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Effects of patin fish-based nutritional intervention on biochemical and physiological recovery in malnourished rats: An in vivo study and its implications for clinical nutrition

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Abstract

Malnutrition is a major global health concern, especially in developing countries. Although patin fish oil and protein offer benefits, their individual and combined effects on maternal physiology remain unclear, particularly during early pregnancy. The aim of this study was to assess the effect of patin-based nutritional intervention on total serum protein, albumin, hemoglobin levels, body weight during pregnancy, body weight during lactation, heart rate, respiratory rate, body temperature, external appearance, behavioral activity, and milk production in malnourished rats. An in vivo study was conducted using *Rattus norvegicus* rats. The rats were divided into six groups: (1) healthy control, receiving standard feed; (2) malnourished control, receiving an 8% low-protein diet; (3) malnourished group, receiving standard feed; (4) malnourished treated with patin oil; (5) malnourished treated with patin meat; and (6) malnourished treated with a combination of patin oil and meat. The treatment consisted of 21 days during pregnancy and 23 days during lactation, for a total of 44 days. Our data indicated that patin-based intervention significantly increased total protein (p=0.044), albumin (p=0.001), and hemoglobin levels (p=0.034) compared to malnourished control group. The malnourished animals treated with patin oil showed the highest increases in total protein (1.67%), albumin (17.75%), and hemoglobin (24.26%). Body weight gain improved significantly in patin-treated group in both pregnancy (p=0.032) and lactation (p<0.001) compared to the malnourished control, with the highest gains observed in the patin oil group. Milk production also increased significantly (p<0.05), reaching its peak in the patin oil and meat combination group (6.97 g). Physiological parameters, including heart rate (p=0.021), respiratory rate (p=0.025), and body temperature (p=0.023), were significantly different among groups, of which patin oil and meat groups had the most optimal parameters compared to malnourished control group. In conclusion, patin-based nutritional intervention effectively enhances protein metabolism, hematological parameters, and physiological health in malnourished maternal rats, with patin oil demonstrating the most pronounced effects.

Keywords: Malnutrition, rat model, total protein, albumin, hemoglobin

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Introduction

Malnutrition remains a critical global health issue, particularly in developing countries, where limited access to good quality nutrition contributes to a high prevalence of undernutrition [1]. It is a major risk factor for various diseases, significantly impairing physical and cognitive development, particularly in vulnerable populations such as children and pregnant women [2]. Limited access to quality nutrition in developing regions results in a disproportionately high prevalence of malnutrition, making it a critical public health concern [3]. Malnutrition is associated with protein deficiency, anemia, and immune dysfunction, leading to increased morbidity and mortality [4,5]. Chronic malnutrition in children is associated with stunted growth, developmental delays, and increased susceptibility to infections due to immune impairment [6,7]. World Health Organization (WHO) reports that 22.3% of children under five experienced stunting, with 13.7 million cases of wasting [8]. In Indonesia, the 2024 nutritional status survey by the Ministry of Health estimated malnutrition prevalence at 21.6% [7].

Malnutrition can result in metabolic disturbances, physiological changes, organ dysfunction, and loss of body mass [9]. Pregnant and lactating women are particularly vulnerable, with malnutrition adversely affecting maternal and fetal health [10]. A major challenge in addressing maternal malnutrition is developing effective, accessible, and affordable nutritional interventions [10]. While traditional multivitamin and mineral supplementation provides benefits, it often lacks the targeted physiological effects needed for severe malnutrition [11]. Fish-based supplements, rich in bioavailable proteins and essential fatty acids, present a promising alternative by addressing key nutritional deficits [11,12]. Although fish oil and protein have demonstrated health benefits, further research is needed to compare their effects on maternal physiology [12].

Limited research has explored the impact of patin fish (*Pangasius hypophthalmus*) oil, meat, and combined supplements on protein and hemoglobin levels, critical indicators of maternal health in malnutrition [13]. Patin fish has potential as a nutritional intervention for malnutrition [13]. It provides high-quality, easily digestible protein and is rich in omega-3, omega-6, and omega-9 fatty acids, which contribute to various biological functions [13]. The protein content enhances albumin and total protein levels, supporting physiological recovery in malnourished rats [13]. Additionally, high-quality protein promotes tissue repair and restores organ function [13]. Omega-3 fatty acids found in patin play a role in reducing systemic inflammation and enhancing metabolic function through increased protein synthesis and tissue repair [14]. Omega-3 fatty acids play a crucial role in reducing systemic inflammation, enhancing metabolic function by stimulating protein synthesis, and supporting immune modulation, thereby improving overall health in malnourished conditions [14].

