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Lima Bean and Patin Fish Flour Improve Brain Function and Systemic Recovery in Malnourished Rats

Rita Maliza[1*], Alimuddin Tofrizal[2], Rahimi Syaidah[3], Bramadi Arya[4,5], Putra Santoso[1], Robby Jannatan[1], Muhammad Hamdi Ibrahim[1], Muhammad Samudra Ilham[1]

[1] Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang 25163, Indonesia,

[2] Department of Anatomical Pathology, Faculty of Medicine, Universitas Andalas, Limau Manis, Padang 25163, Indonesia,

[3] Department of Histology, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia,

[4]Doctoral Program in Biomedical Science, Faculty of Medicine, Universitas Andalas, Padang 25127, Indonesia,

[5]Department of Medical Laboratory Technology, Universitas Syedza Saintika, Padang 25132, Indonesia,

* Corresponding author: ritamaliza@sci.unand.ac.id

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Abstract: Malnutrition during critical developmental periods impairs brain structure, cognition, and oxidative balance. This study investigated the neuroprotective effects of lima bean (*Phaseolus lunatus* L.) and patin fish (*Pangasius* sp.) flour (LBPF) in Wistar rats. Rats were assigned to five groups (P1-P5) receiving low-protein, standard, or LBPF-supplemented diets (40 and 77 g/kg BW) over eight weeks. LBPF treatment significantly improved cortical thickness and reduced neuronal degeneration compared to the malnourished controls (P2). High-dose LBPF (P5) restored cortical thickness (1433 ± 10.63 µm vs. P2: 1191 ± 20.13 µm) and hippocampal neuronal density (85.7 ± 1.06% vs. P2: 15.83 ± 2.36%) while markedly reducing degenerating neurons in cortex (0.9333 ± 0.4372 cells/field vs. P2: 14.30 ± 0.05774) and hippocampus (2 ± 0.94 cells/field vs. P2: 7.4 ± 0.3). Cognitive performance improved, with the discrimination index approaching normal levels (90.33 ± 5.48%), and malondialdehyde (MDA) levels

decreased to near-normal values (8.369 ± 1.08 μM). The combined nutrients in LBPF, including essential amino acids, omega-3 fatty acids, and bioactive compounds, supported neuronal repair, synaptic integrity, and oxidative balance. These findings demonstrate that LBPF effectively mitigates malnutrition-induced brain damage and underscore the importance of early, targeted nutritional interventions for promoting long-term neurocognitive health.

Keywords: Lima Bean Flour, Patin Fish Flour, Malnutrition, Neuroprotection, Cognitive Function, Oxidative Stress.

利马豆与巴丁鱼粉改善营养不良大鼠的脑功能与全身恢复

摘要：
发育关键时期的营养不良会损害大脑结构、认知功能及氧化平衡。本研究探讨了利马豆（*Phaseolus lunatus L.*）和巴丁鱼（*Pangasius* 属）粉混合物（LBPF）对 Wistar 大鼠的神经保护作用。实验将大鼠分为五组（P1－P5），分别给予低蛋白饮食、标准饮食或补充 LBPF 的饮食（40 和 77 g/kg 体重），干预周期为 8 周。与营养不良对照组（P2）相比，LBPF 干预显著增加了大脑皮层厚度并减少了神经元变性。高剂量 LBPF（P5）显著恢复了皮层厚度（1433 ± 10.63 μm，对比 P2：1191 ± 20.13 μm）和海马神经元密度（85.7 ± 1.06%，对比 P2：15.83 ± 2.36%），同时明显降低了皮层（0.9333 ± 0.4372 个细胞/视野，对比 P2：14.30 ± 0.05774）和海马（2 ± 0.94 个细胞/视野，对比 P2：7.4 ± 0.3）中的变性神经元数量。认知功能显著改善，辨别指数接近正常水平（90.33 ± 5.48%），丙二醛（MDA）水平下降至接近正常值（8.369 ± 1.08 μM）。LBPF 中所含的必需氨基酸、ω-3 脂肪酸及多种生物活性成分协同促进神经元修复、突触完整性及氧化平衡。本研究表明，LBPF 能有效缓解营养不良引起的脑损伤，强调了早期、针对性营养干预在促进长期神经认知健康中的重要意义。

关键词：利马豆粉；巴丁鱼粉；营养不良；神经保护；认知功能；氧化应激

1. Introduction

Malnutrition during critical periods of development can have profound and lasting effects on brain health and cognitive function. Inadequate nutrition, particularly in early life, impairs brain development and increases the risk of neurological and psychiatric disorders later in life [1, 2, 3]. Malnutrition disrupts various aspects of brain function, including executive processes, emotion regulation, and overall cognition [4]. Children with a history of malnutrition often present with lower cognitive scores, reduced head circumference, and altered brain structure [5]. These effects involve disturbances in neurotransmitter systems, especially serotonin, and alterations in metabolic pathways such as the kynurenine pathway, which produces neuroactive compounds essential for brain function. Importantly, the consequences of early malnutrition may persist into adulthood, increasing susceptibility to cognitive decline and dementia [6, 7].

Malnutrition and inflammation are closely linked, forming a vicious cycle that worsens overall health and brain function. Malnutrition can induce systemic inflammation, which contributes to neuroinflammation and cognitive decline [8]. Evidence from rodent studies shows that dietary interventions may reduce inflammatory markers and promote brain health [9]. Several alternative protein sources have been investigated for their potential to reverse malnutrition. Edible insects such as *Acheta domesticus* and *Rhynchophorus phoenicis* Fabricius improve body composition and iron levels in malnourished rats [10].

