neuronal function and interneuronal communication, potentially impairing cognitive function. DHA supplementation may provide neurobiological effects by increasing synaptic plasticity, regulating neuroinflammation, and maintaining neuronal membrane integrity, which may ultimately support learning and memory (7, 11, 12).

DHA is also associated with myelination, a crucial part of nervous system maturation. Myelin is the sheath that surrounds axons and increases the speed and efficiency of nerve impulse transmission. Cognitive development depends not only on the number and activity of neurons, but also on myelin integrity and neural network connectivity. Myelin formation involves the proliferation, differentiation, and maturation of oligodendrocytes and requires lipids and fatty acids for myelin membrane formation. Nutritional deficiencies during development can impact white matter formation and neural network integrity (4, 9, 13). DHA is reported to play a role in the process of nerve cell maturation and the development of myelin tissue, while essential fatty acid deficiency can disrupt the formation and maintenance of myelin structure and nerve connectivity (7, 12).

Spatial memory is a cognitive function that can be used to evaluate changes in brain function due to nutritional deficiencies during development. Spatial memory is the ability to acquire, store, and recall information about the location and spatial relationships of objects or the environment. This function is closely related to hippocampal integrity and the neural networks that support learning and memory processes (14, 15). Nutritional deficiencies during development have the potential to affect the structure and function of the hippocampus, leading to changes in spatial memory abilities. Experimental research in animals allows evaluation of spatial memory function using the Y-maze test and its spontaneous alternation parameter. This parameter describes the animal's tendency to sequentially explore different arms of the Y-maze and can indicate spatial working memory function (16, 17).

A research gap remains: few experimental studies simultaneously evaluate the effects of DHA administration on functional and structural aspects of the nervous system in experimentally induced malnutrition. Previous studies have primarily evaluated DHA's benefits on neurological development, synaptic plasticity, myelination, or cognitive function separately (8, 18, 19). Evidence regarding the relationship between DHA administration at different doses and spatial memory as an indicator of cognitive function and changes in myelin markers as an indicator of neural network development or integrity in malnutrition models still requires strengthening (12, 20).

A research approach that integrates behavioral neuroscience assessment through spatial memory evaluation with immunohistochemical examination of myelin markers is needed to obtain a more comprehensive picture of the neurobiological effects of DHA in malnutrition conditions (10, 12, 14-

16). Simultaneous evaluation of functional and structural outcomes is expected to provide a better understanding of the relationship between DHA supplementation, cognitive function, and neural network changes under experimental nutritional deficiencies (10, 12).

This study used Wistar rats (Rattus norvegicus) as an experimental model of malnutrition, supplemented with DHA at different doses. This model represents experimentally induced malnutrition and is not intended as a model for stunting in humans. Spatial memory was evaluated using the Y-maze test using spontaneous alternation parameters, while changes in myelin markers in cerebral tissue were observed using immunohistochemistry. This approach allows simultaneous evaluation of DHA's effects on both functional and structural aspects of the nervous system.

This study aims to analyze the effect of docosahexaenoic acid (DHA) administration on spatial memory and myelin marker expression in the brains of experimentally malnourished Wistar rats (Rattus norvegicus). This study is expected to provide experimental evidence on DHA's potential to support cognitive function and nervous system development during malnutrition. The research findings are also expected to form the basis for further research on the neurobiological mechanisms of nutritional interventions and the development of translational research relevant to growth disorders and neurological development. This study's results are not intended to directly conclude the effectiveness of DHA in preventing or treating stunting in humans, but rather to provide mechanistic evidence from an experimental model of the nervous system response to DHA supplementation in malnutrition.

METHODS

Study Design

This study used a true experimental design with a post-test-only control group design to evaluate spatial memory and myelin profile, and a repeated-measures design to evaluate changes in body weight before and after the intervention.

