In Vivo Study of Fish Meal-Based Ready-To-Use Fish Food (RUFF) on Growth and Cognitive Development

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Abstract

The prevalence of global malnutrition among children under 5 remains far from the SDG 30 target. As a countermeasure, Ready to Use Therapeutic Food (RUTF) was developed as an intervention and recovery measure. However, RUTF formulations use milk as the primary source of protein, which is scarce in some countries. That is why RUFF (Ready to Use Fish Food) is made, as a development of RUTF, using fish meal to create an even paste, improve formulations, and maximize the utilization of natural resources. This study aimed to create mackerel and pangas catfish RUFF and assess the impact of diet on growth and cognitive development in rodents. RUFFs were produced by preparing fish meals, which were then used to prepare RUFF formulations and were administered to rats for 28 days. Throughout the intervention, rats were counted for food consumption, weighed weekly, and subjected to the classic labyrinth. The pangas catfish and mackerel groups had the most significant weight gain. After 28 days, the rats were sacrificed to analyze brain development by weight and volume, as well as fatty acid profiles in blood plasma using GC- FID. The mackerel group had the largest brain weight and volume, at 1.747 g and 1.833 mL. The mackerel group also had the best cognitive performance and fastest average time in the labyrinth test. This supports that the mackerel group had the highest omega-3 content in both fish powder and blood plasma. The study concluded that experimental animals fed mackerel RUFF showed the best growth and cognitive development.

Keywords: cognitive development, growth, mackerel, pangas catfish, RUFF

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Introduction

Malnutrition is a health condition characterized by excess or shortage (imbalance) of calories, proteins, and other nutrients (Soeters et al., 2017). The young population commonly encounters malnutrition, especially children under five, who need sufficient nutrition for cognitive development and healthy growth (Adebisi et al., 2019). An imbalanced diet at crucial ages impacts physical and mental formation and growth and damages cognitive development (Wagh, 2018; Woldehanna et al., 2017). One strategy to meet the nutritional demands of malnourished children is to use Ready-to-Use Therapeutic Food (RUTF).

RUTF products comprise a homogenous combination of fat and water-soluble components that do not require additional preparation steps for ingestion. They are realistically created using sanitary facilities and circumstances (Phuong et al., 2014). The creation of RUTF itself is based on the CODEX Committee on Nutrition and Foods for Special Dietary Uses (2022), with the primary requirements for the development of RUTF being macronutrients necessary for growth, including 50% milk protein and a protein digestibility-corrected amino acid score (PDCAAS) value of 0.9. However, milk is scarce and expensive in some countries. Fish are a rich source of protein and calories and can be used as a substitute for milk. Ready- to-Use Fish Food (RUFF) is the development of RUTF complete nutrition. RUFF is also being widely developed, including Num'Trey, a local product from Cambodia manufactured from fish meal, as an alternative fortification for milk (Sigh et al., 2018).

The prevalence of undernourished children under five years of age in Indonesia, based on the Indonesian Nutrition Status Survey (SSGI) (2023), has declined from 24.4% in 2021 to 21.6% in 2022. Nevertheless, this is still beyond the goal of decreasing the stunting rate to 14% by 2024. As the second largest producer of fish in the world, Indonesia can fulfill its domestic fish consumption demands (FAO, 2020). Fish, also known as an affordable and sustainable source of protein, can alleviate malnutrition worldwide. It offers essential needs for both micro-and macronutrients, is high in fat, and its protein is readily digested. Fish are also high in essential polyunsaturated fatty acids (PUFAs), such as linoleic acid/LA (precursor of omega-6), alpha-linolenic acid/ALA (precursor of omega-3), and long-chain omega-3 fatty acids, which arevital for growth and cognitive development. Based on Sigh et al. (2020) and Yameogo et al. (2017), whole blood profile for malnutrition, especially stunting and Moderate Acute Malnutrition (MAM) with weight under the standard average child weight, is associated with low PUFA, LA, Arachidonic Acid (AA), and omega-6.While omega-3 in malnutrition is associated with poor development and low cognitive ability (Adebisi et al., 2019; Awuchi et al., 2020; Kar et al., 2008; Manary, 2006; Saunders & Smith, 2010; Woldehanna et al., 2017).