A recent study has explored the effects of patin-based supplements in animal models, particularly rats [13]. Patin oil administration significantly increased hemoglobin levels and body weight in anemic Wistar rats [15]. Furthermore, patin oil extract improved high-density lipoprotein (HDL) levels in alloxan-induced diabetic rats [16], suggesting its potential cardioprotective effects in malnourished individuals [17]. Additionally, patin supplementation has been shown to enhance immune function in malnourished rats [13]. Patin concentrate increased albumin levels and immunity in neonatal rats born to lactating mothers on a low-protein diet [13]. Although those studies have highlighted the benefits of patin oil and protein separately, limited research has compared their individual (fish oil) and combined (oil and meat combination) effects on multiple physiological parameters in malnourished maternal physiology [13,18]. Additionally, the effect of patin supplementation during early pregnancy remains unexplored. The aim of this study was to assess the effect of patin-based nutritional intervention on total serum protein, albumin, hemoglobin level, body weight during pregnancy, body weight during lactation, heart rate, respiratory rate, body temperature, external appearance, and behavioral activity level, and milk production in malnourished rats. Findings from animal models may provide valuable insights for designing nutritional interventions to address malnutrition, particularly in regions with high prevalence rates.

Methods

Study design and setting

An in vivo study was conducted using female Sprague Dawley rats to evaluate the effects of patin oil and meat on malnourished pregnancy and lactation at the Laboratory Animal Management Unit, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia. Patin oil was extracted via wet rendering and incorporated into feed. Thirty rats were divided into six groups, including healthy and malnourished controls, as well as those receiving patin oil, fish meat, or both. Malnutrition was induced through a low-protein diet (8%) over four weeks, after which the malnourished rats were mated. Dietary interventions were implemented from early pregnancy until lactation ended. Study outcomes, including physiological parameters, milk production, body weight, and hematological profiles, were assessed. Physiological parameters were evaluated at the end of lactation. Milk production was estimated every five days during lactation, and body weight was monitored throughout pregnancy and lactation. Hematological profiles were assessed before the dietary intervention and one day after lactation ended.

Extraction of patin fish oil and meat

Patin oil was extracted using the wet rendering method. Fresh patin, each weighing approximately 750 g, were cut into small pieces to optimize the extraction process. The fish were then boiled in 500 mL of distilled water at 60°C for 30 minutes with continuous slow stirring. Following boiling, the mixture was filtered to separate the crude oil from the fish meat. The oil and aqueous layers were subsequently separated using a separating funnel [19]. The obtained oil was further purified by centrifugation at 10,000 rpm for 10 minutes at 10°C to eliminate residual sediment [19]. The remaining fish meat from the wet rendering process was dried in an oven at 40°C and subsequently ground into a fine powder. This processed fish meat was incorporated into standard rat feed pellets. The meat treatment was prepared using patin fish meat obtained through boiling during the wet rendering process for patin fish oil extraction. The remaining meat residue from this process was subsequently dried in an oven at 40°C and ground into a fine powder using a blender. This dried meat powder was used as the meat-based intervention model. The standard feed consisted of commercially available vitamin-rich rat food (CitraFeed Rat Bio, Citra IWA Feedwill, Jakarta Indonesia) pellets formulated for healthy laboratory rats, containing balanced amounts of protein, carbohydrates, and other essential nutrients in accordance with established dietary requirements for experimental animals. The patin fish oil extract, patin fish meat powder, and their combination were incorporated into the standard feed to create the respective treatment diets.

Sample size and study groups

The sample size in this study was determined based on a completely randomized design (CRD) with six treatment groups, each comprising four replicates, resulting in a total of 24 rats. The rats were divided into six groups: (1) healthy control group, received standard feed; (2) malnourished control group, received an 8% low-protein diet; (3) malnourished group received standard feed; (4) malnourished + patin fish oil group, received standard feed supplemented with patin oil; (5) malnourished + patin fish meat group, received standard feed supplemented with patin meat; and (6) malnourished + patin fish oil and meat group, received standard feed supplemented with a combination of patin oil and meat.

Animals and preparation

Female Sprague Dawley rats (*Rattus norvegicus*), obtained from the Bogor Life Science and Technology Laboratory, IPB University, Bogor, Indonesia, were used in this study. The inclusion criteria consisted of female rats aged three months, weighing 150–200 grams, nulliparous, and in normal physiological condition. Only rats that successfully underwent malnutrition induction without severe adverse effects were included. Malnutrition was confirmed by a ≥20% reduction in initial body weight. Exclusion criteria included failure to achieve the targeted malnutrition level and signs of extreme stress or adverse reactions during initial handling. Dropout criteria included non-responsiveness to intervention, severe stress, or death during the study. Daily evaluations involved health monitoring, behavioral observations, and nutritional intake assessments.

Acclimatization was conducted for two weeks under controlled environmental conditions, including a 12-hour light/dark cycle, temperature at 22–25°C, and humidity of 50–60%. Rats were housed in standard polypropylene cages with *ad libitum* access to water and a standard diet to ensure baseline nutritional status. During this period, health assessments were performed daily to monitor body weight, general behavior, and signs of illness. Routine parasite treatment was administered, and any rats displaying signs of infection or severe stress were excluded from the study.

Malnutrition and pregnancy induction

The malnourished rat model was established through the administration of a low-protein diet containing 8% protein, combined with a 50% feed restriction in the second week and a 60% restriction in the third week, over a total duration of four weeks. According to the 2023 Food and Drug Supervisory Agency guideline on nutritional status assessment in malnutrition pathophysiology, successful malnutrition induction was defined as a body weight reduction exceeding 20% [20]. Once this criterion was met, female rats were mated with male rats, and dietary intervention was started when the rats were confirmed pregnant.