Participants /Sample

The experimental animals were 30 male Wistar rat pups (Rattus norvegicus) from six healthy Wistar females, obtained from the experimental animal maintenance unit of the Faculty of Veterinary Medicine, Airlangga University. Males were selected to reduce sex-based biological variation associated with hormonal changes. Animal health status was assessed based on physical and behavioral examinations, including activity, ability to suckle or eat, fur and skin condition, mobility, and the absence of anatomical abnormalities or clinical signs of disease. Inclusion criteria included live-born male Wistar rats that were active, clinically healthy, had no congenital abnormalities, and met the nutritional status criteria according to the study group. Animals were excluded if they developed congenital abnormalities, disease, or infection during the study, did not meet the malnutrition model criteria, experienced severe clinical conditions meeting the humane endpoint, or died before the study was completed. A total of 30 animals met the criteria and completed the entire study series. No deaths or dropouts occurred, so all animals were analyzed, with a final sample of six per group for body weight, spatial memory, and myelin profile analyses. To reduce the possibility of a litter effect, rat pups from the same mother were distributed across multiple research groups so that not all pups from one mother were placed in the same group.

Data Collection

The study was conducted in 2024 at the Experimental Animal Cage and Pathology Laboratory, Faculty of Veterinary Medicine, Airlangga University. Here procedure step by step :

Acclimatization and Induction of Malnutrition

Mother rats underwent a seven-day acclimatization period prior to the experimental procedures. During acclimatization, animals were maintained under controlled laboratory conditions at 22–25°C, 50–70% relative humidity, and a 12:12-hour light-dark cycle, with ad libitum access to drinking water. Following parturition, malnutrition was induced in the pups via maternal nutritional restriction during lactation. Mothers in the malnourished group received 50% of the average feed intake of the normal control group, while mothers in the normal control group were provided a standard diet ad libitum. The standard diet contained 18–20% protein, 4–5% fat, and 4–5% fiber, with a metabolic energy content of 2,900–3,100 kcal/kg. All groups had unrestricted access to water. We implemented nutritional restriction from postnatal day (PND) 1 to PND 12. We recorded feed provided and residual feed daily to estimate actual consumption. We monitored the general condition and growth of the rat pups throughout the induction period. On PND 12, successful induction of the malnutrition model was confirmed by growth retardation, as indicated by lower body weight compared to the normal control group. Biochemical biomarker testing for malnutrition status was not performed in this study.

Randomization and Study Groups

On PND 12, animals meeting the study criteria were randomly assigned using a computer-generated random number sequence into five groups of six rats each: K− (normal control group without malnutrition induction or DHA supplementation), K+ (malnutrition group without DHA supplementation), P1 (malnutrition group receiving 10.8 mg/day DHA), P2 (malnutrition group receiving 16.2 mg/day DHA), and P3 (malnutrition group receiving 27 mg/day DHA). To reduce clustering by maternal origin, we distributed pups from the same mother across groups. We measured initial body weight on PND 12 before the intervention.

DHA Administration

The docosahexaenoic acid (DHA) intervention began on PND 13 and was administered once daily for 12 consecutive days until PND 24. DHA was administered orally by gavage at 10.8 mg/day in P1, 16.2 mg/day in P2, and 27 mg/day in P3. The DHA dose was determined based on the dose used in the preliminary study that served as the basis for this study. DHA was administered using the same vehicle, with the administration volume adjusted to maintain comparable administration procedures between groups. The K+ group received a vehicle without DHA, using the same procedures and volume. We carried out the intervention at roughly the same time each day to minimize circadian variation. We measured final body weight on PND 24 after the intervention period. Chronologically, the research series included seven days of maternal acclimatization, offspring birth, malnutrition induction on PND 1–12, monitoring of feed consumption and growth, model confirmation and randomization on PND 12, initial body weight measurement, DHA administration on PND 13–24, final body weight measurement on PND 24, Y-maze test on PND 25, anesthesia and euthanasia, brain removal, tissue fixation and processing, and myelin immunohistochemical examination.

Spatial Memory Examination

Short-term spatial working memory (SRAM) was examined on PND 25 using a Y-maze consisting of three identical arms (A, B, and C) at a 120° angle. Before testing, the animals were acclimated to the testing room conditions. Each rat was then placed in one of the arms and allowed to explore the three arms freely for eight minutes. An arm entry was recorded when all four paws entered an arm. Spontaneous alternation was defined as sequential entry into three different arms without repetition. The percentage of spontaneous alternation was calculated using the formula:

Spontaneous alternation (%) =[actual number of alternations/(total number of arm entries − 2)] × 100.