Mackerel (*Scomberomorus commerson*) is a marine fish typically found in Indonesian seas. It is high in PUFA and almost tantamount to salmon and tuna, especially omega-3 fatty acids. According to Sahari et al. (2009), the omega-3 fatty acid content in fresh mackerel fish is 17.92% of the total fatty acids. *Pangasius hypophthalamus* is a freshwater fish originally found in the Mekong River, Thailand (Roberts & Vidthayanon,1991). The *Pangasius hypophthalamus*, also called the Pangas catfish, is a ubiquitous freshwater fish known as the Dori fish. This fish is also rich in fat compared to other freshwater fishes. Oil from the pangas catfish contains mono- and polyunsaturated fatty acids, which are beneficial to human health (Lestari et al., 2020). Pangas catfish are inexpensive, widespread, and of significant economic value. It is also easy to cultivate because it adapts quickly to its surroundings (Sokamte et al., 2020). As stated above, this study used mackerel as the source of marine fish and pangas catfish as the source of freshwater fish. Furthermore, this research aims to create products based on marine and freshwater fish meals to exploit rich resources while developing RUFF products. We also assessed the effects of diet on growth and cognitive development in rodents.

Material and Methods

Materials

This study used two types of fish: mackerel (a seawater fish) and catfish (a freshwater fish). Mackerel fish fillets (*Scomberomorus commerson*) were obtained from Muara Angke, North Jakarta, Indonesia. Pangas catfish fillets (*Pangasius hypophthalamus*) were obtained from Temanggung (Central Java, Indonesia). The handling processes of both fishes were sent on the same day from the stores using a cool box to prevent degradation of quality. When it was received, the fish were brought to the freezer to maintain quality.

Methods

Production of Ready-to-Use Fish Food (RUFF)

The production of RUFF, made from fish meal, has been modified and adapted from the standard of Ready-to-Use Therapeutic Food (RUTF) main guidelines, as outlined in CODEX (2022). Fish meals were prepared separately to create two products: mackerel meal and pangas catfish meal. These fish meals were produced using the methods of Mervina and Mariiyati (2012) and Noviana (2022) then employed in the optimal formulation based on Noviana's (2022) research. The RUFF formulation between the Pangas catfish and mackerel groups was adjusted to meet the available RUTF specifications for energy, protein, carbohydrate, and fat. Most crucially, it has been changed to correspond to the primary requirement of CODEX (2022), specifically PDCAAS 0.9, by following FAO standards (2017). The procedure started with processing the RUFF composition, which included rice flour, whole milk powder, fish meal, roasted peanuts, coconut oil, sugar, and a combination of vitamins and minerals (Table 1). However, the products themselves were prepared fresh before each feeding schedule in a sanitary manner and stored in sealed containers. This study did not include the packaging process, thus, further studies are needed.

Animal Studies

The animal research protocol was approved by the Institutional Review Board or Ethics Committee (The

Table 1. The formulation used in making RUFF is mackerel meal-based and pangas catfish meal-based

Composition (g/100g sample)	Sample	
	Pangas catfish	Mackerel
Fish meal	10	11
Roasted peanut	13	14
Full cream powder	11	10
Rice flour	27	27
Granulated sugar	10	10
Coconut oil	27	26
Vitamin and mineral mix	2	2
PDCAAS	0.95	0.99

Animal Ethics Committee School of Veterinary Medicine and Biomedical Sciences IPB University) (116/KEH/SKE/IX/2023 on September 18th, 2023). The study employed male Sprague-Dawley rats that were 21 days old and weighed 50–60 g each. The number of rats used in the study adhered to Frederer's formula and was determined to be a minimum of 24 or 6 rats per group, as recommended by Ridwan (2013). The rats were selected based on their uniform body weight and divided into four groups based on different feeding treatments: casein (AOAC, 1995) (Table 2), non-protein (based on AOAC, without adding casein, simulated as protein deficiency or malnourished) (Table 2), RUFF with mackerel fish meal (mackerel), and RUFF with pangas catfish meal (pangas catfish). During the study, the rats were provided with 15 gram of food and water ad libitum every day for 28 days throughout the study period. The RUFF ratio itself does not adjust to isonitrogenous or isocaloric. The administration was given as is to compare with casein as a control group, which, based on AOAC (1995), has met nutritional adequacy.