Lima bean (*Phaseolus lunatus*) flour enhances growth, body weight, and cognitive function, while also offering neuroprotective effects [11]. Recent studies further suggest that lima bean powder regulates the pituitary-liver axis and exerts anti-inflammatory activity [12]. Protein supplementation using fish derivatives also shows promise. Wheat flour enriched with fish protein concentrate (FPC) improved weight gain and protein efficiency in rats, with optimal results at 6% supplementation [13]. Fish protein hydrolysate (FPH) from kuniran fish increased albumin levels in preclinical trials [14]. Eel and Tempe Composite (ETC) flour improved nutritional biomarkers [15], while combined eel and soy-based tempe (CEST) flour produced bioactive peptides that enhanced serum protein, hemoglobin, and IGF-1 levels [16].

This study addresses that gap by investigating a nutritional intervention combining lima bean (*Phaseolus lunatus* L.) and patin fish (*Pangasius* sp.) flour (LBPF) in malnourished rats. These two ingredients were selected based on their complementary nutritional profiles and prior evidence supporting their roles in mitigating protein–energy malnutrition. Lima beans are rich in essential amino acids, minerals, and polyphenols that contribute to antioxidant defense, anti-inflammatory activity, and neuronal repair, making them valuable plant-based proteins for neurocognitive recovery [11, 12, 17, 18]. Patin fish provides high-quality animal protein, long-chain omega-3 fatty acids, vitamin B12, and key micronutrients that support membrane integrity, synaptic development, and brain tissue regeneration [13, 14, 15, 16, 19, 20, 21, 22]. Combining these two food sources allows the formulation of a balanced, nutrient-dense intervention capable of addressing both structural and metabolic deficits associated with malnutrition. The complementary nutrient profile of LBPF is therefore expected to enhance brain health, reduce inflammation, and promote systemic recovery. Building on earlier findings from plant-based and fish-derived nutritional interventions, this study aims to determine whether LBPF can reverse structural and functional impairments in the cortex and hippocampus while improving cognitive performance and biochemical markers in malnourished rats. The novelty of this work lies in evaluating a dual-source protein formulation within a single dietary strategy an approach not previously examined in relation to neurocognitive outcomes. By elucidating the biological effects of this formulation, the study provides practical and mechanistic insights that may inform the development of affordable, nutrient dense interventions for populations vulnerable to malnutrition.

2. Methods and Materials

2.1. Materials and Sample Authentication

Lima bean (*Phaseolus lunatus* L.) samples were collected from the Air Dingin area, Solok District, West Sumatra, Indonesia. To ensure species authenticity, a sample was formally identified and confirmed as *P. lunatus* L. at the Herbarium of Andalas University (ANDA). Patin fish (*Pangasius* sp.) were acquired from a sustainable aquaculture facility (CV Sipujuk, Padang, Indonesia).

2.2. Preparation of Lima Bean Flour

The lima beans were cleaned, rinsed with running water, and soaked in distilled water (1:5 bean-to-water ratio, w/v) for 12 hours at room temperature. Following soaking, the beans were boiled in distilled water (2:1 water-to-bean ratio, v/w) for 60 minutes. The boiled beans were then dried in a ventilated oven at 60°C to a constant weight. Finally, the dried beans were cooled and ground into a fine flour using a 20-mesh sieve, following a modified procedure from [23].

2.3. Preparation of Patin Fish Flour

The patin fish were cleaned, washed, and filleted, with only the flesh being used for processing. The fish flesh was dried in a food-grade drying oven at 60°C for 15 hours to preserve its nutritional quality, as described by [24]. The dried flesh was subsequently ground into a fine flour. Both the processed lima bean and patin fish flours were stored in sterilized, airtight polyethylene bags at 8°C until required for experimental use.

2.4. Animals

Young male Wistar rats (5–6 weeks old, n = 25) were obtained from a licensed supplier ("Pondok Tikus," West Sumatra, Indonesia). The animals were housed individually in a temperature-controlled environment (22 ± 1°C) under a 12-hour light/dark cycle, with lights on from 07:00 to 19:00. Prior to the experiment, all rats underwent an acclimatization period with *ad libitum* access to standard food and water.

2.5. Experimental Design and Dietary Groups

Following acclimatization, the rats were randomly assigned to one of five experimental groups. The study duration was eight weeks. Malnutrition was induced in groups P2 through P5 for the initial four weeks. The dietary regimens for each group were as follows:

- P1 (Normal Control): Received standard diet for the entire 8-week period.
- P2 (Malnutrition Control): Received a 5% low-protein diet for the entire 8-week period.
- P3 (Recovery Control): Received a 5% low-protein diet for 4 weeks, followed by a standard diet for the final 4 weeks.
- P4 (LBPF Low-Dose): Received a 5% low-protein diet for 4 weeks, followed by a standard diet supplemented with LBPF (40 g/kg BW) for the final 4 weeks.

- P5 (LBPF High-Dose): Received a 5% low-protein diet for 4 weeks, followed by a standard diet supplemented with LBPF (77 g/kg BW) for the final 4 weeks.