We conducted all tests under standardized environmental and lighting conditions. After each test, the Y-maze surface was cleaned with 70% ethanol and dried before the next animal used it to minimize the influence of odor traces.

Brain Tissue Collection and Processing

After the Y-maze test was completed, the animals were anesthetized and euthanized according to ethical procedures for animal research. A craniotomy was then performed, and brain tissue was carefully removed. Cerebral tissue was fixed in 10% neutral buffered formalin for 24–48 hours. Samples were then processed through dehydration using graded alcohols, clearing with xylene, paraffin infiltration, and paraffin embedding. Paraffin blocks were cut with a microtome at approximately 4 µm and placed on glass slides for immunohistochemical examination.

Myelin Immunohistochemical Examination

Cerebral myelin profiles were evaluated using immunohistochemistry with Myelin Basic Protein (MBP) as a marker of myelination. Tissue preparations underwent deparaffinization, rehydration, antigen retrieval, blockade of endogenous peroxidase activity, incubation with anti-MBP primary antibody, incubation with secondary antibody, and visualization using 3,3′-diaminobenzidine (DAB) chromogen. Positive MBP expression was indicated by the formation of a brownish color in the immunoreactive area. Preparations were observed using a light microscope at 400× magnification in five fields of view for each preparation. The results were then used to obtain cerebral myelin profile values, which were analyzed quantitatively between study groups

Data Analysis

Numerical data are presented as mean ± standard deviation (SD). We examined data distribution using the Shapiro–Wilk test, and evaluated homogeneity of variance using Levene's test. A p-value < 0.05 was set as the limit of statistical significance. Body weight measured in the same animal at two times, namely before and after the intervention, was analyzed using a mixed-design repeated-measures ANOVA. Measurement time (pre-post) was treated as a within-subject factor, while study group (K−, K+, P1, P2, and P3) was a between-subject factor. We analyzed the main effect of time, the main effect of group, and the time × group interaction to assess differences in body weight change patterns between groups. If a significant effect or interaction was found, we performed further comparative analyses with Bonferroni correction to control for errors due to multiple comparisons. Spatial memory and myelin profile, each measured once at the end of the study, were analyzed using a one-way ANOVA if parametric assumptions were met. If there were significant differences and homogeneity of variance was met, we performed Bonferroni-adjusted post hoc comparisons between groups. If the assumption of homogeneity of variance was not met, we used the Games–Howell post hoc test. Effect sizes in ANOVA analyses were reported using partial eta squared (ηp^2).

Ethical Consideration

All research procedures were carried out after obtaining ethical approval from the Health Research Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University with number 195/UN24.9/LL/2024. Animal care, malnutrition induction, intervention administration, behavioral testing, anesthesia, euthanasia, and tissue collection were carried out with due regard to animal welfare principles and reported referring to the ARRIVE 2.0 guidelines (21).

RESULT AND DISCUSSION

RESULT

Characteristics and Final Sample

A total of 30 Wistar rats (Rattus norvegicus) were included in the study and randomly allocated into five groups: K−, K+, P1, P2, and P3, with six rats in each group. All animals completed the study, and the final analysis included six rats per group.

Body Weight Before and After Intervention

Body weight was measured before and after the intervention. The Shapiro–Wilk test indicated that body weight data were normally distributed at both measurement points ($p>0.05$).

Table 1. Body Weight of Mice Before and After Intervention

Group	n	Before (g), Mean ± SD	After (g), Mean ± SD	Mean difference (g)
K−	6	87.33 ± 6.35	132.33 ± 1.86	+45.00
K+	6	65.33 ± 6.15	123.17 ± 2.23	+57.84
P1	6	67.50 ± 5.39	114.83 ± 4.36	+47.33
P2	6	62.50 ± 4.09	119.17 ± 2.48	+56.67
P3	6	64.33 ± 7.42	125.33 ± 5.39	+61.00

Note: Values are presented as mean ± SD. K−, normal control; K+, malnourished control; P1, DHA 10.8 mg/day; P2, DHA 16.2 mg/day; P3, DHA 27 mg/day. Mean difference = after − before; positive values indicate weight gain. n=6 per group.

