Development of mackerel meal-based RUFF (*Ready-to-Use Fish Food*) product with the addition of sardine, soybean, and red palm oil in malnourished rat models

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Abstract. RUFF (Ready-to-Use Fish Food) has been formulated to address severe acute malnutrition (SAM) in children under five. However, its effect on malnourished models has not been reported. This study explains how mackerel meal-based RUFF with sardine, soybean, and red palm oil affects body weight in malnourished rats. Using a completely randomized design, 25 male Sprague-Dawley rats were split into five groups: reference (standard diet), control (protein deficiency 5%), protein-deficiency + sardine oil treatment (PD FO; RUFF A), protein-deficiency + soybean oil treatment (PD SO; RUFF B), and protein deficiency + red palm oil treatment (PD RO; RUFF C). After 14 days of inducing malnutrition, the treatment groups received RUFF. The study found that the RUFF formulation met the nutritional standards set by FAO and WHO for RUTF (Ready-to-Use Therapeutic Food). In terms of growth, the body weight of the treatment group increased significantly. Statistical analysis showed significant differences in the PD FO (p = 0,001), PD SO (p = 0,007), and PD RO (p = 0,001) treatment groups. This study shows that RUFF products, adding sardine, soybean, and red palm oil, can improve the nutritional status of malnourished animal models, which can be further studied in human subjects.

1 Introduction

Malnutrition is a condition that occurs when the intake of nutrients is inappropriate, in the form of excess or deficiency. Malnutrition is particularly prevalent in children under five years old. Malnutrition manifests as underweight or low weight for height (acute malnutrition), underweight or low weight for age (chronic malnutrition). Malnutrition during child development and growth can lead to acute conditions [1]. Acute malnutrition (wasting) is classified as severe acute malnutrition (SAM) or moderate acute malnutrition (MAM) based on anthropometric measurements and clinical status [2].

The World Health Organisation (WHO) indicates that the prevalence of wasting among children under five is 52 million, and SAM is 17 million. Basic health research (Riskesdas) by the Ministry of Health reported that the rate of acute malnutrition in Indonesia in 2018 was 10.2% [3], then the survey of the nutritional status of Indonesian toddlers (SSGBI) showed the number of children experiencing acute malnutrition in 2019, 2021, and 2022 was 7.4%, 7.1%, and 7.7% respectively [4]. These prevalence rates indicate that the problem of acute malnutrition in Indonesia is still relatively severe and far from the target in 2025 of less than 5%.

Malnutrition has negative impacts such as increasing the risk of infection, reducing the immune system, affecting the function and recovery process of organ systems [5,6], disrupting physical and mental growth, cognitive development [7-9]. Meanwhile, severe acute malnutrition will create a 5-20 times greater chance of death in children under five [10]. Therefore, severe acute malnutrition is classified as a serious disease that needs to be treated immediately to prevent further severity.

SAM rehabilitation treatment can utilize appropriate food processing approaches to improve nutrient absorption, such as *ready-to-use therapeutic food* (RUTF). RUTF is intended to fulfill the nutritional needs of SAM children with high energy and protein content. As one of the sources of protein and high calories, fish can produce RUTF, thus called RUFF. Indonesia has a diverse marine and fisheries sector that has the potential to be optimally developed. RUFF is a development product of RUTF, with similar nutritional value and energy content as RUTF. One of the RUFF products developed is Num'Trey, a local fishmeal-based product alternative to fortification from milk-in products [11].

Mackerel (*Scomberomorus commerson*) is a seawater fish found easily in Indonesian fishery, producing 203,759.46 tonnes in 2021 [12]. Mackerel

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fish is high in protein (21.4 g in 100 g) and one of the rich sources of omega-3 fatty acids at 32.08% [13]. Based on studies, fishmeal's protein content is significant and high in macro- and micro-minerals [14]. In this case, mackerel fish processed as a meal can increase protein digestibility and improve growth nutrition.

In preliminary research, mackerel meal-based RUFF was developed in paste form and administered to male Sprague-Dawley rats for 28 days. The mackerel meal-based RUFF intervention group produced a higher final body weight (142.45 ± 10.88 g) than the normal group with a standard diet (113.56 ± 11.20 g), resulting in body weight growth of 3.19 ± 0.36 g and 2.01 ± 0.38 g for the RUFF intervention group and the standard group, respectively. However, research on the effect of variations in adding oil as a lipid source in RUFF products on malnutrition model rats has not been studied.