2.6. Brain Tissue Collection and Preparation

At the conclusion of the 8-week experimental period, all animals were euthanized using an overdose of ketamine anesthetic. Brains were immediately harvested, and the wet weight of each was recorded as a measure of structural development. Subsequently, the brain tissues were fixed in 10% formal saline for 72 hours before further processing.

2.7. Tissue Processing and Histological Staining

The fixed brain tissues were processed through a graded series of alcohol solutions for dehydration, cleared with xylene, and embedded in paraffin wax. Using a microtome, coronal sections of 4–5 μm thickness were prepared and mounted onto glass slides. The sections were then stained with hematoxylin and eosin (H&E) for histological examination.

2.8. Microscopic Analysis and Morphometry

Stained sections were analyzed under a light microscope. The thickness of the cerebral cortex was measured in predetermined regions using calibrated imaging software. Hippocampal neuron density was quantified using imaging software. Neuronal damage was qualitatively assessed based on morphological criteria, including cell shrinkage, pyknosis (nuclear condensation), and cytoplasmic eosinophilia.

2.9. Behavioral Assessments

All behavioral assessments were conducted during the light phase of the cycle. The order of testing for each animal in the Y-Maze, Novel Object Recognition Test (NORT), and Morris Water Maze (MWM) was randomized to prevent sequence bias. Animal movements were recorded using a video camera (GoPro HERO), and the resulting videos were analyzed by a blinded observer or with ANY-Maze tracking software to ensure unbiased scoring.

2.9.1. Morris Water Maze (Latency Time)

The MWM was used to evaluate spatial learning and memory, following the methods of [25]. The apparatus consisted of a circular pool (116 cm in diameter, 55 cm in height) filled with water maintained at 21–23°C. The pool was conceptually divided into four quadrants (North, South, East, West), with a hidden escape platform submerged in one quadrant. Rats were placed in the water, and the latency to locate the submerged platform was recorded across several trials. After each trial, rats were removed, gently dried, and returned to their home cages.

2.9.2. Y-Maze Test (Spontaneous Alternation)

The Y-Maze test was used to assess spatial working memory through spontaneous alternation behavior, based on the protocol by [26]. The maze consisted of three identical arms (40 cm length x 8 cm width x 15 cm height) positioned at a 120° angle from each other. Each rat was placed in the center of the maze and allowed to freely explore all three arms for 5 minutes. An arm entry was recorded when all four paws of the rat were within the arm. Spontaneous alternation was defined as successive entries into the three different arms and was calculated as a percentage.

$$SA = \frac{\text{sABC} + \text{sACB} + \text{sBCA} + \text{sBAC} + \text{sCAB} + \text{sCBA}}{(\text{eArm A} + \text{eArm B} + \text{eArm C}) - 2} \times 100$$

SA (%) = Spontaneous Alternation, tendency animal model to alternate between different paths when exploring maze

sABC = ABC sequence, sequence of arms that entered respectively (A→B→C) during test

eArms A = Entry to arms A, number of total entries to arms A during test

2.9.3. Novel Object Recognition (Discrimination Index)

The NORT was conducted to assess recognition memory, as described by [27]. The test was performed in an open-field arena. The test involved three phases. During the habituation phase, rats were allowed to explore the empty arena. In the training phase, two identical objects were placed in the arena, and rats were allowed to explore them. Following a rest period, the testing phase was conducted, where one of the identical objects was replaced with a novel object. A blinded observer recorded the time spent interacting with each object, with interaction defined as sniffing or touching the object with the nose.

$$DI = \frac{\Sigma\ \text{tNov}}{\Sigma\ (tNov + tFam)} \times 100\%$$

DI = Discrimination Index, the percentage of time spent with the novel object with respect to the total exploration time

Σ tNov = total time spent with Novel object

Σ tFam = total time spent with Familiar object

2.10. Malondialdehyde (MDA) Levels Measurement

The levels of malondialdehyde (MDA), a key marker of lipid peroxidation, were measured in brain tissue homogenates according to the methods described by [28]. Immediately following euthanasia, a 0.25 g sample of brain tissue was collected from each rat and stored at -80°C until analysis. For the assay, samples were homogenized in phosphate-buffered saline (PBS)

using a tissue homogenizer (Tissueruptor II, Qiagen). The homogenates were then centrifuged at 5,000 rpm for 15 minutes, and the resulting supernatant was collected. MDA levels in the supernatant were quantified using a commercial lipid peroxidation assay kit (Abcam) following the manufacturer's protocol. The absorbance of the final reaction product was measured with a UV-Vis spectrophotometer (Raptor UV1100N).

2.11. Statistical Analysis

All quantitative data are presented as mean ± standard error of the mean (SEM), unless otherwise specified. Statistical analyses were conducted using one-way analysis of variance (ANOVA). When significant effects were detected, Duncan's post hoc test was applied for multiple pairwise comparisons. A p-value less than 0.05 was considered statistically significant. Significance levels in figures and tables are indicated as $p < 0.05$. Non-significant (ns) differences are noted as such.