Mean body weight increased in all groups after the intervention. The greatest mean increase was observed in P3 (61.00 g), followed by K+ (57.84 g), P2 (56.67 g), P1 (47.33 g), and K− (45.00 g). However, baseline body weight differed across groups; therefore, the intervention effect cannot be interpreted solely from post-intervention values or mean changes. Further inferential analysis using individual-level data, such as mixed-design repeated-measures ANOVA, is required to assess the effects of time, group, and the time × group interaction

Effect of DHA on Cerebral Myelin Profile

Table 2. Cerebral Myelin Profile

Group	n	Mean ± SD	Normality (p)
K−	6	0.883 ± 0.496	0.947
K+	6	0.467 ± 0.250	0.459
P1	6	0.517 ± 0.232	0.801
P2	6	1,000 ± 0.219	0.783
P3	6	1,050 ± 0,579	0.211

Note: Data are presented as mean ± standard deviation (SD). Normality was assessed using the Shapiro–Wilk test. K−, normal control; K+, malnourished control; P1, DHA 10.8 mg/day; P2, DHA 16.2 mg/day; P3, DHA 27 mg/day. n=6 per group.

The mean cerebral myelin profile was 0.883 ± 0.496 in K−, 0.467 ± 0.250 in K+, 0.517 ± 0.232 in P1, 1.000 ± 0.219 in P2, and 1.050 ± 0.579 in P3. The Shapiro–Wilk test showed that the data were normally distributed in all groups ($p>0.05$), and Levene's test confirmed homogeneity of variance ($p=0.240$). One-Way ANOVA demonstrated a significant overall difference in cerebral myelin profiles among the groups ($p=0.037$; $\eta p^2=0.325$). Descriptively, P3 showed the highest mean myelin profile, whereas K+ showed the lowest. Higher mean myelin profiles were observed in P2 and P3 than in the malnourished control group; however, specific between-group differences should be interpreted based on Bonferroni-adjusted post hoc comparisons.

Effect of DHA on Spatial Memory

Spatial memory analysis showed a mean of 0.576 ± 0.031 in K−, 0.460 ± 0.041 in K+, 0.483 ± 0.116 in P1, 0.562 ± 0.050 in P2, and 0.609 ± 0.026 in P3.

Table 3. Spatial Memory Based on Y-Maze

Group	n	Mean ± SD	Normality (p)
K−	6	0.576 ± 0.031	0.203
K+	6	0.460 ± 0.041	0.224
P1	6	0.483 ± 0.116	0.585
P2	6	0.562 ± 0.050	0.773
P3	6	0.609 ± 0.026	0.762

Note: Values are presented as mean ± SD. Normality was assessed using the Shapiro–Wilk test. K−, normal control; K+, malnourished control; P1, DHA 10.8 mg/day; P2, DHA 16.2 mg/day; P3, DHA 27 mg/day. Spatial memory was assessed using the Y-maze test; n=6 per group.

Levene's test indicated that the assumption of homogeneity of variance was violated (p=0.0001). One-Way ANOVA showed a significant overall difference in spatial memory among the groups (p=0.001; ηp^2=0.499). Given the unequal variances, Games–Howell post hoc comparisons were performed to identify specific between-group differences.

Table 4. Games–Howell Post Hoc Spatial Memory

Group comparison	p-value	Interpretation
K− vs K+	0.002	Significant
K+ vs P2	0.024	Significant
K+ vs P3	<0.001	Significant

Note: Games–Howell post hoc comparisons were used due to unequal variances. K−, normal control; K+, malnourished control; P2, DHA 16.2 mg/day; P3, DHA 27 mg/day. Statistical significance was set at p<0.05.