Variations of the addition oils in the preparation of RUFF are expected to optimize the improvement of nutritional status in malnourished rat models, especially in growth and development. This potential can be found in sardine, soybean, and red palm oil. Sardine oil is an animal source of omega-3, soybean oil is an alternative omega-3 from plants, and red palm oil is a source of antioxidants such as tocopherols and carotenoids. Therefore, the research aimed to assess the potential of developing RUFF based on mackerel flour with the addition of sardine, soybean, and red palm oil to recover growth and development in malnourished subjects.

2 Method

2.1 Time and Materials

This research was conducted for three months, from March 2024 to May 2024, at the Joint Laboratory of Experimental Animals, Department of Food Science and Technology, Faculty of Agriculture (FATETA) and SEAFAST Center, IPB University, Bogor, West Java.

The main ingredient of this research is the development of RUFF with mackerel and mung bean meals to optimize protein sources. The ingredients for RUFF were developed by FAO and WHO based on CODEX CXG 95-2022 [15]. The composition of RUFF includes mackerel meal, mung bean meal (Lingkar Organik), casein (Tatua 100, New Zealand), peanut paste, vitamin-mineral mix (Fortitech, Malaysia), tapioca meal (Gunung Agung), sugar (Rose Brand), sardine oil (PT. Sumber Yala Samudera, Muncar), soybean oil (Mazola), and red palm oil (Palm Oil Research Center, North Sumatra).

Mackerel meat filets were used in the production of fishmeal. This study used standard (S) and protein-deficient (PD) diets. The S feed followed the AOAC method [AOAC 2005]. The composition of S feed was casein (10%), corn oil (8%), mineral mix (5%), vitamin mix (1%), CMC (1%), water (5%), and corn starch (70%). PD feed is a modified S feed with a protein restriction of 5% of the day's total energy [Lewicki et al. 2018]. Based on previous research, the PD feeding experiment with a protein percentage of 5% for 21 days caused the growth of rats to decrease, marked by a decrease in body weight, thus proving that the adequacy of energy and nutrients was not met [16].

2.2 Procedure

Male Sprague-Dawley rats (*Rattus norvegicus*) aged 21 days weighing approximately 50-60 g were obtained from Breeding Rat and Mice, Dramaga Agri Satwa. The Animal Ethics Commission of IPB University approved the research protocol with number 260-2024 IPB. Rats used as experimental animals have fulfilled the procedures of the Guidelines for Reporting Animal Experiments (ARRIVE).

Five rats were used for each group based on Federer's formula. Therefore, 25 rats were needed for the five different groups. The rats were acclimatized for a week to adjust to consistent physical and psychological conditions. Next, the rats were grouped into one reference group (N), one control group (C), and three protein-deficient treatment groups given the RUFF product intervention, namely the protein deficiency group with sardine oil-based RUFF (PD FO), the protein deficiency group with soybean oil-based RUFF (PD SO), and the protein deficiency group with red palm oil-based RUFF (PD RO).

Group N and Group C were fed with S and PD in powder form for 28 days. The treatment groups that had been malnourished with PD feeding for 14 days were continued with RUFF product intervention. RUFF was given for 14 days in paste form. Feed and water were served ad libitum during the experiment.

2.3 Fishmeal dan RUFF Production

The method to produce mackerel meal begins with selecting and cleaning fresh fish, slicing the fish, drying, and sieving [17]. Fish cleaning involves removing non-edible parts of the fish, and then rinsing it until there is no blood or slime. The next step is drying the fish filets at 50°C for around nine hours to remove the water. The temperature is set at 50°C to retain nutritional value, especially the protein content. Following drying, the fish meat was crushed with a food processor and sieved with a 60-mesh sieve to produce fine fishmeal. The fishmeal was packed in a double pouch seal, and food-grade silica was added.