Study interventions

Intervention feed in the form of pellets was administered daily from the second day of pregnancy until the end of lactation, 23 days of postpartum. The duration of intervention consisted of 21 days during pregnancy and 23 days during lactation, for a total of 44 days. Patin fish meat was administered at 2.1 gram per day [13], while patin oil was given at 0.036 gram per day [19]. This treatment is carried out every day during pregnancy and breastfeeding in rats. The treatments involving patin fish oil extract, patin fish meat extract, and their combination were mixed with the standard feed for laboratory rats. Feed was provided *ad libitum*, allowing unrestricted access, with an average intake of 30 g per day. Water was also supplied *ad libitum* to ensure adequate hydration. Feed pellets were formulated to meet the nutritional requirements of the rats and were placed in designated feeding containers within the cages. These containers were refilled daily to maintain continuous feed availability. The feeding and monitoring procedures were conducted by trained researchers or laboratory technicians experienced in handling laboratory animals and ensuring proper care. Blinding was not deemed necessary, as feed was provided *ad libitum*, and no specialized handling or dosing adjustments were required.

Study outcomes

Blood collection was performed before the initiation of the treatment diet and one day after lactation ended. For blood sampling, rats were restrained using a specialized restrainer to minimize stress. The caudal vein was disinfected with 70% ethanol before venipuncture, and blood was collected using a sterile syringe before being transferred to appropriate tubes for biochemical analysis. The blood samples were used to measure protein, albumin, and hemoglobin analysis. Total serum protein level was measured using the biuret spectrophotometric method with a UV-Vis spectrophotometer, Shimadzu UV-1800 (Shimadzu, Kyoto, Japan), while serum albumin level was assessed using the bromocresol green (BCG) method with a GENESYS 10S UV-Vis spectrophotometer set to 620 nm (Thermo Fisher Scientific, Waltham, MA, USA). Hemoglobin level was analyzed using colorimetric hemoglobin test strips with the HemoCue Hb 201+ Analyzer (HemoCue AB, Ängelholm, Sweden).

Body weight was recorded every five days throughout pregnancy and lactation, with a final measurement conducted at the end of lactation (day 23 postpartum). Physiological parameters, including heart rate, respiratory rate, body temperature, external appearance, and physical activity level were assessed at the end of lactation. Heart rate was measured using a Littmann Classic III stethoscope (3M, Saint Paul, MN, USA) placed on the thoracic region, while respiratory rate was determined by counting abdominal movements (expansion and contraction). Body temperature was recorded using a rectal thermometer. External appearance was evaluated based on fur texture, skin elasticity, and eye clarity, while behavior activity was assessed through behavioral observations in a controlled environment. Milk production was estimated every five days during lactation using a weight-based method, in which fasted pups were weighed before and after suckling, with the weight difference representing estimated milk intake. All measurements were conducted at a consistent time of day to ensure reliability and minimize variability.

Statistical analysis

Data obtained from the physiological responses of rats were tested for normality and homogeneity using the Kolmogorov-Smirnov test and Levene's test [21]. If the data were normally distributed and homogeneous, parametric analysis was performed using analysis of variance (ANOVA) [22], followed by post hoc Duncan's test. If the data did not meet normality and homogeneity assumption, non-parametric analysis was conducted using the Kruskal-Wallis test [23], followed by the Mann-Whitney U test for pairwise comparisons. Statistical analyses were performed using SPSS IBM version 23 (IBM, Armonk, NY, USA), while GraphPad Prism 10.4.1 (GraphPad Software, San Diego, CA, USA) was utilized for graphical representation [24].

Results

Effect of patin fish-based nutritional intervention on blood protein levels in malnourished rats

Patin fish-based nutritional intervention including oil, meat, and a combination, significantly increased blood protein levels compared to the malnourished control group fed a low-protein diet ($p<0.05$) (**Table 1**). A significant difference was observed in final total protein levels and total protein gain (Δ total protein) across all groups ($p=0.044$). Among the interventions, the malnourished animals treated with patin oil group had the highest increase in total protein (1.67%), followed by those treated with patin meat group (1.63%) and the combination of both oil and meat group (0.63%). The percentage increase in total protein differed significantly between the healthy control and malnourished control groups ($p=0.043$). The healthy control group showed a 7.42% increase in total protein, whereas the malnourished control group, maintained on a low-protein diet, had an 11.40% reduction.

Effect of patin fish-based nutritional intervention on serum albumin levels in malnourished rats

Patin fish-based nutritional intervention, including oil, meat, or a combination, significantly increased serum albumin levels in the malnourished group compared to the malnourished control group receiving a low-protein diet ($p<0.05$) (**Table 2**). However, the albumin level remained lower than those in the healthy control group ($p>0.05$). Among the intervention groups, patin oil group demonstrated the greatest increase in albumin (17.75%), followed by the patin meat group (17.50%) and combination of oil and meat group (9.71%). The percentage change in albumin levels differed significantly between the healthy control and malnourished control groups ($p=0.001$). The healthy control group had a 33.35% increase in albumin, whereas the malnourished control group maintained a low-protein diet experienced a 21.22% reduction.