The Games–Howell results showed that the spatial memory of the malnourished group without DHA (K+) was significantly lower than that of the normal control group (K−) in P2 and P3. The P3 group showed the highest mean of 0.609 ± 0.026, while the K+ group showed the lowest mean of 0.460 ± 0.041. These findings indicate that DHA administration at 16.2 mg/day and 27 mg/day is associated with higher spatial memory performance than in the malnourished group without DHA. In contrast, the 10.8 mg/day dose did not show a significant difference based on the reported comparison.

DISCUSSION

Characteristics and Final Sample

The characteristics and size of the final sample are important in assessing the validity of an animal study. This study used an equal number of animals in each group, and all groups were represented in the final analysis. This helps maintain balance in the experimental design and reduces the potential for bias from unequal attrition between groups. In animal studies, differential attrition between groups can affect estimates of intervention effects and reduce the validity of result interpretation. ARRIVE 2.0 (21). Emphasizes the importance of reporting the number of animals allocated, the number analyzed for each outcome, and the reasons for any exclusion or loss of samples. Using Wistar rats in early life is also relevant for evaluating the consequences of malnutrition on nervous system development. The prenatal and early postnatal periods are periods when brain growth, neuronal differentiation, oligodendrocyte development, synapse formation, and myelination are actively occurring. Disruptions in nutrient availability during this period can change not only body growth but also brain structure and function. Previous experimental studies have shown that protein malnutrition during development can

affect various aspects of the nervous system, from neuronal structural organization to physiological and behavioral functions.

Uniform sample sizes across groups are also important because this study compared multiple DHA doses. With a balanced number of animals, patterns of change between groups can be interpreted more proportionally. However, the relatively small sample size still warrants consideration. In animal studies, p-values should not be the sole basis for interpretation; effect size and biological relevance are also important. ARRIVE 2.0 recommends reporting descriptive statistics, effect sizes, and, where possible, confidence intervals so readers can assess the magnitude and precision of the effects.

Another consideration is the possibility of litter effects, especially since the intervention was conducted on rat pups during the early developmental phase. Pups from the same mother are not completely biologically independent due to shared genetics and maternal environment. Distributing pups from one mother across multiple groups and reporting the mother of origin help reduce the risk of pseudoreplication. ARRIVE 2.0 also emphasizes that the experimental unit must be clearly defined, as the unit may be an individual animal, a litter, or a pen. The study sample characteristics provide a sufficient basis for comparing the effects of multiple DHA doses. Interpretation should still consider sample size, litter origin, and animal characteristics as biological factors that can influence responses to malnutrition or DHA supplementation.

Body Weight Before and After Intervention

The difference in growth between the normal and malnourished groups supports the successful establishment of a nutritional disorder model (22, 23). Body weight is a simple but important indicator in experimental malnutrition research because energy and protein restriction during the growth period can be readily reflected in somatic growth. Research data indicate growth inhibition in the model group before intervention, while growth continued throughout the subsequent period. Weight gain during the intervention period should be interpreted with caution. This growth does not automatically indicate a specific effect of DHA because the animals are in an active growth phase. The malnourished group started at a different weight than the normal group. In such conditions, catch-up growth can occur, i.e., accelerated growth after a period of nutrient restriction when nutrient availability improves. Weight gain alone is not sufficient to determine the success of DHA intervention (4, 5).

These findings also confirm that recovery of somatic growth is not necessarily synonymous with recovery of neurological development. The nervous system has a critical developmental period, and several processes, including myelination, are highly sensitive to early-life nutritional deprivation. Previous experimental research shows that severe malnutrition during lactation can disrupt myelination, and some studies in protein malnutrition models also suggest that the consequences of early nutrition may involve more complex changes in brain development and maturation than weight constraints alone. This matters in this study because the effects of DHA on the nervous system are best assessed through direct neurological outcomes. In contrast, weight is better used as a supporting indicator of growth conditions (24, 25). From a statistical perspective, differences in baseline conditions also limit the ability to analyze body weight adequately by comparing final means. Because measurements were taken on the same animals before and after the intervention, changes in body weight must account for both intra-individual relationships and baseline conditions. A repeated-measures or mixed-model approach is more appropriate to determine whether patterns of change over time differ between groups. Interpretation should not preclude concluding that the group with the greatest weight gain automatically had the best response to the intervention. Biologically, the body weight findings in this study support a model of malnutrition and growth during the recovery period rather than proving a direct effect of DHA on growth. This also strengthens the rationale for using myelin profiles and spatial memory as primary outcomes to evaluate DHA's neurological effects.