The rats were weighed, and consumption was counted weekly to measure physical growth during the intervention days. Neurobehavioral testing for cognitive development was conducted using the classic labyrinth test (Gasmi, 2018). Observations of activity and behaviour were carried out on all rats in repetition for three tests. Observations of activity and behaviour were carried out on all rats in repetition for three tests. The activity category based on Mirza (2012) was divided into four categories with a score of 1-4, whereas: very active = 4, active = 3, less active = 2, and inactive = 1. The description of each category score is as follows:

- Very Active (4): After entering the maze, continuously perform activities such as smelling and walking, climbing the maze walls, and reaching the finish point in less than 5 min.
- Active (3): After entering the maze, continue smelling and walking; climb the maze wall but do not reach the finish point.
- Less active (2): After entering the maze, the activity of smelling and walking is carried out for around 0.5 minutes; climbing the maze wall is rarely done, then it is silent and does not reach the finish point.
- Inactive (1): After entering the maze, the activities of smelling and walking are carried out for around 0.5 minutes; climbing the maze wall is not carried out, and then it is silent and does not reach the finish point.

After the intervention, termination was performed to collect blood plasma samples and brain organs as a follow-up to determine cognitive development. Blood plasma was collected through the heart, and the rat was previously anaesthetised using xylazine-ketamine. Next, the weight and volume of the brain were measured. Fatty acid profiling of blood plasma samples was carried out using GC-FID.

Fatty Acid Profiling in Fish Powder and Rat's Blood Plasma

Fatty acid profiling in fish meal samples was performed using a GC-FID (Gas Chromatography-Flame Ionisation Detector) GC-2010 Shimadzu (Kyoto, Japan). The samples were extracted using the direct extraction technique, referring to SNI 01-2891-1992. Meanwhile, the sample preparation used the AOAC (2012) 969.33 (Preparation of Methyl Ester BF3 Method: GC-FID) method. The injection procedure was performed using 007 cyanopropyl methyl silane (capillary column) from QUADREX with an injector temperature of 220oC and a detector temperature of 240oC; each injection volume was one µL. In this study, the FAME standard mixture was injected before the sample was injected into the column.

Data Analysis

The effect of different feeding treatments was observed using one-way analysis of variance (ANOVA)

Table 2 The formulation for casein and non-protein groups

Compositions (g/100 g sampel)	Sampel	
	Casein	NP
Casein	11.24	0
Corn oil	7.85	8
Mineral Mix	4.61	5
Vitamin Mix	0.94	1
CMC	1	1
Water	4.33	5
Maizena	70.03	80

with a confidence level of 5%. Tukey's post-hoc test was used to compare significant differences. An independent t-test was used to compare the proximate composition differences between mackerel andpangas catfish meal.

Results and Discussion

Physical Growth and Consumption Rate

The data revealed that allgroups experienced an increase in body weight every week (Table 3), and the highest growth rate throughout the intervention period was experienced by RUFF with pangas catfish meal. Its increase starts at an average of 57.9 grams and ends at 158.70 grams. At the same time, the pangas catfish group had an almost steady consumption rate, with the lowest average. It is noteworthy that RUFF is a high-calorie product that increases the weight of malnourished children. Based on a review by Tutunchi et al. (2020), a diet high in oleic acid or omega-9 fatty acid generally enhances fullness and lowers feelings of hunger because it can control appetite and food consumption. Furthermore, pangas catfish meal is known to have a high amount of oleic acid, not to mention the use of peanuts in the RUFF formulation, which is also high in omega-9 tissue and is the major PUFA in most internal organs (Hadley et al., 2016).

The rat's whole body primarily comprises saturated fatty acids (48.4% of total fatty acids), monounsaturated fatty acids were present in the second greatest amount (34.8%). monounsaturated fatty acids were present in the second greatest amount (34.8%). The total amount of n- 6 PUFA is 12.0%, which is more than 5-fold greater than the total n-3 PUFA. The n-6 PUFA with the highest content is LA (10.1%), followed by AA (1.4%). AA is the major PUFA in nearly every tissue, including brown adipose tissue (Hadley et al., 2016).

According to Rahmawaty and Meyer (2020), RUTF has considerable benefits in supporting the recovery process of malnourished patients in terms of weight and height gain to return them to normal growth. The RUFF, along with the RUTF guidelines, is designed to achieve the same aims. This can be attributed to the effect of high saturated fatty acids (SFA), which significantly contribute to weight gain. As fish meals are utilised in formulations, coconut oil and milk are also rich in SFA. Djordjevic et al. (2019) and Dupont et al. (1990) stated that coconut oil is high content is dominated by SFA content. Not to mention the usage of peanuts that are high in linoleic acid.