3. Results

3.1. Brain weight

Malnutrition caused a substantial reduction in brain weight, with the malnutrition control group (P2) exhibiting a mean value of 1.407 ± 0.07965 g compared with 1.767 ± 0.02728 g in the normal control (P1). Partial recovery was observed in the recovery control group (P3), which reached 1.576 ± 0.03844 g. LBPF supplementation produced a clear, dose-dependent improvement; the P4 group achieved 1.625 ± 0.05783 g while the high-dose P5 group reached 1.632 ± 0.01202 g. Although the brain weight in P5 did not completely return to the level of normal controls, both LBPF-treated groups demonstrated significant restoration relative to the malnutrition control, indicating that the combined formulation effectively counteracted malnutrition-induced brain mass loss (Figure 1).

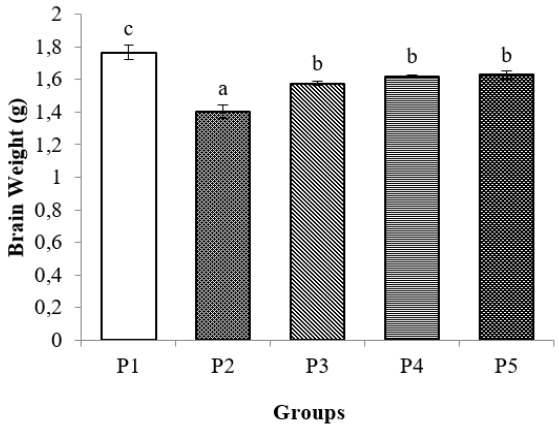

Figure 1. Effects of lima bean and patin fish flour (LBPF) on brain weight in experimental groups. (P1) normal control, (P2) malnutrition control, (P3) recovery control, (P4) LBPF 40 g/kg BW, and (P5) LBPF 77 g/kg BW. Different lowercase letters indicate significant differences among groups (Duncan's post-hoc test, $p < 0.05$).

3.2. Cerebral Cortex Thickness and Cortical Neuron Degeneration

Histological examination of the cerebral cortex revealed clear morphological differences among the experimental groups (Figure 2). Malnourished rats (P2) showed pronounced neuronal degeneration, characterized by nuclear pyknosis, shrunken cell bodies, and eosinophilic cytoplasm. In contrast, animals supplemented with lima bean and patin fish flour (LBPF) demonstrated substantial improvements in cortical morphology. Both LBPF-treated groups, particularly the high-dose group (P5), exhibited markedly fewer degenerating neurons, with overall neuronal appearance closely resembling that of the normal control (P1).

Malnutrition induced a significant thinning of the cerebral cortex, with the malnutrition control group (P2) displaying a cortical thickness of 1191 ± 20.13 μm compared with 1559 ± 11.71 μm in the normal control (P1) (Figure 3A). Only minimal improvement was observed in the recovery control group (P3; 1204 ± 13.78 μm). Supplementation with lima bean and patin fish flour restored cortical structure in a dose-dependent manner, as the low-dose (P4) and high-dose (P5) groups exhibited significantly greater cortical thicknesses of 1340 ± 41.57 μm and 1433 ± 10.63 μm, respectively, both markedly higher than P2.

Quantitative analysis also revealed pronounced neuronal degeneration in malnourished animals (P2), which showed the highest number of degenerating neurons (14.30 ± 0.05774 cells/field), nearly 27-fold greater than the normal control (P1; 0.53 ± 0.05774 cells/field) (Figure 3B). The LBPF-treated groups displayed substantial reductions in degenerating neurons, with P4 showing 3.267 ± 0.5457 cells/field and P5 showing 0.9333 ± 0.4372 cells/field. The values in P5 were statistically comparable to the normal control, indicating near-complete reversal of malnutrition-induced cortical cell degeneration.

3.3. Hippocampal Neuronal Damage and Neuronal Viable Density

Histological examination of the hippocampus revealed marked neuronal degeneration in the malnutrition group (P2), characterized by pyknotic and shrunken neurons indicated by red arrows (Figure 4). Minimal improvement was observed in the recovery control (P3), whereas LBPF-treated groups, particularly the high-dose group (P5), showed visibly reduced neuronal damage with cellular morphology approaching that of the normal control (P1).