Effect of DHA on Cerebral Myelin Profile

The findings on myelin profiles suggest that malnutrition during development may impair myelination, while DHA supplementation showed a stronger trend toward recovery at higher doses. The ANOVA results support an overall difference between groups, although interpretation of paired group findings should be based on post hoc analysis corrected for multiple comparisons. Biologically, the association between malnutrition and myelin disruption may be explained by the high nutritional requirements during brain development. Myelin formation requires oligodendrocyte proliferation and maturation, as well as significant membrane synthesis. Protein and energy deficiencies during this period may impair the availability of substrates for myelin formation. Previous experimental studies have shown that malnutrition can decrease myelin deposition in the rat brain. Other studies in postnatal protein deficiency models have found decreased myelin content and lipid components, including cholesterol, galactolipids, and phospholipids. More recent findings also show that protein malnutrition during development can disrupt oligodendrocyte genesis, differentiation, maturation, and the expression of proteins associated with myelination. Thus, the impaired myelin profile in the malnutrition model in this study has a strong biological basis and is consistent with previous research (26, 27).

DHA's potential to improve these conditions can be explained by its role as a key structural component of nervous tissue. DHA is a long-chain omega-3 fatty acid that accumulates in brain tissue and is a crucial component of neuronal membrane phospholipids (12, 28). DHA supports nerve cell growth and differentiation, membrane organization, and neuronal function. Basak et al. also emphasize that DHA is a crucial component for the structural and functional development of the brain during early development. The relationship between DHA and myelin extends beyond its availability as a lipid component. The fatty acid composition of membranes can influence the characteristics of neurons, oligodendrocytes, astrocytes, and subcellular structures, including myelin (8). Omega-3 fatty acid deficiency has been reported to alter the membrane composition of neural tissue and contribute to changes in neurological function. This provides a basis for explaining why DHA supplementation in malnutrition can create a metabolic environment more conducive to myelin maintenance and formation. A more pronounced response pattern at higher DHA doses may indicate that DHA's biological effects require a certain level of availability. However, this pattern is not sufficient to prove a linear dose-response relationship. The lack of clear changes at the lowest dose may reflect the dose not reaching biologically effective levels, the relatively short intervention duration, or individual variation in tissue response.

Effect of DHA on Spatial Memory

The effect of DHA on spatial memory was the most consistent finding in this study. Malnutrition models showed impaired memory function, while medium and high doses of DHA resulted in better performance compared to the malnutrition condition without supplementation. Unlike the myelin profile, these results were supported by the Games–Howell post hoc test, which is consistent with the non-homogeneity of variance condition. Memory impairments due to early-life malnutrition have long been observed in experimental studies. The hippocampus is one structure vulnerable to nutritional deficiencies during development and plays a crucial role in learning and spatial memory. Research by Jordan et al. showed that early-life malnutrition can lead to deficits in spatial memory performance in mice (14, 15). Other studies have shown that early nutritional deficiencies can impair the development of spatial learning abilities and short-term memory capacity, even when animals are tested later in development. Several interrelated mechanisms may explain DHA's ability to improve memory performance. DHA supports neuronal membrane integrity, synaptic transmission, neurogenesis, membrane receptor function, synaptic plasticity, myelination, and modulation of neuroinflammation. These functions are crucial for forming and storing information, particularly in the highly plastic hippocampal tissue (17, 29).