This study also suggests that the higher the omega-6 content in food, the higher the growth performance in terms of weight. Wang (2015) also indicated that omega-6 promotes adipogenesis and lipogenesis, thereby increasing fat mass. Indeed, a high content of omega-3, especially Docosahexaenoic Acid (DHA), could increase weight gain but not fatness. Instead, it boosts protein muscle generation and increases height (Cardino et al., 2023; Rahmawaty & Meyer, 2020). It could be concluded that mackerel meal-based RUFF and pangas catfish meal-based RUFF were high in calories, which affected weight gain (Table 5).

All groups received 15 g of food daily for the entire intervention day, and the residual rations were counted the next day. Table 3 shows that the non-protein (NP) group had the lowest growth rate. This trend in the non-protein group was affected by intake because it was only sufficient to sustain body weight. Insufficient protein leads to the storage of extra energy, such as fat, glycogen, or excretion (Escriva et al., 1991). Brown adipose tissue, which helps regulate body temperature, is formed by energy storage (Meyer et al., 1959; Swick & Gribskov, 1983; Yagi et al., 2019). Further increases in intake are limited when the energy storage capacity reaches a certain limit (de França et al., 2009; Escriva et al., 1991). As a result, the average ratio of consumption in the NP group was 6.84 ± 0.753 g, with a declining trend. This was why, despite an increase in body weight, even though it was not high enough compared to casein (as a control group), the average food consumption in the NP group was considered low and not significantly different.

Table 3. Rat’s growth parameters based on the weight and volume of the brain, also labyrinth test performance

Samples	Average Consumption (g)	Average Initial Weight (g)	Average Final Weight (g)	Growth Rate (g/day)
NP	6,84±0,753^{a}	57,80±6,015^{a}	78,10±11,444^{a}	0,73±0,380^{a}
Casein	9,02±0,737^{b}	57,22±4,055^{a}	113,56±11,195^{b}	2,01±0,380^{b}
Mackerel	7,15±0,717^{ab}	53,18±4,750^{a}	142,45±10,883^{bc}	3,19±0,362^{c}
Pangas catfish	6,64±0,733^{a}	57,90±7,430^{a}	158,70±11,139^{c}	3,60±0,370^{c}

Notes: Values in the same column followed by different letters (a, b, c) indicate significant differences at α = 0.05.

Brain Development

According to Stevenson et al. (2017), the brain accounts for only 2-3% of the body's mass but is responsible for the highest level of energy consumption. Various factors, including age and nutrient intake, influence brain mass. Malnutrition due to a deficiency of one or more nutrients can harm the energy storage process, not only slowing weight gain, but also affecting brain development. This is also found in the study by Santos de Souza et al. (2008), where malnourished children tend to have lower body and brain weights. As indicated in Table 4 and state before, notably, the non-protein group exhibited the lowest brain weight and weight gain in this study.

According to Silva et al. (2021), brain development is completed during the early phases of weaning and infancy. The brain growth spurt phase occurs in mice on the 7th–10th day. On the 20th day, the brain has attained 90–95% of its adult weight. Compared to humans, this occurs throughout infancy until early childhood. It will then achieve the maximal density of nerve cells with the myelination process in cognition, which is still happening on the 35th to 49th days of a rat's age or 12–18 years of human age. Subsequently, the brain attains adult brain weight with neurotransmitter maturation on days 60+ of rat age or 20+ of human age (Semple et al., 2013). Keeping brain areas on the developmental trajectory is necessary not only for encouraging behaviours provided by the individual regions but also, more critically, to promote time-coordinated growth of brain areas meant to operate together as circuits mediating complex behaviours (Cusick & Georgieff, 2016).

In addition, known as a critical period of cognitive development, early childhood (from birth to 6 years) is defined as an intensive phase of brain development throughout the human lifespan. Dynamic overproduction and pruning of synapses occur during this phase, initially in regions of the brain that receive information and then in association areas engaged in higher-level tasks, such as working memory, attention, and planning. Healthy brain growth during this era enables optimal cognitive development andbuilds a framework for future cognitive development (Carson et al., 2016; Cusick & Georgieff, 2016). If nutrition is unbalanced, such as in the non-protein group with the lowest brain weight (Table 4), children may have smaller brains because of the influence of energy storage. Therefore, weight decreases with less stored energy, causing brain mass to decline owing to reduced dendrites and increased oxidative stress (Ryzhavskii et al., 2019; Semple et al., 2013; Stevenson et al., 2017). Simpler dendrites reduce the concentrations of growth factors and neurotransmitters, causing weight loss in the brain and body. In addition, most early childhood intervention studies that have combined nutritional and psychosocial stimulation have reported long-lasting effects on cognition that sometimes last into adolescence (Cusick & Georgieff, 2016; Schneider et al., 2018).