Effect of patin fish-based nutritional intervention on hemoglobin levels in malnourished rats

Patin fish-based nutritional intervention also significantly increased hemoglobin levels in the malnourished group compared to the malnourished control group receiving a low-protein diet ($p<0.05$) (**Table 3**). The patin oil group had the greatest increase in hemoglobin (24.26%), followed by the combination of oil and meat group (20.19%) and patin meat group (10.03%) ($p=0.034$). Post-intervention, hemoglobin levels differed significantly between the healthy control and malnourished control groups ($p=0.049$). The healthy control group had the highest hemoglobin level (21.73±1.34 g/dL), whereas the malnourished control group maintained a low-protein diet and had a significant reduction (14.3±0.53 g/dL). Although all patin-based interventions led to increased hemoglobin levels, the values remained lower than those in the healthy control group. Final hemoglobin level was 19.58±0.43 g/dL in the patin oil group, 18.13±0.38 g/dL in patin meat group, and 18.97±0.67 g/dL in fish oil and meat combination group (**Table 3**).

Effect of patin fish-based nutritional intervention on body weight during pregnancy in malnourished rats

Patin fish-based nutritional intervention improved body weight gain in pregnant rats by the 20th day of gestation ($p<0.05$) (**Table 4**). The patin oil group had the highest weight gain, with a 36.61% increase, followed by the patin meat group (35.69%) and patin oil and meat combination group (31.77%). The change in body weight (Δ weight) differed significantly between the healthy control and malnourished control groups ($p=0.032$). The healthy control group had a weight gain of 52.33±2.52 gram, whereas the malnourished control group, maintained a low-protein diet, and had a lower gain of 12.67±2.52 gram. Among the patin-based intervention groups, the patin oil group achieved the highest weight gain at 74.33±5.28 gram (**Table 4**).

Effect of patin fish-based nutritional intervention on body weight during lactation in malnourished rats

Patin fish-based nutritional intervention significantly influenced body weight gain in lactating rats ($p<0.001$) (**Table 5**). The patin oil group showed the highest weight gain, with a 27.23% increase, followed by patin meat group (22.74%) and combination group (26.61%). At weaning, body weight differed significantly between the healthy control and malnourished control groups ($p<0.001$). The healthy control group had the highest body weight (249.33±18.01 gram), whereas the malnourished control group, maintained on a low-protein diet, had a lower body weight (136.67±5.03 gram). All patin-based nutritional intervention groups showed higher final body weight, with the patin oil group having the highest weight (231.00±10.15 gram) (**Table 5**).

Effect of patin fish-based nutritional intervention on milk production of lactating malnourished rats

Patin fish-based nutritional intervention significantly influenced estimated milk production in malnourished lactating rats ($p<0.05$) (**Figure 1**). The patin oil and meat group had the highest estimated milk production, averaging 6.97 grams, exceeding the values observed in the patin oil group (4.98 grams) and the patin meat group (5.23 grams). However, despite this improvement, milk production in the patin oil and meat group remained lower than in the healthy control group (9.04 grams), indicating that maternal malnutrition during pregnancy had a lasting impact on lactation capacity. Milk production in malnourished low protein group (1.43 grams) was lower than in the malnourished standard feed (3.42 grams)

Effect of patin fish-based nutritional intervention on heart rate, respiration rate, body temperature, and physical appearance in malnourished rats

Patin fish-based nutritional intervention improved heart rate, respiratory rate, body temperature, physical appearance, and activity levels in malnourished maternal rats compared to those fed a low-protein diet (**Table 6**). Heart rate differed significantly among the groups ($p=0.021$), with the highest mean value observed in the patin oil group (324±23.81 beats per minute). Similarly, respiratory rate varied significantly ($p=0.025$), with the patin oil group showing the highest rate (111±9.00 breaths per minute). Body temperature also differed significantly ($p=0.023$), with the highest temperature recorded in the patin oil and meat group (35.23±0.15°C).

Physical appearance assessments revealed distinct differences across groups. The healthy control group and those receiving patin oil, meat, or their combination had clean, smooth fur, red eyes, and active behavior. In contrast, the malnourished control group had dull, rough fur, pale eyes, and inactivity. The malnourished group fed a standard diet showed a better physical condition than the malnourished control group but remained suboptimal compared to the healthy control group.

Table 1. Effect of patin fish-based nutritional intervention on blood protein levels in malnourished pregnant rats

Variables	Healthy control	Malnourished control	Malnourished group	Patin oil	Patin meat	Patin oil and meat	*p*-value[1]
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Initial protein total (g/dL)	7.35±0.26^{b}	6.18±0.40^{a}	6.25±0.31^{a}	6.22±0.19^{a}	6.29±0.16^{a}	6.27±0.06^{a}	<0.001**
Final protein total (g/dL)	7.84±0.64 ^{a}	5.48±0.08 ^{c}	6.32±0.07^{b}	6.32±0.20^{b}	6.39±0.29^{b}	6.31±0.11^{b}	<0.001**
Δ protein total (g/dL)	0.53±0.40^{a}	-0.70±0.06^{b}	0.07±0.02^{c}	0.10±0.01^{c}	0.09±0.02^{c}	0.04±0.02^{c}	0.044*
Increase in total protein (%)	7.42	-11.40	1.46	1.67	1.63	0.63	0.043*
p-value[2]	0.307	0.009**	0.772	0.606	0.729	0.252	