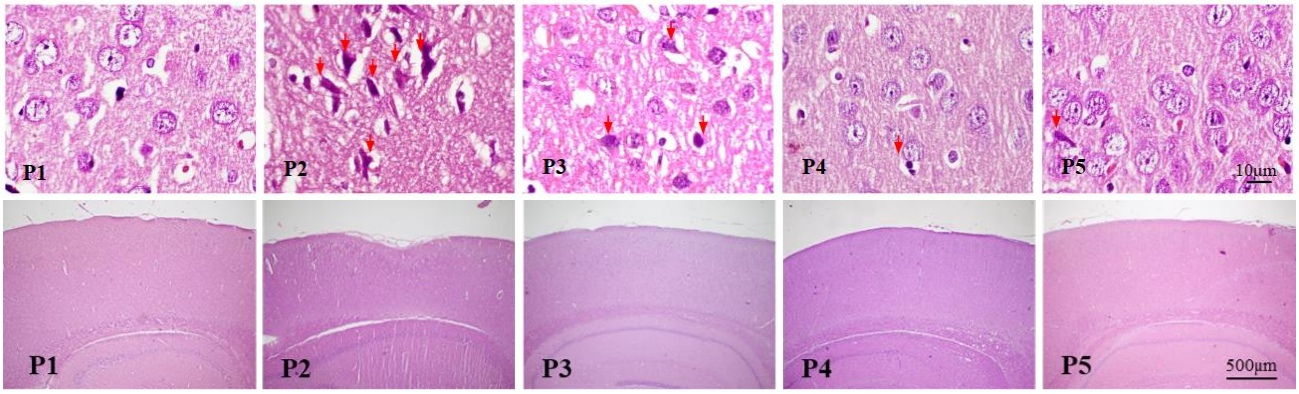

Figure 2. Histological appearance and cortical thickness of the cerebral cortex in experimental groups. Upper panels show representative cortical histology from (P1) normal control, (P2) malnutrition control, (P3) recovery control, (P4) LBPF 40 g/kg BW, and (P5) LBPF 77 g/kg BW. Malnutrition-induced animals exhibit signs of neuronal degeneration in the cerebral cortex, characterized by pyknotic, shrunken neurons, or neurons with eosinophilic cytoplasm and lysed cells (red arrows ↓). Lower panels depict cortical thickness measurements across groups, showing dose-dependent restoration with LBPF. Hematoxylin–eosin staining; upper panels 400× and 1000×, lower panels 100× magnification. Scale bar: 500 μm.

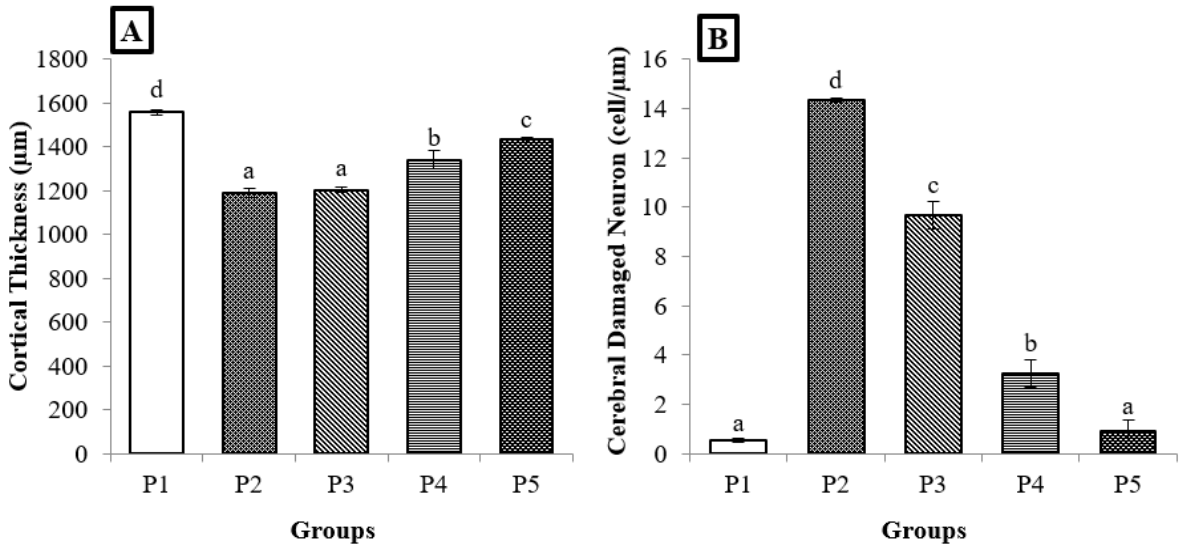

Figure 3. Effects of lima bean and patin fish flour (LBPF) on cerebral cortex thickness (A) and the number of degenerating neurons (B). Experimental groups include (P1) normal control, (P2) malnutrition control, (P3) recovery control, (P4) LBPF 40 g/kg BW, and (P5) LBPF 77 g/kg BW. Different lowercase letters indicate significant differences among groups (Duncan's post-hoc test, $p < 0.05$).

Malnutrition caused a marked reduction in hippocampal neuronal density (Figure 5A). The malnutrition control group (P2) showed a viable neuron density of 15.83 ± 2.36%, substantially lower than the normal control (P1; 94.06 ± 0.74%). Only partial recovery was observed in the recovery control (P3; 40.8 ± 1.83%). Supplementation with LBPF improved neuronal density in a dose-dependent manner, with P4 reaching 72.96 ± 2.46% and P5 85.7 ± 1.06%, the latter showing values statistically comparable to P1. Consistent with these findings, malnutrition markedly increased the number of degenerating hippocampal neurons (Figure 5B). The P2 group displayed 7.4 ± 0.3 cells/field, far higher than the normal control (P1; 0.4 ± 0.06 cells/field). LBPF supplementation reduced degeneration significantly, with P4 showing 4.8 ± 0.85 cells/field and P5 2 ± 0.94 cells/field, with P5 approaching normal levels.

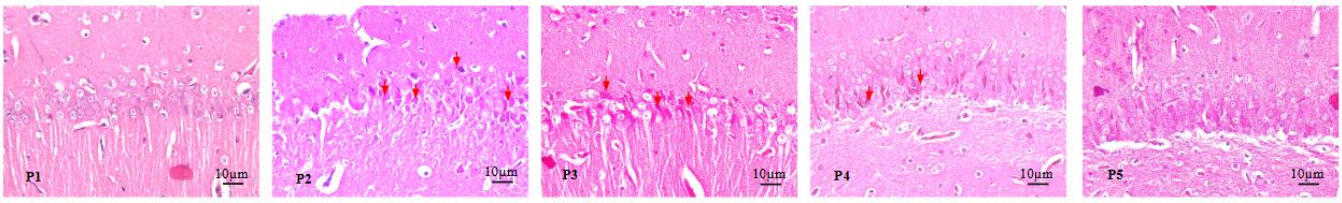

Figure 4. Histological changes in the hippocampus of experimental rats. (P1) normal control, (P2) malnutrition control, (P3) recovery control, (P4) LBPF 40 g/kg BW, and (P5) LBPF 77 g/kg BW. Damaged neurons are indicated by red arrows. Hippocampal Damaged Neuron that aims by red arrow (↓). Hematoxylin eosin staining; images captured at 4× and 40× magnification using an Olympus CX33 microscope.