Cognitive Performance

During the classic labyrinth test, experimental animals are assessed for their learning ability and ability to build spatial memories through a repeating procedure (Ranade et al., 2008; Sharma et al., 2010). In general, memory is classified into two categories: declarative memory (to answer 'what' and include knowledge of facts, locations, objects, and many more) and non-declarative memory (to respond 'how' and include habits). Declarative memory is then separated into spatial and semantic memories (facts). Spatial memory is produced during a specific event and generally contains detailed information, such as directions to a particular area (Sharma et al., 2010). Spatial memory is also divided into two terms: short-term memory (working memory), which is based on events from a specific trial and has a limit (within the day), and long-term memory (reference memory), which is formed over repeated attempts from the unchanging circumstances of a task (day to day) (Wang & Xu, 2007).

As early childhood is known to have the fastest spatial memory development from the learning process, the classic labyrinth test or complex alley maze is a suitable test. It is a neurobehavioural test with a stable route from trial to final testing. This test observes how the learning process significantly affects experimental

Table 4. Rat's cognitive development parameters based on the weight and volume of the brain, also labyrinth test performance

Sample	Parameter			
	Brain weight (g)	Brain volume (mL)	Labyrinth test score	Labyrinth test completion time
NP	1,382 ± 0.11[a]	1,567 ± 0.10[a]	3.56 ± 0.344[a]	235.17 ± 58.19[a]
Casein	1,630 ± 0.16[b]	1,567 ± 0.10[a]	3.62 ± 0.344[a]	205.28 ± 64.57[a]
Mackerel	1,747 ± 0.17[b]	1,833 ± 0.26[b]	3.83 ± 0.272[a]	176.39 ± 93.55[a]
Pangas catfish	1,617 ± 0.18[b]	1,600 ± 0.22[ab]	3.63 ± 0.298[a]	233.89± 39.63[a]

Notes: Values in the same column followed by different letters (a, b, c) indicate significant differences at α = 0.05.

animals' neurobehavioral, like exploratory behaviour, notably in building spatial memory, which is a reference memory in particular (Olton, 1977; Sharma et al., 2010). The advantages of this test are that it is easy to use, cheap.

According to Table 4, the mackerel RUFF group demonstrated the highest test scores over time, with an average score of 3.83. While this score was not statistically different from the others, it indicated the highest level of performance among all groups. These findings indicate that the mackerel group generally exhibited superior performance in cognitive tests relative to the other groups. In contrast, the labyrinth test results for the non-protein group were the lowest among all the groups. Unbalanced nutrients can impede brain development, leading to less complex dendrites and reduced concentrations of growth factors and neurotransmitters (Cusick & Georgieff, 2016; Schneider et al., 2018). Patro et al. (2019) also discovered that malnutrition can induce a decline in brain function and increase oxidative stress, particularly in the brain. In addition, malnutrition can inhibit myelination and brain maturation, reducing neuronal cells and causing lower brain volume, especially in the hippocampal region. This limits memory and causes a memory deficit because the hippocampus is responsible for cognitive development, especially memory and spatial storage (Wang & Xu, 2007).

Memory deficits affect cognitive performance. Valadares et al. (2010) reported that malnutrition, particularly protein malnutrition, could lead to behavioral rigidity, causing longer response times in maze tests despite ineffective responses. Table 4 shows that the performance was consistent with being active, but gradually became less active. This is supported by McGaughy et al. (2014) and Rushmore et al. (2021), who suggest that protein malnutrition may also result in cognitive rigidity, making it harder to adapt without failing to reconsolidate memory. This can also be caused by the repetition of fasting before a neurobehavioural test, followed by an unbalanced nutritional diet, which may induce stress. However, further research is required in this regard.

