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Research Article

Formulation of stick laor (*Eunice viridis*) as an alternative food supplement for toddlers in stunting prevention

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Abstract

Adequate nutrition is needed as the basic capital of the child's growth and development process, especially protein. The occurrence of malnutrition conditions will affect the achievement of perfect growth. Based on this phenomenon, it is necessary to fulfill nutritional needs, especially the consumption of foods rich in energy and protein as well as micronutrients. Local food sources of energy and protein produced from Maluku Province are enbal, laor and moringa leaves. Laor (*Eunice viridis*) contains 29.8% protein, contains essential and non-essential amino acids, 229.26 mg calcium, 2.439 mg zinc and 3.149 mg iron per 100 grams of laor flour. The purpose of this study was to analyze the Physicochemical and sensory evaluation of stick laor (*eunice viridis*) as an alternative food supplement for toddlers in stunting prevention. The research method used in the first year of research was a RAL experimental design (Completely Randomized Design) which consisted of four treatments, namely the difference in comparison with the use of 5% laor flour was coded F1, for the use of 10% laor flour was coded F2 and the use of 15% laor flour coded F3. The variables measured were levels of protein, fat, carbohydrates, energy, zinc, Fe, calcium. Organoleptic test for taste, aroma, color and texture. The results showed that the highest content of energy, protein, fat, calcium, iron and zinc was F3, F2 had the best acceptability in terms of color, taste, aroma and texture. Sticks Laor increase the intake of energy, protein, and calcium for toddlers and increase serum calcium levels.

Keywords: Sticks laor, food supplement, toddlers, stunting prevention

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INTRODUCTION

The process of growth and development is an important process in life. At that time the growth of the brain and all parts of the toddler's body rapidly and the quality of a child can be seen from the process of growth and development. The growth of toddlers has increased rapidly at an early age, namely from 0-5 years. This period is also often referred to as the "golden age" phase. Golden age is a time to attention the growth process of toddlers.¹⁻³

The growth process is often associated with a continuous process of human physical change (aspects of shape and size) until it reaches a certain functional level of biological maturity. Growth follows a sigmoid curve (growth curve) so that there is a process of acceleration, deceleration and accumulation. This growth curve as a tool that describes a linear growth pattern according to gender and age. Linear growth is an important part of growth and is related to the increase in height. Height will form a normal distribution according to normal criteria, namely if it is between -2SD and +2SD.^{4,5}

The process of growth and development of toddlers is also influenced by nutrition. Before birth, the child is dependent on nutrients in the mother's blood. After birth, the child depends on the availability of food and the ability of the digestive tract. Lack of food intake or the occurrence of infection can cause growth disorders. Growth disorders can occur in the "window of opportunity" period, namely when a toddler occurs in a state of malnutrition that is not treated as early as possible so

that it becomes permanent in the first 2 years of life. If this condition occurs during the golden period of brain development (0-3 years), the brain will not be able to develop properly. This results in a decrease in intellectual ability and productivity, an increase in the ratio of degenerative diseases and the birth of children under five with low birth weight or premature in the future.^{6,7}

The prevalence of stunting under five nationally increased by 0.4%, namely 36.8% in 2007 to 37.2% in 2013, and decreased to 30.8% in 2018. However, this prevalence rate is still higher than the figure. the prevalence of underweight and very thin nutrition is 12.1% in 2013 and 10.2% in 2018.^{8,9}

Adequate nutrition is needed as the basic capital of the child's growth and development process, especially protein. The occurrence of malnutrition conditions will affect the achievement of perfect growth. Based on this phenomenon, it is necessary to fulfill nutritional needs, especially the consumption of foods rich in energy and protein as well as micronutrients.¹⁰⁻¹²

One of the many micronutrients that have an important role is zinc. Zinc has the effect of cell replication and nucleic acid metabolism. The role of zinc is also a mediator of growth hormone activity, especially on bones (body skeletal system) at the age of growth and development of toddlers.^{10,13}

Zinc plays a role in taste and appetite. Giving animal protein to children can increase zinc levels in the body so that it will affect toddler food consumption. The introduction and provision of food in the form of animal protein to toddlers who are late (after

breastfeeding) will cause zinc deficiency in the body. This situation will trigger disturbances in the process of growth and development.^{14,15}

Zinc deficiency causes growth retardation (growth retardation) by interfering with the function of the hormone IGF-1. IGF-1 as a factor mediating the cellular effects of growth hormone. Studies have shown that serum levels of IGF-1 are reduced in zinc-deficient animals. One possible mechanism is that zinc deficiency leads to reduced cellular levels of the IGF-1 receptor. In addition to zinc deficiency, the role and adequacy of protein intake in the body also affects the growth process.¹⁶⁻¹⁸

