



## Effects of Supplementation with Furikake *Ulvamina* Made of Algae (*Ulva sp.*) and Tuna (*Thunnini*) on Cognitive Function of Malnourished Mice (*Rattus norvegicus*)

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### Abstract

**Background:** Algae (*Ulva sp.*) and tuna (*Thunnini*) are edible marine resources rich in protein, essential amino acids, zinc, and iodine that can be used to improve the growth of malnourished children including their cognitive functions. The combination of both resources may produce a supplementation (Furikake *Ulvamina*) with nutritional quality to support the acceleration of cognitive functions of malnourished children.

**Objective:** This study aimed to develop a marine-based product/supplement to accelerate cognitive functions in malnourished mice (*Rattus norvegicus*).

**Methods:** A product called "Furikake *Ulvamina*" was used in an experimental study of 25 malnourished mice. The Morris Water Maze test was adapted into a "speed time to survive" to test the cognitive functions of each group. One-way Anova tests were performed to statistically analyze the mean differences in the speed of time to survive before and after the intervention.

**Results:** All five groups showed an increase in the speed of time to survive after treatment was given with an average increase of speed of 26.9 seconds. Statistically, there was no significant difference in this cognitive function acceleration indicator between groups ( $p > 0.05$ ). Furthermore, weight and length were also increased after four weeks in all groups.

**Conclusions:** There was no significant difference in cognitive function acceleration in each group of mice after the supplementation of Furikake *Ulvamina* for four weeks. This product may potentially accelerate growth in



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malnourished mice. Furikake *Ulva* can also promote the consumption and benefits of Indonesian marine local commodities (algae and tuna).

## Introduction

The health and nutritional status of children especially those with undernutrition in Indonesia remains problematic and should be properly addressed and solved. In 2013, the morbidity of children under five years old was 32 per 1000 births and 20% of the population is considered undernourished.<sup>1</sup> Furthermore, in the years between 2000-2016, the global prevalence of stunting decreased from 32.7% to 22.9% but the Indonesian National Health Survey (*Riskesdas*) data showed that until 2016 in Indonesia, 38.9% of toddlers had nutrition problems such as stunting (23.4%), malnutrition (3.4%), and undernutrition (14.4%).<sup>1</sup>

Malnutrition during the first 1000 days of life can cause an irreversible condition of stunting. An epidemiological study showed that the interactions between maternal nutrition, hormonal disruption, and placental development are determinants of stunting. Stunting involves chronic malnutrition that starts from pregnancy up to 24 months after birth. The main causes are inadequate intake and chronic infection. Other causes are inadequate breastfeeding, improper complimentary food, bad sanitation, lack of availability of clean water, low mother education, and poverty.<sup>2-6</sup>

The optimal growth of undernourished/malnourished children can be reached by increasing the quality and quantity of food. Supplementation to prevent stunting can be done with food changes and improvements that are actively and continuously given during the first two to three years after birth.<sup>7-9</sup>

Indonesia is rich in food sources that have great nutrition such as green algae (*Ulva* sp.) and tuna (Thunnini). Local foods can also be used as a staple food or supplementary food. *Ulva* is a food source that is rich in proteins, fiber, and minerals. As much as 10-26% of dry based *Ulva* contains complete essential amino acids.<sup>10,11</sup> Tuna is also a food source that is low in fat (5%) and very high in protein (20%). Yellow-fin tuna contains approximately 22.2% of proteins which includes essential amino acids such as threonine, tryptophan, valine, and

histidine.<sup>12-14</sup> The combination of both resources may produce a supplementation (Furikake *Ulva*) with nutritional quality to support the growth acceleration of malnourished children.

Based on the abovementioned studies, it is known that algae (*Ulva* sp.) and tuna (Thunnini) are edible marine resources that are rich in protein, essential amino acids, zinc, and iodine that can be used to improve the growth (including cognitive functions) of malnourished children. Before this product will be given to children, we studied how it affects cognitive acceleration in malnourished mice through an experimental animal study. Ethical clearance was obtained from Universitas Gadjah Mada before the data collection started.