Although brain development is already complete during pregnancy, myelination is only fully complete after birth. The formation of myelin from oligodendrocytes is a prolonged process that continues from childhood to adolescence in humans, where myelin build-up continues into adulthood, although at a reduced rate (Dinicolantonio & O'keefe, 2020; Patro et al., 2019). In the rodent brain, myelination events are programmed predominantly postnatally, beginning at birth in the spinal cord and being achieved throughout the brain by days 45–60. Myelination tends to occur during the development of different brain areas (Patro et al., 2019). Both concepts rely on the observation that the young, rapidly developing brain is more vulnerable than theolder brain, but also retains a greater degree of plasticity (e.g., recoverability) (Cusick & Georgieff, 2016).Myelin thickness has been reported to affect conduction velocity, which is pivotal for proper brain function and synchronous action potential firing (Patro et al., 2019).

As per Table 4, the mackerel RUFF group had an average time to complete the test of 176,39 seconds, the fastest average time among the four groups. At the same time, the non-protein (NP) group tends to have the lowest average completion time, with an average completion time of 235.17 seconds. The mackerel group's best cognitive performance, with the fastest average time to complete the labyrinth, parallels the fact that mackerel meal contains high omega-3 levels, especially DHA. High levels of omega-3 in the PUFA ratio describe the ability of the mackerel group because the main constituent of the brain consists of 60% fat, with the composition of omega-3 reaching 20% (Stevenson et al., 2017). Omega-3 consumption promotes myelination as a direct factor in oligodendrocytes. This affects brain abilities, such as improving neurotransmission regulation and neuroplasticity, and enhancing membrane fluidity (Knöchel et al., 2015; Mukhametov et al., 2022; Stonehouse, 2014). In addition, omega-3 also maintains nerve cells, modulates neuroinflammatory mechanisms, and regulates emotion or mood (Knöchel et al., 2015; Spencer et al., 2017). DHA plays an essential role in neurodevelopment, is abundant in the central nervous system, and is needed for the transcription factors CREB-1 and caspase-3 in synaptogenesis, which creates complex dendrites and increases brain weight (Cardino et al., 2023; Yaméogo et al., 2017). The mackerel group tends to have the heaviest brain weight and volume.

Fatty Acid Fish Meals and Rat's Blood Plasma Profiling

The fatty acid profiling of fish meal results using GC-FID in Table 5 shows that mackerel fish meal had a lower PUFA value but was dominated by omega-3. Meanwhile, pangas catfish meal had high monounsaturated fatty acids (MUFA) and high PUFA values, which were dominated by omega-6 fatty acids.

As for the blood plasma fatty acid data, the mackerel fish meal group had the highest omega-3 levels, according to other data showing cognitive performance in neurobehavioral tests and brain weight. The pangas catfish group consistently has the highest MUFA values compared to other groups as stated in the study by (Lestari et al., 2020; Sokamte et al., 2020). This was also supported by the data from pangas catfish meal fatty acid profiling, which had high MUFA.

Table 5. The results of fatty acid profiling in rat's blood plasma

Fatty acids	Mackerel Meal	Pangas catfish Meal	NP	Casein	Pangas Catfish	Mackerel
C4:0	0.07	0.08	Nd	Nd	Nd	Nd
C12:0 (Lauric acid)	0.13	0.16	Nd	Nd	0.55	Nd
C14:0 (Myristic acid)	1.55	1.85	0.91	0.72	1.44	1.2
C15:0 (Pentadecanoic Acid)	0.31	0.11	Nd	Nd	Nd	Nd
C16:0 (Palmitic acid)	13.83	15.53	25.8	26.69	27.21	27.27
C16:1 ω7 (Palmitoleic acid)	2.68	0.52	1.39	1.69	1.64	1.48
C18:0 (Stearic acid)	4.41	4.24	19.14	17.12	13.86	15.67
C18:1 ω9 (*Oleic acid*)	4.48	16.59	14.69	16.19	21.42	17.59
C18:2 ω6 cis (Linoleic acid)	0.59	6.22	14.64	15.6	16.42	15.91
C18:3 ω6 (Linolenic acid)	0.03	0.13	Nd	Nd	Nd	Nd
C18:3 ω3 (Linolenic acid)	0.26	0.3	Nd	Nd	Nd	Nd
C20:0 (Arachidic acid)	0.21	0.12	Nd	Nd	Nd	Nd
C20:1 ω9 (Cis-11-ecosenoic acid)	0.22	0.59	Nd	Nd	Nd	Nd
C20:2 ω6 (Cis-11,14-Eicosedienoic Acid)	0.45	0.33	Nd	Nd	Nd	Nd
C20:3 ω6 (Eicosatrienoic acid)	Nd	0.56	Nd	Nd	Nd	Nd
C20:3 ω9 (Mead acid)	Nd	Nd	2.09	1.98	1.46	2.25
C20:4 ω6 (Arachidonic acid)	0.88	0.77	18.71	17.89	13.7	14.17
C20:5 ω3 (EPA)	1.74	0.33	Nd	Nd	Nd	Nd
C22:0 (Behenic Acid)	0.4	0.09	Nd	Nd	Nd	Nd
C22:6 ω3 (DHA)	3.04	0.82	2.32	2.13	2.32	4.46
∑SFA	48.91	45.74	46.15	44.52	43.07	44.15
∑MUFA	1.22	0.90	16.08	17.88	23.06	19.07
∑PUFA	26.42	31.95	37.76	37.6	33.88	36.79
∑ ω3	5.04	1.45	2.32	2.13	2.32	4.46
∑ ω6	24.35	29.21	35.44	35.47	31.56	32.32
∑ ω9	Nd	Nd	14.69	16.19	21.47	17.59
Ω6 / ω3	16.79	13.03	15.28	16.65	13.60	7.25
AA/DHA	5.9	5.19	8.06	8.39	5.91	3.18