The preparation of RUFF begins with the preparation of ingredients and weighed according to the formulation. Roasted peanuts were crushed with a blender until they had a peanut butter-like texture. Dry ingredients (mackerel flour, mung bean flour, casein, sugar, tapioca flour) were then mixed. Sardine oil, soybean oil, red palm oil, and peanut butter were added and homogenized with a blender for 30 seconds until all ingredients were homogeneous. Finally, the remaining ingredients (vitamin-mineral premix) were added to the mixture while homogenized at low speed to stabilize the particle size and texture.

2.4 Data Collection and Data Analysis

The data collected throughout the research were rat feed leftover and rat body weight. Feed leftovers were calculated every day, and the body weight of the rats was weighed every three days.

The research design used was completely randomized. Research results were computed using Microsoft Excel for simple calculations and IBM SPSS Statistics 29 software for one-way analysis of variance (ANOVA) test and independent paired samples T-test. Data are shown as mean ± standard deviation (SD). If there were significant results, Duncan's test was conducted with a significance level of α 0.05 for further analysis.

3 Result and Discussions

This study developed RUFF to fulfil the need for amino acids to overcome malnutrition. Essential amino acids cannot be synthesized from the body, so they must be obtained through food and have an essential role for children who are malnourished. Mackerel and mung bean meals with potential protein content are utilized to increase local products and the availability of RUFF in Indonesia. However, using mackerel and mung bean meal has partially replaced the protein needs of milk powder, thus the need to use casein. The RUFF formula in this study uses ingredients such as mackerel meal, mung bean meal, peanut paste, and casein as protein sources. FAO and WHO have established requirements for the amount of amino acids in RUFF products in mg/g protein [15].

Table 1. Amount of protein in RUFF per 100 g

Raw Materials	Protein (%)	Protein (g)
Mackarel meal	80.16	8.02
Mungbean meal	24.76	2.23
Peanut paste	24.00	2.40
Casein	93.00	5.46
Total		18.10

Table 1 shows how the contribution of each ingredient to the total amount of protein. Based on previous studies, mackerel meal (86.48%) and mung bean meal (24.76%) have high protein content, contributing 8.02 g and 2.23 g of protein per 100 g of RUFF, thus providing the primary source of protein and amino acids in the overall composition. Peanut paste, with 24% protein and essential amino acids, complemented the contributions of mackerel and mung bean meals with an addition of 2.40 g protein per 100 g RUFF. The highest casein protein content, at 93%, enriched the amino acid profile and ensured that the RUFF formulation met or exceeded FAO and WHO standards, contributing 5.46 g of protein per 100 g of RUFF.

Table 2 presents RUFF's estimated essential amino acid content, calculated based on the amount of protein from ingredients such as mackerel meal, mung bean meal, peanut paste, and casein. Based on the amino acid composition and comparison with FAO and WHO standards, the RUFF in this study has the potential as a milk protein supplement in nutrition programs for children with SAM. RUFF can effectively fulfil the protein and amino acid requirements needed to meet the weight gain target of 10 g/kg BW/day in infants and children aged 6-59 months [18].

Table 2. Estimation of amino acid profile in RUFF per 100 g

Amino acid	Total (mg/18.10 g)	Total (mg/g protein)	FAO standard (mg/g protein)
Histidine	607.34	33.55	24
Isoleucine	759.79	41.97	34
Leucine	1461.19	80.71	70
Lysine	1198.75	66.21	65
SAA*	686.62	37.93	31
AAA**	1417.85	78.32	63
Threonine	692.96	38.28	36
Tryptophan	244.07	13.48	10
Valine	951.69	52.57	46

*SAA (sulphur amino acid) = methionine + cysteine
**AAA (aromatic amino acid) = phenylalanine + tyrosine

This study was conducted in three stages over five weeks. The first stage occurred in the first week (7 days) and was called the adaptation phase, where all rats were fed a standard diet containing 10% protein. Then, in the second stage (weeks 2 and 3), the malnutrition phase was induced by feeding a protein deficiency (PD) so that the PD FO, PD SO, and PD RO groups experienced nutritional deficiency with a protein content of 5%. This phase simulates the condition of malnutrition, characterized by significant weight loss, dull and yellowish fur, and hair loss. Rats receiving the PD diet showed symptoms of malnutrition [19,20], whereas rats on the standard diet experienced normal growth and development. Finally, in the third stage of the intervention phase (weeks 4 and 5), rats were fed RUFF products based on mackerel meal to assess the recovery of growth and development.