[a-c]Analyzed using Duncan post-hoc test; different superscript letters within the same row indicate a significant difference at $p<0.05$
[1]Analyzed using ANOVA test, comparison among groups
[2]Analyzed using paired student t-test, comparison between initial and final total protein
*Statistically significant at $p<0.05$
**Statistically significant at $p<0.001$

Table 2. Effect of patin fish-based nutritional intervention on serum albumin levels in malnourished pregnant rats

Variable	Healthy control	Malnourished control	Malnourished group	Patin oil	Patin meat	Patin oil and meat	*p*-value[1]
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Initial albumin (g/dL)	2.42±0.63^{a}	2.31±0.12^{a}	2.32±0.19^{a}	2.37±0.39^{a}	2.33±0.40^{a}	2.59±0.07^{a}	0.421
Final albumin (g/dL)	3.05±0.21^{a}	2.29±0.45^{b}	2.47±0.64^{c}	2.72±0.21^{a}	2.67±0.19^{c}	2.87±0.07^{a}	0.015*
Δ albumin (g/dL)	0.72±0.04^{a}	-0.62±0.04^{b}	0.15±0.04^{a}	0.35±0.05^{a}	0.34±0.05^{a}	0.28±0.01^{a}	0.012*
Increase in total albumin (%)	33.35	-21.22	5.53	17.75	17.50	9.71	0.001*
p-value[2]	0.012[2]*	0.034*	0.456	0.067	0.089	0.123	

[a-c]Analyzed using Duncan post-hoc test; different superscript letters within the same row indicate a significant difference at $p<0.05$
[1]Analyzed using ANOVA test
[2]Analyzed using paired student t-test, comparison between initial and final albumin
*Statistically significant at $p<0.05$

Table 3. Effect of patin fish-based nutritional intervention on hemoglobin levels in malnourished pregnant rats

Variables	Healthy control	Malnourished control	Malnourished group	Patin oil	Patin meat	Patin oil and meat	*p*-value[1]
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Initial hemoglobin (g/dL)	19.13±0.65^{b}	15.97±1.57^{a}	16.97±1.54^{a}	15.4±0.6^{a}	16.37±0.55^{a}	16.4±1.497^{a}	0.048*
Final hemoglobin (g/dL)	21.73±1.34^{a}	14.3±0.53^{b}	17.97±0.76^{c}	19.58±0.43^{d}	18.13±0.38^{c}	18.97±0.67^{e}	0.049*
Δ hemoglobin (g/dL)	2.6±0.95^{b}	-1.63±1.3^{a}	1.00±0.8^{c}	4.18±2.1^{d}	1.63±0.46^{c}	3.27±0.78^{d}	0.032*
Increase hemoglobin (%)	13.55	-10.04	6.23	24.26	10.03	20.19	0.034*
p-value[2]	0.031*	0.307	0.772	0.013*	0.606	0.253	

[a-e]Analyzed using Duncan post-hoc test; different superscript letters within the same row indicate a significant difference at $p<0.05$
[1]Analyzed using ANOVA test
[2]Analyzed using paired student t-test, comparison between initial and final hemoglobin
*Statistically significant at $p<0.05$

Table 4. Effect of patin fish-based nutritional intervention on body weight during pregnancy in malnourished pregnant rats

Variables	Healthy control	Malnourished control	Malnourished group	Patin oil	Patin meat	Patin oil and meat	*p*-value[1]
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Day 1 weight (g)	231.33±44.41^{b}	171.00±25.24^{a}	181.00±41.39^{a}	202.67±12.58^{a}	197.67±25.15^{a}	207.00±12.29^{a}	0.021
Day 20 weight (g)	283.67±42.36^{a}	183.67±22.72^{b}	215.00±41.07^{c}	271.00±25.98^{d}	268.33±19.66^{e}	283.46±11.85^{a}	0.048*
Δ weight (g)	52.33±2.52^{a}	12.67±2.52^{b}	34.00±2.00^{c}	74.33±5.28^{d}	70.67±9.29^{e}	65.67±8.02^{f}	0.032*
Increase weight (%)	23.39	7.66	19.44	36.61	35.69	31.77	0.025*
p-value[2]	0.016*	0.035*	0.074*	0.021^{2*}	0.008^{2*}	0.004^{2*}	

[a-f]Analyzed using Duncan post-hoc test; different superscript letters within the same row indicate a significant difference at $p<0.05$
[1]Analyzed using ANOVA test
[2]Analyzed using paired student t-test, between day 1 and day 20
[*]Statistically significant at $p<0.05$

Table 5. Effect of patin fish-based nutritional intervention on body weight during lactation in malnourished pregnant rats