Experimental evidence also supports a direct link between DHA supplementation and spatial memory function. In rats, DHA administration has been reported to improve performance on a radial maze task and is associated with increased Fos expression in the CA1 area of the hippocampus. These findings are relevant to this study because they suggest that DHA's effects on spatial behavior may be related to changes in neuronal activity in brain regions involved in memory processing. DHA is also a crucial component of neural network development and function, and low DHA levels have been linked to impaired cognitive performance and behavior (10, 11). Its roles include neurogenesis, neurotransmission, and protection against oxidative stress. In malnutrition, DHA supplementation likely goes beyond replacing deficient lipid components and may help create membrane and synaptic conditions that better support the recovery of neuronal function (7, 12).

The more pronounced response patterns at the medium and high doses compared to the low dose are intriguing. These findings could suggest a threshold effect, whereby a certain level of DHA availability is required before functional benefits can be detected through behavioral testing. However, this study was not designed to determine a dose threshold or a formal dose-response relationship. The highest dose should not be called the "optimal dose"; a more appropriate formulation is that doses of 16.2 and 27 mg/day showed more consistent effects on spatial memory under these experimental conditions (15, 30). The relationship between memory outcomes and myelin profiles also provides an interesting biological perspective. The direction of change in both outcomes suggests that improved myelin condition tends to be accompanied by improved memory performance. DHA influences neurological function through multiple pathways, including myelination and synaptic plasticity. This study did not correlate individual myelin levels with Y-maze performance. Therefore, increased myelin cannot be considered a causal mechanism for improved spatial memory (29).

Overall, these findings strengthen the possibility that DHA intervention after nutritional deficiencies may still benefit the developing nervous system, with more consistent evidence for spatial memory outcomes. However, translation of these findings to humans should be approached with caution. Recent systematic reviews and meta-analyses show that DHA has an important biological role in brain development. Still, the additionstudies have not consistently shownbenefits of supplementation for human neforodevelopmental outcomes have not bults are best viewed as preclinical evidence supporting DHA's mechanisms and potential, rather than as a direct basis for determining supplementation dosages in malnourished children.

CONCLUSION

Docosahexaenoic acid (DHA) administration has been shown to affect spatial memory and cerebral myelin profiles in malnourished Wistar rats (Rattus norvegicus). We found significant differences in myelin profiles between groups ($p=0.037$; $\eta p^2=0.325$), with the highest mean in the group receiving 27 mg/day of DHA. However, interpretation of differences between pairs of groups requires post hoc analysis corrected for multiple comparisons. DHA administration also significantly affected spatial memory ($p=0.001$; $\eta p^2=0.499$). The malnourished groups receiving 16.2 mg/day and 27 mg/day DHA showed higher spatial memory performance compared to the malnourished group without DHA. In contrast, the 10.8 mg/day dose did not differ significantly. The 27 mg/day DHA group had the highest mean spatial memory performance.

Body weight increased in all groups throughout the study period, but repeated-measures analyses that account for baseline body weight and the time-by-group interaction are needed to determine DHA's specific effect on weight change. The results suggest that DHA supplementation, particularly at doses of 16.2–27 mg/day, may support improvements in spatial memory function and myelination in malnutrition, with effects on spatial memory being more consistent than on myelin profiles. These

findings support the need for further research with longer intervention durations, larger sample sizes, and validation of malnutrition status and myelination using biochemical and molecular parameters.

ACKNOWLEDGEMENT

The authors thank the Faculty of Veterinary Medicine, Airlangga University, for providing laboratory facilities and support during this research. They also appreciate all laboratory staff, animal cage managers, and others who assisted with the research process, data collection, and manuscript preparation. The authors thank the University of Palangka Raya for its academic support throughout the research.

AUTHOR'S CONTRIBUTION STATEMENT

TW contributed to the research concept, data collection, laboratory experiment implementation, data analysis, and interpretation of the results. IJ contributed to the writing, drafting, editing, and revision of the manuscript. All authors have read and approved the final version of the manuscript before publication.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The authors acknowledge the use of ChatGPT, Scopus AI, and Grammarly to support language improvement, improve clarity, and improve the overall readability and structure of the manuscript.

FUNDING

None.

DATA AVAILABILITY

The dataset generated and analyzed during the current study is available from the corresponding author upon request.

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