Assessment of health conditions due to imbalances in energy, protein, and other nutrients by measuring growth and development rates. Long-term nutritional imbalances during critical ages, such as in children, can weaken the immune system and affect organ development [5,6]. The effectiveness of RUFF on rat growth was observed by body weight change by administering RUFF in three types: RUFF A based on

sardine oil, RUFF B based on soybean oil, and RUFF C based on red palm oil. Previously, the groups of rats were malnourished and fed recovery diets during the intervention phase.

Table 3. Changes in feed intake and body weight during malnutrition (n = 25)

Group	Total feed intake (g)	Initial weight (g)	Final weight (g)	p^2
N	112.70 ±33.65	56.00 ±3.54	58.60 ±3.97	0.073
C	84.42 ±10.07	49.00 ±2.00	44.00 ±1.87	0.007*
PD FO	84.54 ±21.74	49.60 ±1.34	44.20 ±1.10	0.003*
PD SO	85.99 ±21.25	51.00 ±2.65	44.80 ±2.77	0.001*
PD RO	96.84 ±14.11	53.40 ±2.30	49.00 ±2.83	0.001*
p^1	0.218	0.001*	0.000*	

Values shown are mean ± standard deviation, *There is a significant difference ($p \leq 0.05$) $p^{1)}$ ANOVA between groups, followed by Post Hoc Duncan's test at $\alpha = 5\%$, $p^{2)}$ Paired T-test at $\alpha = 5\%$

Table 3 shows the feed intake and body weight changes during malnutrition. Over 14 days, the reference group (N) showed a gradual weight gain of 2.60 ± 2.41 g, but the change was not statistically significant (p=0.073). In contrast, the control group (C) fed the PD diet had a significant weight loss of -5.00 ± 2.24 g, and the PD FO, PD SO, and PD RO treatment groups also showed significant reductions of -5.40 ± 1.95 g, -6.20 ± 1.79 g, and -4.40 ± 1.67 g.

Figure 1 shows the trend of weekly body weight changes in all groups of rats. The trendline in group C (fed PD) was lower than that of group N and the treatment groups (PD FO, PD SO, and PD RO). Consistent with Table 4, group C had a significant weight loss of -4.60 ± 2.61g. The low protein intake in group C, which was fed a diet with a protein intake of less than 6%, was unable to support normal growth, resulting in growth retardation [21]. Earlier studies have shown that malnutrition caused by nutrient deficiencies can inhibit organ growth and development [7,8,22].

Table 4 shows rats' total change in feed intake and body weight before and after RUFF intervention (n = 25). In a malnourished state, decreased feed intake for 14 days can contribute to weight loss in group C [23]. Skeletal muscle tissue is sensitive to protein deficiency because it acts as the protein reserve of the body [19,24]. In another case, although the PD diet was fed on an isocaloric basis with the standard diet, caloric reduction and micronutrient deficiencies occurred.

Long-term protein deficiency can affect calorie absorption and energy metabolism in the body. Furthermore, this condition affects the CoA-synthetase enzyme and increases fatty acid oxidation [25,26]. If energy storage capacity is maximal, intake of supplemental feed is restricted [27], further reducing feed consumption trends.

Table 4. Changes in feed intake and body weight during before and after intervention (n = 25)

Group	Total feed intake (g)		Body weight (g)	
	Before	After	Before	After
N	112.70 ±33.65	93.76 ±11.27	58.60 ±3.97	60.20 ±5.36
p^2	0.364		0.298	
C	84.42 ±10.07	86.60 ±9.27	44.00 ±1.87	39.40 ±3.65
p^2	0.424		0.020*	
PD FO	84.54 ±21.74	88.69 ±21.00	44.20 ±1.10	89.20 ±12.07
p^2	0.414		0.001*	
PD SO	85.99 ±21.25	101.06 ±26.74	44.80 ±2.77	92.40 ±23.14
p^2	0.095		0.007*	
PD RO	96.84 ±14.11	83.52 ±13.76	49.00 ±2.83	84.40 ±11.06
p^2	0.043*		0.001*	
p^1	0.218	0.564	0.000*	0.000*