Nd = not detected.

Plasma PUFAs reflect the intake and metabolic processes involved in PUFA mobilization and utilization. When PUFA levels at intake tend to be low or imbalanced, they can cause irreversible growth abnormalities in the long term. Apart from that, PUFA also has a direct impact on insulin growth factors, which influence bone development, fat metabolism, and inflammation (Cardino et al., 2023; Sigh et al., 2018). The decrease in PUFA in growing animals, especially EFA, in a study conducted by Brenna et al. (2015), shows average growth with functional problems such as neurocognitive deficits. EFA depletion leads to reduced conversion of EFA to LC-PUFA, which may result from the limited intracellular availability of energy and acetate units required for EFA conversion. Low LC-PUFA levels may adversely affect membrane integrity, growth immunity, and neural system function.

As shown in Table 5, the amount of PUFA is influenced by omega-6 and omega-3 fatty acids. Both have competitive metabolic processes because they use the same enzymes. Therefore, the fatty acid profile ratio is a marker of growth and cognitive development (Andruchow et al., 2017). However, the affinity of desaturase and elongase enzymes for fatty acids is ordered as omega-3 > omega-6 > omega-9. Omega-3 will get 'priority' in the fatty acid metabolism pathway.

Additionally, if ω-3 fatty acids are insufficient, they can be replaced by other fatty acids (Dong et al., 2020). Because of the complex metabolism process within the body, there may be a difference in the amount of omega 6/omega 3 and AA/DHA ratios of fish meals compared to blood profiles in the Pangas and Mackerel groups. Furthermore, the changes in content may be due to the small amount of blood that is not properly recognized.

One of the effects of the fatty acid ratio on growth is that low omega-3 PUFA cause an increase in lipogenic enzyme activity (Jean-Baptiste et al., 2018). Meanwhile, the higher the omega-3 level, the greater the height increase (Sigh et al., 2020). The influence of the fatty acid profile ratio as a marker of cognitive development, based on Babirekere-Iriso et al. (2016) and Sheppard and Cheatham (2013), is the balance of enzymatic affinity and supply. The highest n-3 level will support enzymatic balance, whereas n-3 has a higher affinity and will support cognitive abilities. However, if n-3 and n-6 intakes were both high, this would cause an imbalance in enzymatic activity, which would cause the cognitive abilities to suffer. The higher omega-6 fat content in blood plasma correlates with a neurocognitive deficit in malnourished growing rats (Brenna et al., 2015). This can be shown by the NP group, in which omega-6 was quite high, which made the ratio of both omega-6 and omega-3 high. It was also correlated with other data on growth and cognitive development, from brain weight to cognitive performance.

The FAO itself recommends that the omega6/omega3 ratio in a balanced diet, which has the potential to have a good impact on children, is LA/ALA between 5:1 and 10:1. Meanwhile, based on studies, Rahmawaty & Meyer (2020), Simopoulos (2002), is 2.5:1 or 1:1. This ratio is known to be consistent in various studies to maintain health and improve aspects of neurodevelopment and genetics. However, based on the regulation of CODEX (2022), the guidelines foressential fatty acids (EFA) in RUTF for omega-6 fatty acid content is a maximum of 780 mg/100 kcal, and omega-3 fatty acid content is a minimum of 110 mg/100 kcal.