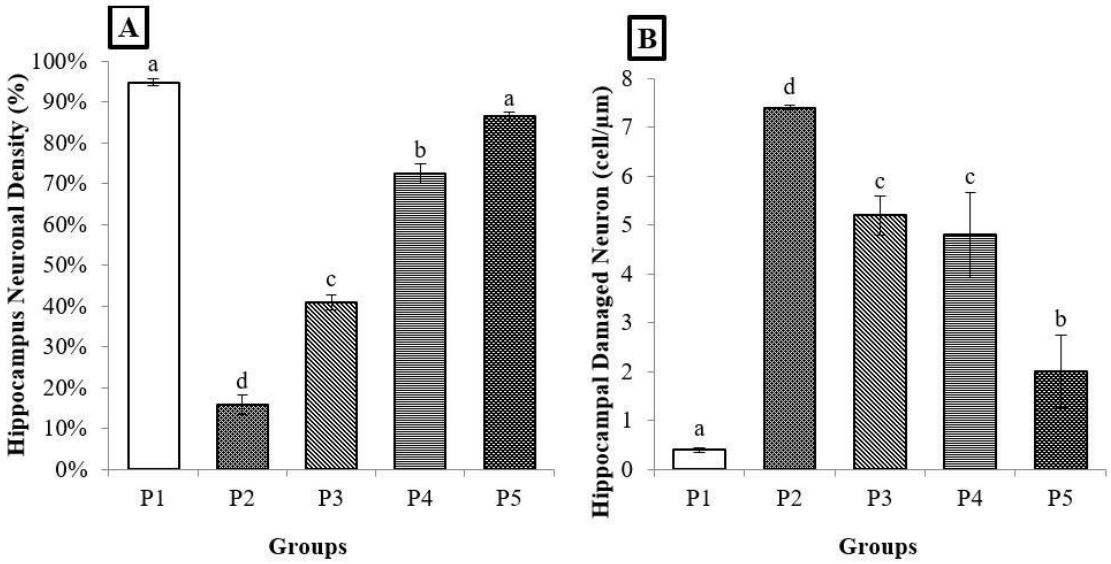

Figure 5. Effects of lima bean and patin fish flour (LBPF) on hippocampal neuron density. (A) and number of damaged hippocampal cells (B). (P1) normal control, (P2) malnourished control (8 weeks), (P3) malnourished 4 weeks + normal feed 4 weeks, (P4) malnourished 4 weeks + LBPF 40 g/rat for 4 weeks, and (P5) malnourished 4 weeks + LBPF 77 g/rat for 4 weeks. Different lowercase letters indicate significant differences among groups (Duncan's post-hoc test, $p < 0.05$).

3.4. Behavioral Assessment

The effects of lima bean and patin fish flour (LBPF) on cognitive and memory function are presented in Figure 6. In the Y-Maze test (Figure 6A), malnutrition markedly reduced spontaneous alternation, with the P2 group achieving only 53 ± 10.14% compared to 97 ± 3.07% in the normal control (P1). LBPF supplementation improved performance in a dose-dependent manner, as P4 reached 83.67 ± 8.56%, and the high-dose P5 group achieved 95.66 ± 4.33%, closely matching normal control levels.

A similar pattern was observed in spatial learning performance in the Morris Water Maze (Figure 6B). Malnourished rats (P2) required the longest latency to locate the hidden platform (10.98 ± 0.48 s), whereas the P1 group performed fastest (4.81 ± 0.46 s). LBPF supplementation enhanced spatial learning, with P4 (6.13 ± 0.49 s) and P5 (6.24 ± 0.64 s) showing latency times approaching those of normal rats.

Recognition memory assessed using the Novel Object Recognition Test (NORT) (Figure 6C) was also severely impaired by malnutrition. The P2 group showed a markedly reduced discrimination index (28 ± 4.53%) relative to P1 (92 ± 4.61%). LBPF supplementation improved recognition performance, with P4 rising to 66 ± 1.52% and P5 reaching 90.33 ± 5.48%, indicating substantial restoration of recognition memory. Together, these findings show that LBPF supplementation effectively reverses the cognitive and memory deficits induced by malnutrition.

3.5. Malondialdehyde Levels

Malondialdehyde (MDA) levels were quantified in brain tissue to evaluate oxidative stress status across treatment groups (Figure 7). Malnutrition resulted in elevated lipid peroxidation, as indicated by the increased MDA concentration in the malnutrition control group (P2; 10.27 ± 1.842 μM) compared with the normal control group (P1; 9.073 ± 0.9536 μM). The recovery control group (P3) showed no improvement, maintaining similarly high MDA levels (10.16 ± 1.855 μM).