Variables	Healthy control	Malnourished control	Malnourished group	Patin oil	Patin meat	Patin oil and meat	*p*-value[1]
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Day 1 weight (g)	211.00±37.07^{a}	173.67±2.98^{a}	210.67±12.10^{a}	183.00±21.66^{a}	179.00±25.51^{a}	161.67±21.73^{a}	0.021
Weaning weight (g)	249.33±18.01^{a}	136.67±5.03^{b}	201.00±18.03^{d}	231.00±10.15^{c}	219.33±31.18^{a}	203.67±18.01^{a}	0.048*
Δ weight (g)	38.00±16.52^{b}	-37.00±15.72^{a}	-9.67±4.62^{d}	48.00±18.03^{c}	40.33±13.58^{b}	42.00±10.00^{b}	0.032*
Increase weight (%)	19.85	-20.92	-4.69	27.23	22.74	26.61	0.025*
p-value[2]	0.012*	0.002*	0.049*	0.011*	0.001**	0.011*	

[a-d]Analyzed using Duncan post-hoc test; different superscript letters within the same row indicate a significant difference at $p<0.05$
[1]Analyzed using ANOVA test
[2]Analyzed using paired student t-test, between day 1 and weaning weight
[*]Statistically significant at $p<0.05$
[**]Statistically significant at $p<0.01$

Table 6. Effect of patin fish-based nutritional intervention on heart rate, respiration rate, body temperature, and physical appearance in malnourished pregnant rats

Variables	Healthy control	Malnourished control	Malnourished group	Patin oil	Patin meat	Patin oil and meat	*p*-value[1]
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Heart rate (beats/minutes)	342.00±9.00^{a}	216.00±8.00^{c}	291.00±18.73^{b}	324.00±23.81^{a}	300.00±44.40^{a}	318.00±13.75^{a}	0.021^{1*}
Respiration rate (breaths/minutes)	112.00±12.49^{a}	69.00±3.00^{c}	102.00±9.00^{b}	111.00±9.00^{a}	108.00±3.00^{a}	110.00±11.36^{a}	0.025^{1*}
Body temperature (°C)	36.00±0.50^{a}	33.83±0.76^{c}	34.67±0.58^{b}	35.10±0.17^{a}	35.03±0.56^{ab}	35.23±0.15^{a}	0.023^{1*}
Fur condition	Clean, smooth	Dull, rough	Slightly dull	Clean, smooth	Clean, smooth	Clean, smooth	
Eyes	Red	Pale	Slightly pale	Red	Red	Red	
Activity	Active	Inactive	Less active	Active	Active	Active	

[a-c]Analyzed using Duncan post-hoc test; different superscript letters within the same row indicate a significant difference at $p<0.05$
[1]Analyzed using ANOVA test
[2]Analyzed using paired student t-test
[*]Statistically significant at $p<0.05$

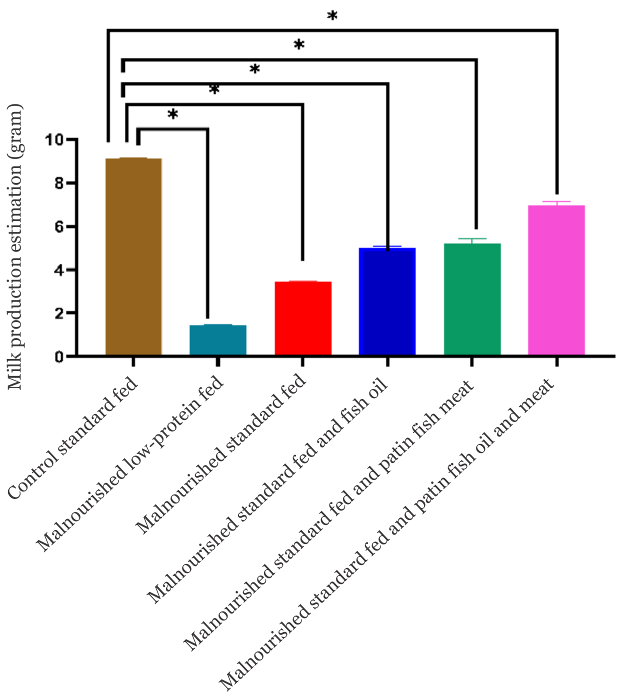

Figure 1. Effect of patin fish-based nutritional intervention on milk production of lactating malnourished rats. *Statistically significant at $p<0.05$.

Discussion

Malnutrition had significant and long-term effects on social, medical, economic, and developmental aspects at individual, familial, community, and national levels [25]. Malnutrition is frequently associated with impaired immune function due to inadequate nutrient availability, which weakens immune responses and increases susceptibility to infections [26]. This cycle of malnutrition and illness further exacerbated nutritional deficiencies through reduced appetite and impaired nutrient absorption [26]. At the cellular level, malnutrition disrupted homeostasis by impairing key signaling pathways involved in energy metabolism, inflammation, and protein synthesis [27]. Deficiencies in proteins, amino acids, and essential fatty acids inhibited the activation of the mechanistic target of rapamycin (mTOR), a critical regulator of protein synthesis and tissue growth [28]. Reduced mTOR activity promoted muscle catabolism, contributing to maternal weight loss [29]. During lactation, insufficient essential amino acids impaired milk protein synthesis, leading to decreased milk production and potentially compromising neonatal nutrition [29,30].