## Materials and Methods

In a parallel experimental study with a control group, 25 mice (*Rattus norvegicus*) aged 4 weeks weighing  $88.50 \pm 22.83$  grams were allocated into 5 groups (A0/control, B0, B1, B2, B3) following an adaptation phase with a standard diet (50 grams of AD II). Subjects in B0, B1, B2, and B3 were malnourished by giving a 50% standard diet for 2-3 weeks until they weighed below 70% of the control group (A0). During the intervention period, subjects in A0 and B0 received standard diet while the malnourished mice were given standard diet supplemented with Furikake *Ulva* with the following doses; 10% (B1), 15% (B2), and 20% (B3) of the standard diet. Furikake *Ulva* was made of 60% tuna and 40% algae. Supplementation was mixed into the standard feed for 4 weeks.

In this study, the "speed of time to survive" was used to determine the cognitive function. This aspect was measured before and after the intervention using the Morris Water Maze method. The Morris Water Maze protocol was supposed to be using a pond in the form of a circular tank of 1.5 m in diameter, and 0.45 m high. However, the circular tank in this study was 1.2 m diameter and 0.45 m high.

Statistical analysis was done using SPSS program. Normality test was performed before the hypothesis

test. One-way ANOVA tests were performed to statistically analyze the mean differences in the speed of time to survive before and after the intervention period. The data were expressed as mean ± standard deviation (SD).

**Results**

The treatment was given in the form of daily supplementation in the regular diet of the mice. Table 1 provides data on the supplementation dose received by each group. The highest dose of supplementation (20%) was received by group B3 and the groups with the least supplementation of the product were A0 and B0 (standard diet only).

To obtain the cognitive data, measurements were taken before and after treatment to record the time needed by the mice to survive the water maze. Figure

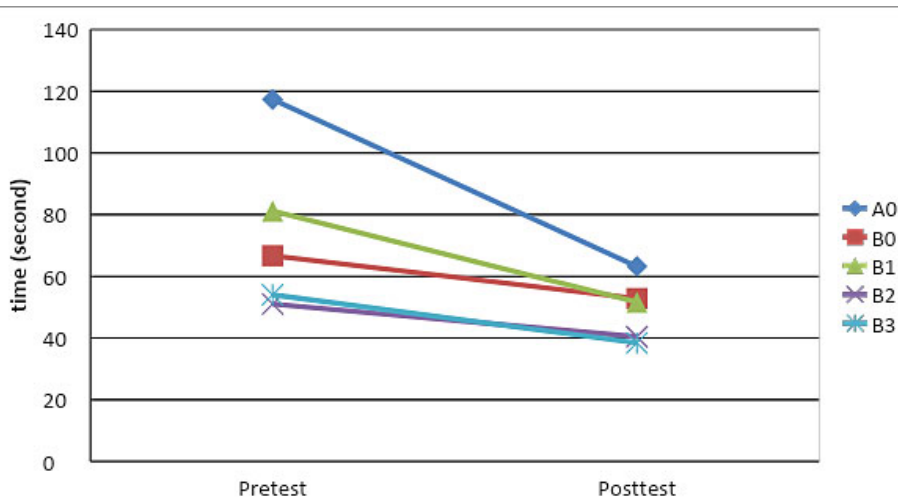
1 shows the increase in the speed of time to survive. Cognitive function acceleration was observed from the deviation of time on the post and pre-test. In general, all groups had an increase in speed after the supplementation was given. On average, the speed to survive in the water maze was faster than before the treatment period conducted.

The cognitive scores were measured from the results of the time spent to survive before and after the supplementation. The average scores were analyzed with one-way ANOVA tests to see if there was a significant difference between each group. All data also had a normal distribution based on the Kolmogorov-Smirnov test. The results showed that there was no statistically significant difference in the time change to survive after the supplementation between both groups of treatment ( $p= 0.711$ ).