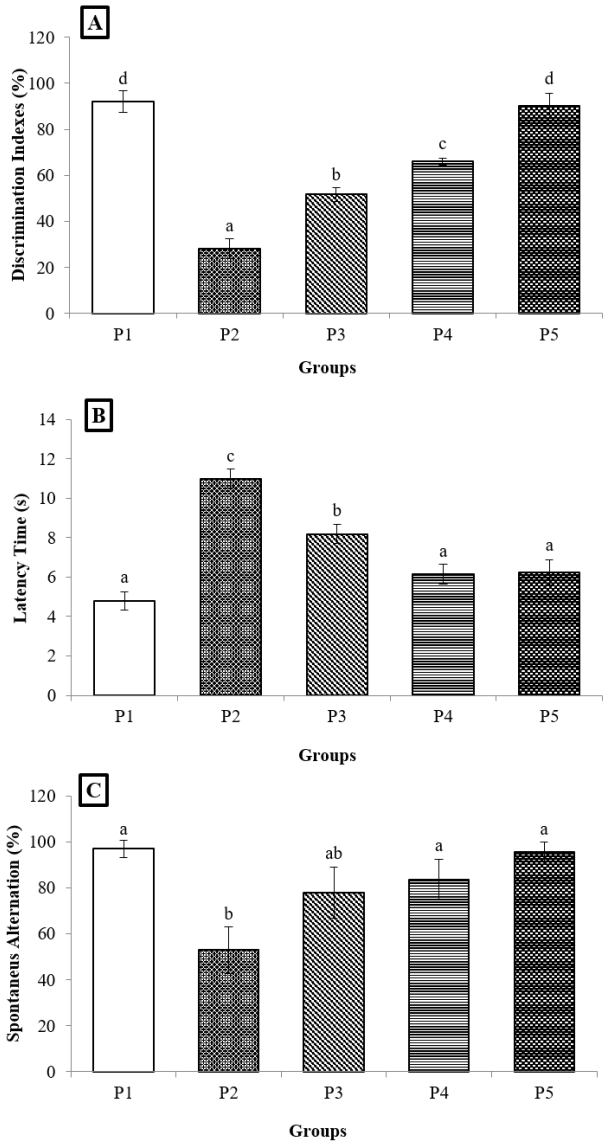

Figure 6. Behavioral assessment of malnourished rats, including (A) spontaneous alternation (SA) in the Y-Maze test, (B) latency time to locate the hidden platform in the Morris Water Maze, and (C) discrimination index (DI) in the Novel Object Recognition Test. Different lowercase letters indicate significant differences among experimental groups based on Duncan's post-hoc test ($p < 0.05$).

LBPF supplementation produced a dose-dependent decrease in oxidative stress markers. The P4 group exhibited lower MDA levels (8.614 ± 1.141 μM), while the high-dose P5 group showed the greatest reduction (8.369 ± 1.0862 μM). Although these reductions did not reach statistical significance, the overall trend indicates that LBPF administration, particularly at the higher dose shifts MDA values toward the normal baseline.

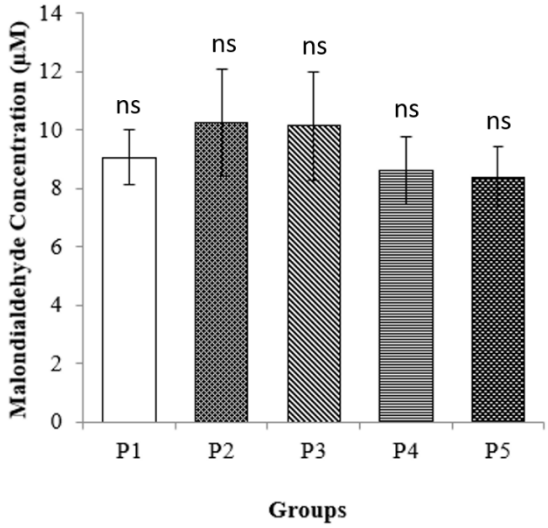

Figure 7. Effects of lima bean and patin fish flour (LBPF) on brain malondialdehyde (MDA) levels. Experimental groups were (P1) normal control, (P2) malnutrition control, (P3) recovery control, (P4) LBPF 40 g/kg BW, and (P5) LBPF 77 g/kg BW. Data are presented as mean ± SE. No significant differences were detected among groups by one-way ANOVA ($p > 0.05$).

4. Discussions

Malnutrition is widely recognized as a major contributor to structural and functional impairments in the developing brain. In this study, four weeks of protein–energy deficiency resulted in a significant decline in cortical integrity, hippocampal neuronal survival, and overall brain weight, findings that align with previous reports showing that malnutrition increases oxidative stress and accelerates neuronal loss [29]. Consistent with earlier work in Wistar rats, malnourished groups in this study exhibited marked cortical thinning, a reduction typically detectable as early as day 20 postnatally [30, 31]. These structural deficits are of particular concern because the cerebral cortex and hippocampus are highly vulnerable during early development, and damage in these regions has been associated with long-term cognitive and behavioral impairment [32, 33]. Although early-stage injury can remain reversible with timely intervention [34, 35], prolonged nutritional deficits may cause permanent alterations in brain architecture.