Values shown are mean ± standard deviation, *There is a significant difference ($p \leq 0.05$) $p^{1)}$ ANOVA between groups, followed by Post Hoc Duncan's test at $\alpha = 5\%$, $p^{2)}$ Paired T-test at $\alpha = 5\%$

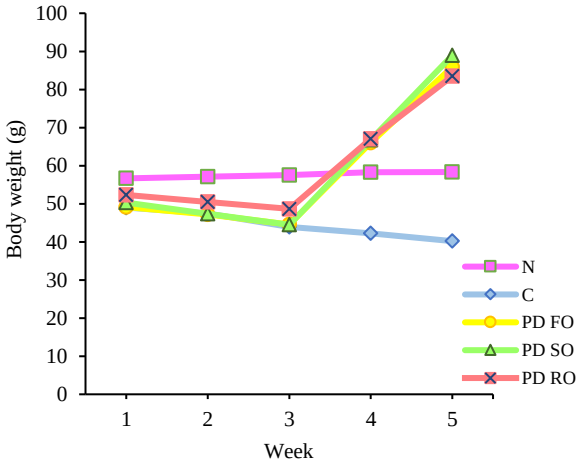

Figure 1. Average rat's body weight changes during experiment

Figure 1 presents the effect of RUFF type and rat condition on body weight gain. The average body weight of all groups increased to twice the initial weight after 14 days of RUFF intervention. This study's mackerel meal-based RUFF product showed almost the same effect as milk-based RUFF. As a high-calorie product designed according to RUTF guidelines, RUFF can help children recover from malnutrition [28]. The RUFF in this study had caloric values of 524,020 kcal

(RUFF A), 521,680 kcal (RUFF B), and 523,786 kcal (RUFF C), respectively. The increase in body weight in the three RUFF groups showed no significant difference.

The high omega-3 content from the sardine and soybean oil used in RUFF can increase body weight without increasing fat mass. Omega-3s, especially DHA, can contribute to muscle mass and height gain [28-30]. FAO recommended an omega-6/omega-3 ratio of 5:1 to 10:1 in a balanced diet to support child growth and development. Based on previous studies, the omega-6/omega-3 ratio is 2.5:1 or 1:1 [28,31], which can adequately support neuronal and genetic development. In the RUTF requirements, omega-6 should be a maximum of 780 mg/100 kcal, while omega-3 should be a minimum of 110 mg/100 kcal [15].

RUFF C based on red palm oil (RPO) contains various phytonutrient compounds such as carotene, tocopherols, tocotrienols, phytosterols, squalene, and ubiquinone, which have potential antioxidant and other biological activities. The antioxidants in RPO can help increase the body weight of malnourished rats. Oxidative stress due to nutrient deficiency can damage body tissues and inhibit metabolism. Carotene in RPO reduces oxidative stress by neutralizing free radicals, repairing damaged tissues, and supporting cell regeneration [32]. Thus, the body's recovery process and weight gain can be optimized. Carotene also helps strengthen the immune system so that available energy can be more effectively utilized for tissue repair.

RUFF C fed to the PD RO group contains tocoromanols in the forms of tocopherols and tocotrienols, which function as vitamin E with antioxidant activity. Vitamin E helps to improve the efficiency of limited protein metabolism and supports muscle and bone formation [33,34]. Also, the carotenoids in RUFF C contribute to increased appetite, resulting in increased feed intake in the PD RO group. Vitamin A derived from carotenoid conversion also supports digestive function, improves nutrient absorption, and thus ultimately contributes to increased body weight.

4 Conclusion

A ready-to-use fish food (RUFF) based on mackerel meal was developed to help treat SAM. RUFF promotes recovery and growth by providing a product rich in energy, protein, omega-3, and antioxidants. The essential amino acid profile of RUFF has been compared with the standards set by FAO and WHO, and the results show that RUFF exceeds FAO and WHO requirements to support optimal growth. In-vivo test results showed a significant increase in body weight in the PD FO, PD SO, and PD RO groups during the intervention phase, indicating that RUFF is an effective product.

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