The mTOR pathway functioned as a central regulator of protein synthesis and cell proliferation, primarily activated by amino acids, insulin, and growth factors [31]. Nutrient deficiencies, particularly amino acid insufficiency, suppressed mTOR activation, thereby impairing protein synthesis and cell growth. The activation of mTORC1 required adequate amino acid availability and growth factor signaling [32]. Once activated, mTOR promoted protein synthesis by phosphorylating downstream targets, including S6 kinase (S6K) and eukaryotic translation initiation factor 4E-binding protein (4E-BP1), facilitating ribosomal function and translation initiation [29].

Malnutrition increases oxidative stress and inflammation by activating the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, a key regulator of cellular inflammatory responses [33]. Under nutrient deficiency or stress conditions, NF-κB induces the expression of pro-inflammatory cytokines, including interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), contributing to tissue damage and impaired cellular function [34]. Additionally, malnutrition reduces insulin-like growth factor-1 (IGF-1) production, disrupting cell and tissues growth. IGF-1 signaling through insulin-like growth factor receptor 1 (IGF-1R) activates the phosphatidylinositol 3-kinase/protein kinase B (PI3K-Akt) pathway, which plays a critical role in cell proliferation and survival [35]. Deficiencies in this pathway further exacerbate tissue dysfunction and metabolic dysregulation [34].

Patin-based nutritional interventions, including oil, meat, and their combination, effectively increased total protein levels in malnourished mother rats. Recent study has increasingly explored the effects of patin-based supplementation in animal models, particularly in rats [13]. The administration of patin oil has been shown to elevate hemoglobin levels and body weight in anemic Wistar rats, an effect attributed to its high-quality protein content and omega-3, -6, and -9 fatty acids [15]. Fish-derived proteins are rich in essential [36]. Additionally, the high bioavailability of protein from freshwater fish, such as patin, facilitates efficient absorption and utilization for protein synthesis [37]. However, although protein levels improved following the intervention in this study, the values remained lower than those in the healthy control group. Physiologically, essential amino acids derived from digested patin protein enter the bloodstream and are transported to tissues requiring repair, thereby supporting protein synthesis [38]. Patin protein supplementation may accelerate recovery in malnourished conditions by providing adequate amino acids to restore tissue integrity [39]. These amino acids contribute to the restoration of structural proteins, enzymatic functions, and immune responses, ultimately improving tissue repair and metabolic homeostasis. Additionally, the bioavailability of amino acids from patin protein may enhance the efficiency of protein synthesis, further promoting tissue recovery in malnourished states.

Patin-based nutritional interventions, including oil, meat, and their combination, effectively increased albumin levels in malnourished mother rats in this study. In those fed a low-protein diet, albumin levels declined due to insufficient protein intake necessary for hepatic albumin synthesis [40]. This reduction was mitigated by supplementation, as the fatty acids in patin oil provided essential energy for metabolic activation, while the protein from patin meat supplied amino acids critical for albumin production [40]. Improved nutrient availability restored mTOR pathway activity at the cellular level, thereby enhancing protein synthesis [41]. Patin oil-based nutritional intervention also provides omega-3 fatty acids, particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are essential for fetal development and maternal health [42]. These fatty acids facilitated lipid metabolism, reduced inflammation, and enhanced nutrient absorption, contributing to improved body weight [43]. At the cellular level, omega-3 fatty acids enhanced membrane fluidity and modulated gene expression related to lipid metabolism and inflammation [44]. At the tissue level, it promoted healthy adipose tissue formation and enhanced protein synthesis, supporting muscle and tissue growth [45]. During lactation, these physiological adaptations played a critical role in sustaining milk production and facilitating postpartum recovery [46].

Patin oil, rich in energy-dense lipids, supported recovery from nutrient deficiencies in malnourished rats by enhancing energy metabolism and promoting weight gain [39]. Omega-3 fatty acids from fish oil regulated fatty acid synthesis and storage, contributing to improved body composition [47]. During lactation, energy demands increase substantially as mammary gland activity intensifies, requiring higher nutrient and energy intake for milk production [48]. In this phase, metabolism shifts from fat storage to fat utilization as a primary energy source [49]. However, excessive fatty acid intake from patin oil may suppress glucose utilization, potentially impairing lactose synthesis in milk [50].

In the low-protein diet group in this study, reductions in heart rate, respiration rate, and body temperature indicated compromised cardiac and respiratory function, as well as impaired thermoregulation [51]. These changes likely resulted from decreased metabolic activity, leading to diminished organ function [51]. Patin nutritional intervention mitigated these declines, restoring physiological parameters closer to those observed in the healthy control group. In healthy rats, heart rate typically ranges from 300–450 beats per minute [51], respiration rate from 85–115 breaths per minute [52], and body temperature from 36.5–38.0°C [51]. Patin-based supplementation, rich in omega-3 and omega-9 fatty acids, enhanced essential amino acid intake, directly activating the mTOR pathway [53]. Additionally, omega-3 and omega-9 modulated inflammation by downregulating pro-inflammatory gene expression via the NF-κB pathway, creating an anti-inflammatory environment that optimized mTOR function [53]. Fish-based supplementation, particularly with omega-3 and omega-6, restored mTOR activity by supplying essential amino acids and fatty acids, which are critical for maintaining muscle mass and supporting milk production [54].