LBPF supplementation produced a clear restorative effect on the cerebral cortex. The high-dose group (P5) showed cortical thickness approaching that of normal animals, accompanied by a marked reduction in degenerating neurons. These findings suggest that the combined nutrient composition of lima beans and patin fish supports membrane repair, synaptic maintenance, and extracellular matrix stabilization. Lima beans supply plant-based protein, folate, iron, magnesium, and polyphenols that collectively promote antioxidant defense, neuronal repair, and metabolic regulation [36–

38]. Patin fish provides high-quality animal protein, DHA, EPA, vitamin B12, selenium, and phosphorus, nutrients that play essential roles in membrane fluidity, synaptogenesis, and tissue regeneration [39–43]. Together, these nutrients appear to enhance the brain's capacity for structural recovery, explaining the improved cortical thickness and reduced degenerative features observed in P4 and P5.

A strong trend toward recovery in brain weight was also observed following LBPF treatment. Malnourished controls (P2 and P3) exhibited marked reductions consistent with impaired cell proliferation and decreased neuronal and glial mass. Similar reductions have been reported previously, where early protein deficiency diminished hippocampal stem cell proliferation and reduced the formation of newly generated neurons [44]. LBPF supplementation in groups P4 and P5 supported the restoration of brain mass, likely through enhanced availability of essential amino acids, omega-3 fatty acids, and micronutrients needed for neurodevelopmental processes. Although the statistical differences were not significant, the biological trend indicates meaningful recovery, particularly in animals receiving higher doses.

The hippocampus, a key region for learning and memory [45, 46], showed marked vulnerability to malnutrition but exhibited substantial recovery following LBPF supplementation. Neuronal degeneration decreased in a dose-dependent manner, with P5 (2 ± 0.94 cells/field) approaching normal levels. The improvement aligns with the roles of DHA in stabilizing synapses and promoting neuronal integration within hippocampal circuits [47], while glutamic acid from lima beans supports neurotransmitter synthesis, including glutamate and GABA, which are essential for maintaining excitatory–inhibitory balance [48, 49]. The complementary actions of these nutrients help explain the higher viable neuron density observed in P4 and P5.

Malnutrition resulted in consistent impairments across all behavioral tests, confirming its wide-ranging impact on cognitive processing. In the Y-Maze test, reduced spontaneous alternation indicated compromised working memory, while the low discrimination index in the NORT reflected impaired recognition memory. The prolonged latency in the Morris Water Maze further demonstrated deficits in spatial learning functions that depend on the integrity of both cortical and hippocampal networks. LBPF supplementation produced substantial and dose-dependent improvements across all tests. The P4 group showed partial cognitive recovery, whereas the P5 group achieved performance levels comparable to healthy controls. These functional improvements correspond with histological restoration in the cortex and hippocampus, suggesting that structural recovery facilitated by LBPF directly contributed to enhanced learning and memory capacity.

The cognitive restoration observed in LBPF-treated rats is likely mediated by the complementary nutrient profile of lima beans and patin fish. Polyphenols and antioxidant compounds from lima beans reduce oxidative stress and support neuronal function [55–58], while omega-3 fatty acids and micronutrients from patin fish contribute to membrane integrity, synaptogenesis, and anti-inflammatory pathways [59]. Together, these components create a neurochemical environment conducive to neuronal resilience and efficient synaptic transmission, ultimately restoring cognitive performance. The behavioral improvements following LBPF supplementation therefore reflect both structural recovery and enhanced neurobiological function, supporting the potential of this dual-source nutritional intervention in mitigating cognitive deficits caused by early-life malnutrition.

Malnutrition markedly elevated MDA levels, reflecting increased lipid peroxidation and heightened oxidative stress within brain tissue. This pattern is consistent with previous findings that nutrient deficiency disrupts antioxidant defense systems and promotes the accumulation of reactive oxygen species [60]. LBPF supplementation produced a clear dose-dependent reduction in MDA levels, with the high-dose P5 group approaching the normal physiological range. Although the decrease did not reach statistical significance, the downward trend suggests a potential protective effect that warrants further investigation with larger sample sizes or additional oxidative stress markers. The antioxidant effect of LBPF is likely attributable to the combined action of polyphenols from lima beans and omega-3 fatty acids from patin fish, which together reduce lipid peroxidation and support a more favorable biochemical environment for neuronal recovery [60, 61].

5. Conclusions

This study demonstrates that lima bean–patin fish flour (LBPF) effectively restores brain structure, improves cognitive function, and reduces oxidative stress in malnourished rats, with the high-dose group achieving recovery levels comparable to healthy controls. These findings highlight the synergistic value of combining plant- and fish-based nutrients for neurodevelopmental repair and support the potential use of LBPF as a practical functional food for mitigating the long-term effects of malnutrition. Future research should investigate the molecular pathways underlying LBPF's neuroprotective effects, assess long-term outcomes beyond the recovery phase, and evaluate its applicability in human populations to strengthen its translational potential.

Declarations

Author Contributions

Conceptualization, R.M., A.T., P.S., and R.S.; methodology, R.M., A.T., P.S., and R.S.; formal

analysis, R.M. and B.A.; investigation, R.M., R.J., M.H.I., and M.S.I.; writing original draft preparation, R.M., M.H.I., and B.A. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Data available on request due to restrictions, e.g., privacy or ethical: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ethical considerations.

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Institutional Review Board Statement

The animal study protocol were reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Andalas University. Ethical number: No.985/UN. 16.2/KEPFK/2022

Conflicts of Interest

The author declares that there are no conflicts of interests regarding the publication of this manuscript.

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