**Table 1: Intake data for each group during the intervention period**

Nutrients	Groups				
	A0 (n=5)	B0 (n=5)	B1 (n=5)	B2 (n=5)	B3 (n=5)
Calories (kcal)	163.5	163.5	179.9	188.0	196.2
Protein (gram)	7.5	7.5	10.4	11.9	13.3
Fat (gram)	3.5	3.5	3.6	3.6	3.6
Carbohydrate (gram)	25.5	25.5	26.4	26.9	27.3

Note: daily group intake



**Fig 2: Change in the Speed of Time to Survive**

**Table 2: Analysis of the cognitive scores of each group**

Groups	The Average Speed of Time to Survive (in seconds)		
	Before intervention	After intervention	Changes after intervention (time $\pm$ SD (p-value))
A0	130.0	63.2	66.8 $\pm$ 41.2 (p= 0.022)
B0	74.4	52.8	21.6 $\pm$ 86.8 (p= 0.608)
B1	65.4	51.6	13.8 $\pm$ 80.1 (p= 0.720)
B2	57.2	40.4	16.8 $\pm$ 57.6 (p= 0.550)
B3	54.0	38.4	15.6 $\pm$ 67.9 (p= 0.634)

Note: p<0.05, significant difference; SD, standard deviation

### Discussion

The present study aimed to investigate the benefit of supplementation with Furikake *Ulvamina*, a multimicronutrient powder made of algae (*Ulva* sp.) and tuna (Thunnini), on early cognitive development in malnourished mice. Using the Morris Water Maze method, cognitive functions in all groups improved albeit not significantly. However, no significant difference was found between all groups. Thus, our supplementation could not support early cognitive developmental catch-up in mice with malnutrition.

Furikake *Ulvamina* was deemed to accelerate cognitive development in malnourished rats. However, with three different dosages used in our study, we found no significant effect of 4-week supplementation with Furikake *Ulvamina* on the time to escape on the Morris Water Maze test. There was also no significant difference in the change of the time to escape in all supplemented groups when compared with groups of healthy mice and non-supplemented malnourished mice. The insignificant effect of supplementation might be affected by the age of the mice (less than a month), while the recommended age to give supplements for mice is around two months wherein that age is considered as a mature age. Other possibilities that can cause altered cognitive functions that might happen to the mice is the environmental stressors<sup>15</sup>. Furthermore, the method used to measure the speed of time to escape was the Morris Water Maze which is very simple and without any computerized system as a helping tool.

It is worth-noting that impaired brain development as a result of restricted diet might carry long-lasting effects as concluded in a review of several animal studies due to altered morphological, biochemical, and physiological characteristics<sup>16</sup>. Malnourished mice in our study went through 50% restriction of food intake for 2-3 weeks which was sufficient to induce essential deficits in cognitive and behavioral functions.<sup>17,18</sup> It was further stated that although some deficits could be reversed by nutritional rehabilitation, the reduction in grown cell number could not be reversed even with a long period of supplementation including deficiency in learning and retention of information<sup>19</sup> as well as social behavior.<sup>20</sup> Similarly, in children, malnutrition before the age of 12 months might lead to delayed motoric and language development as well as low intelligence due to impaired hippocampus and cortex.<sup>21</sup> It can be implied from this body of evidence that early undernutrition persisting through the first 2 years of life might induce some long-lasting adverse effects of impaired cognitive and behavioral functions that cannot be restored even with supplementation.

Furikake *Ulvamina* was initially developed with the potential benefits to provide physical growth and cognitive development acceleration for children below 2 years old with malnutrition issues. According to previous studies, this product is rich in essential nutrients for growth and development<sup>10-12,14</sup> and may replace the previously implemented supplementation program by the national government called Taburia, a multi-micronutrient powder. Despite containing

less amounts of essential micronutrients such as iron, zinc, iodine, and magnesium, Furikake *Ulvamina* contains macronutrients such as energy (320 kcal/100 grams product) and protein (57 gram/100 grams product) which the *Taburia* lacks.<sup>22</sup> It was also reported in a previous study that *Taburia* introduced a bitter taste when mixed into meals<sup>23</sup> while the use of real food in the production of Furikake *Ulvamina* may lead to better sensory characteristics.

In conclusion, Furikake *Ulvamina* can also promote the consumption and benefits of Indonesian marine local commodities (algae and tuna). The utilization of marine local products in developing countries to support the health and nutritional status is widely

acknowledged and encouraged.<sup>2,3</sup> Therefore, with further improvements, Furikake *Ulvamina* is believed to offer an alternative solution from natural food commodities in tackling child malnutrition issues in Indonesia.

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#### Conflict of Interest

There is no conflict of interest to disclose.

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