Omega-3 enhances adenosine monophosphate-activated protein kinase (AMPK) activity, regulating energy metabolism, promoting fatty acid oxidation, and improving cellular energy balance [55]. AMPK activation also stimulates mitochondrial biogenesis, increasing oxidative capacity and overall cellular energy production [57]. Additionally, omega-3 and omega-9 from patin-based nutritional intervention demonstrate anti-inflammatory properties by suppressing the NF-κB pathway, reducing pro-inflammatory cytokine levels, and mitigating oxidative stress [53]. Omega-3 further interacts with peroxisome proliferator-activated receptor gamma (PPAR-γ), which inhibits NF-κB activation and promotes anti-inflammatory gene expression, contributing to improved metabolic and inflammatory homeostasis [45].

Patin-based nutritional intervention enhances essential amino acid availability, promoting IGF-1 synthesis and facilitating tissue growth recovery [54]. Omega-3 further supports IGF-1 signaling by improving receptor sensitivity and reducing insulin resistance, thereby optimizing phosphatidylinositol 3-kinase (PI3K)-Akt pathway activation for tissue repair [54]. Leptin and ghrelin, key regulators of satiety and food intake, are disrupted in malnutrition, with leptin levels decreasing to conserve energy [54,57]. Patin-based supplementation, rich in healthy fats, restores leptin levels, aiding in appetite regulation and preventing muscle catabolism for energy utilization [58]. Additionally, omega-3 and omega-9 fatty acids from fish-based supplements contribute to autonomic nervous system restoration by improving neuronal membrane fluidity and enhancing neurotransmission [45]. Omega-3 plays a critical role in neurotransmitter regulation, influencing cardiovascular and respiratory function. Moreover, omega-3 and omega-9 activate peroxisome proliferator-activated receptor gamma (PPAR-γ), improving lipid metabolism and mitigating excessive AMPK activation [59]. Consequently, these effects collectively enhance energy production, improve physical activity levels, and restore body temperature homeostasis [60]. A previous study reported that patin contains 37% omega-9, which provides additional health benefits [61]. The composition and quality of patin are influenced by environmental conditions, diet, and tissue type, resulting in variations in fatty acid profiles, stability, and overall effectiveness [62].

These findings hold significant clinical implications for the development of targeted nutritional interventions aimed ats addressing malnutrition in pregnant women. Pregnancy imposes increased metabolic and nutritional demands to support fetal growth, maternal tissue expansion, and hormonal regulation. Malnutrition during this critical period can lead to adverse maternal and fetal outcomes, including intrauterine growth restriction, low birth weight, preterm birth, and long-term developmental deficits in offspring. Patin-based, rich in essential amino acids, omega-3, and omega-9 fatty acids, have the potential to enhance maternal physiological adaptation by improving protein synthesis, lipid metabolism, and energy homeostasis [37]. The anti-inflammatory and metabolic regulatory properties of these nutrients may contribute to reducing pregnancy-related complications, including gestational hypertension and insulin resistance, thereby supporting overall maternal health. Furthermore, this research underscores the need for expanding nutritional strategies beyond conventional interventions such as fortified staple foods and synthetic supplements. The bioavailability and synergistic effects of naturally derived nutrients from patin could offer a superior alternative to isolated micronutrient supplementation. Incorporating such dietary interventions into maternal healthcare programs may provide a cost-effective, culturally acceptable, and sustainable approach to mitigating malnutrition in resource-limited settings.

Despite the promising results observed in this study, several limitations should be considered. The assessment of physiological parameters focused primarily on metabolic and inflammatory responses without evaluating critical aspects of reproductive physiology, such as ovarian function, placental development, and fetal growth trajectories. Additionally, micronutrient status, including levels of iron, folic acid, and vitamin D, which are essential for maternal and fetal health, was not examined. Given these gaps, further research is warranted to comprehensively evaluate the impact of patin supplementation on maternal and fetal outcomes. Future studies should prioritize well-controlled clinical trials in human populations, particularly among pregnant women experiencing malnutrition, to validate these findings. Such trials should include rigorous assessments of maternal nutritional biomarkers, fetal development parameters, and long-term neonatal health outcomes. Understanding the optimal dosage, duration, and safety profile of patin supplementation will be critical in determining its clinical applicability. Additionally, exploring its integration with existing prenatal nutritional programs could enhance maternal healthcare strategies, ultimately improving pregnancy outcomes and reducing the burden of malnutrition in vulnerable populations.

Conclusion

Patin fish-based nutritional intervention effectively enhanced protein metabolism, hematological parameters, and overall physiological health in malnourished maternal rats, with patin oil demonstrating the most pronounced effects. This intervention significantly improved total protein, albumin, and hemoglobin levels while promoting weight gain, milk production, and physiological recovery. Additionally, enhancements in heart rate, respiratory rate, body temperature, physical appearance, and activity indicated a positive impact on systemic homeostasis. These findings highlight the potential of patin-based supplementation in addressing maternal malnutrition.

Ethics approval

Protocol of this study was reviewed and approved by Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia (Approval number: 147/KEH/SKE/XII/2023).

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author.

Declaration of artificial intelligence use

This study used artificial intelligence (AI) tool and methodology of which AI-based language model ChatGPT was employed in the language refinement (improving grammar, sentence structure, and readability of the manuscript). We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

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