The most prevalent n-6 LCPUFA in the brain, arachidonic acid (AA), is known to play a crucial role in brain growth and quickly builds up in the brain throughout development, which occurs in the brain from the start of the third trimester of pregnancy until the child is approximately two years old. This fatty acid is involved in numerous signaling pathways related to cell division (Hadley et al., 2016; Sambra et al., 2021). It is also a direct precursor of a fatty acid required for brain growth and adrenic acid and myelinic lipid enrichment (C22:4n-6, AdA). In animal models, approximately 50% of the adult amounts of ARA and DHA accumulate in rat brains before myelination and at 15 days after birth when myelination has just started. However, some metabolic derivatives of AA have a pro-inflammatory effect; AA has been linked to and widely perceived as having the opposite effect. ARA produces eicosanoids, which act as mediators and regulators of inflammation, and AA has been controversially linked to neuroinflammation and cellular damage. Metabolic processes produce eicosanoids from ARA and other oxidized derivatives (Hadley et al., 2016; Matsuo et al., 2021; Sambra et al., 2021).

Based on Table 5, it is known that AA: DHA ratio in blood plasma from lowest to the highest is 3.18 (mackerel group), 5.91 (pangas catfish group), 8.06 (NP group), and 8.39 (casein group). Because AA is a pro-inflammatory agent, DHA is required as an anti-inflammatory agent. According to certain studies, assessing the blood AA: DHA or AA: EPA ratio can help determine a number of health issues, including inflammation and essential fatty acid deficiency (EFAD). According to previous research, combining 2.1% of daily calorie intake with a 20:1 AA: DHA ratio in different ratios can avoid the biochemical signs of EFAD (Le et al., 2013). According to a different study, supplementation with ARA and DHA at a ratio higher than 1:1, up to 0.65% of total fatty acids from ARA, improves cognitive function and reduces the possibility of competition from high DHA levels (Hadley et al., 2016; Sambra et al., 2021). In contrast, a high AA: DHA ratio increases oxidative stress, causes triglyceride accumulation, and interferes with mitochondrial processes (Ghazali et al., 2020).

Furthermore, the data of the NP group showed a slightly better fatty acid profile than casein. Based on studies that have been carried out, it is known that protein-energy malnutrition sufferers have a higher amount of omega-3 compared to well-nourished or recovered malnourished people. A study conducted by Koletzo et al. (1986) PEM group was 0.079, the recovered group was 0.064, and the well-nourished group was 0.049.

Meanwhile, the omega3/omega6 ratio profile of TGA in every group, that is, the PEM group was 0.171, the recovered group was 0.149, and the well-nourished group was 0.183. This study is also supported by Marin et al. (1991), who reported that infants aged 2-5 months who suffer from PEM will experience a decrease in the relative percentage of omega-6 and an increase in omega-3. Another study that supports this finding is by Santos de Souza et al. (2008), who found that the amount of omega-3 in the organs of mice in the growth phase and suffering from malnutrition tends to be higher than in those undergoing the recovery process.

As the data show, the abundance of DHA as an omega-3 in blood plasma was detected in fatty acid profiling. DHA tends to reduce more slowly to maintain higher basal values in the plasma due to competition between ALA and LA. DHA also plays a vital role in the body because it is a constituent of phospholipids or membrane lipids in nerves and blood cells (Knöchel et al., 2015; Spencer et al., 2017). However, further research on cognitive development and the impact of RUFF on malnutrition is required.

Conclusion

Mackarel meal-based RUFF is also considered the best in animal experiment growth because it is high in SFA and omega-3. The cognitive development analysis results demonstrated that the RUFF group of mackerel fish meal had the largest brain size with the best neurobehavioral performance. This is supported by the fact that the RUFF mackerel meal-based group had the highest omega-3 content in both fish meal and blood plasma, with 4.46%. Based on these research findings, it can be concluded that the RUFF group of mackerel fish meals exhibited the best cognitive development and growth performance in terms of weight gain.

Limitation Study

Because this study is a preliminary study to determine the effect of omega-3 on RUFF products using mackerel and Pangas catfish, providing feed that is not isocaloric and isoproteinous may cause imbalances in the results. Therefore, further research is needed to support these findings.

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