Another mechanism of zinc can have a positive effect on bone growth through stimulation of the hormone thymulin, a hormone needed for the differentiation, proliferation and maturation of T lymphocytes. T lymphocytes have a role as cells that can induce TNF α , IFN γ , IL-1, IL-6 and IL-11 which plays a role in the regulation of Growth Hormone (GH) to induce Insulin Like Growth Factor-1 (IGF-1). IGF-1 requires Insulin Receptor Substrate 1 (IRS 1) on the proliferation of bone cells or osteoblasts.¹⁹⁻²¹

Protein is one of the important macronutrients that the body needs. Amino acids contained in protein can stimulate growth hormone. Growth hormone itself functions in the process of body cell division, especially in toddlers in the golden period of 0-5 years of growth and development. Protein, especially amino acids dominate the body's metabolic processes. Amino acids consist of essential and non-essential.²²⁻²⁴

Maluku is an archipelago that is rich in natural resources such as laor, cassava and moringa leaves. Laor is one of the unique biota of Maluku waters which for generations has been in great demand by the inhabitants of this archipelago. In March or April, on a full night or a few days after, this biota undergoes swarming, which is an event when certain types of sea worms swarm in abundance around the surface of the water to mate externally. Laor flour (*Eunice viridis*) contains higher protein than skipjack tuna, which is 29.8% protein, contains essential and non-essential amino acids, 229.26 mg calcium, 2.439 mg zinc and 3.149 mg iron per 100 grams of laor flour. The results of amino acid testing of laor flour produced 10 essential amino acids and 8 non-essential amino acids. The essential amino acids contained in laor flour are histidine 0.98%, threonine 2.40%, arginine 19.39%, thryptophan and methionine 1.35%, valine 2.09%, phenylalanine 1.58%, isoleucine 1, 84%, leucine 3.07%, lysine 5.45%. Non-essential amino acids contained in laor flour are aspartic 3.96%, glutamic 5.57%, asparagine 0.14%, serine 2.43%, glutamine <0.05, glycine 3.39%, alanine 3.08 %, tyrosine 4.50%. The content of essential amino acids in laor flour is higher than eggs and milk.²⁵

Enbal (*Manihot esculenta* Crantz) is a local food for the people of Southeast Maluku which contains more than 96 ppm of cyanide acid. Processing of "enbal" has been carried out from generation to generation with simple methods and equipment and to date, various marketable products have been produced, such as flower jelly, peanut sugar, butter cheese.^{26,27}

Moringa leaves grow a lot in yards, gardens and roadsides. Moringa leaves contain elements of multi micronutrients that are needed by children such as beta carotene, thiamin (B1), riboflavin (B2), niacin (B3), calcium, iron, phosphorus, magnesium, zinc, vitamin C.^{28,29} Researchers are interested in developing the local food into a stick laor formula as a food supplement for toddlers in preventing stunting.

MATERIALS AND METHODS

The making of the stick laor formula (*Eunice viridis*) used a RAL experimental design (Completely Randomized Design)

which consisted of four treatments, namely the difference in comparison with the use of 5% laor flour was coded F1, for the use of 10% laor flour was coded F2 and the use of 15% laor flour g is coded F3.

The experiment of making laor sticks was carried out in the laboratory of the Department of Nutrition at the Maluku Health Polytechnic, while the examination of the nutritional content of protein, fat, carbohydrates at the Basic Chemistry Laboratory of the University of Pattimura and examination of the content of iron, calcium, and zinc in the Maluku Provincial Health Laboratory. Organoleptic test was carried out at the Laboratory of the Department of Nutrition in May - July 2021.

The experimental unit in the research on making the stick laor formula is the stick laor formula and the experimental unit in the organoleptic test research is the panelist. The number of panelists used in the organoleptic testing of the stick laor formula based on the 2015 National Standardization Agency on sensory testing guidelines for fishery products was 30 non-standard (untrained) panelists, namely students nutrition. Panelists' criteria include: willing to participate, in good health, not refusing the food to be tested (not allergic).

The variables in the study, namely the independent variable was the stick laor formula, while the dependent variable was the levels of protein, fat, carbohydrates, energy, zinc, Fe, organoleptic tests for taste, aroma, color and texture.

The stages of making sticks using enbal flour, laor flour and moringa leaf flour are carried out in several stages. The first stage of preparation is preparing the equipment and materials needed in making sticks, weighing the materials needed in making sticks. The two stages of implementation are the implementation starting from mixing the ingredients used, all the ingredients are kneaded until smooth, thinning the dough by grinding it using a noodle grinder, then the dough is cut using a knife or a noodle grinder with a width of 7 mm - 1 cm and a length of 8 cm. 10 cm, then the sticks are fried until golden brown. The third stage of completion is the last stage of the process of making sticks with a combination of enbal flour, laor flour and moringa leaf flour. Starting from the process of removing the ripe sticks from the frying pan and allowing them to cool, the cold sticks are put in packages according to their groups, after which organoleptic testing is carried out.

Organoleptic assessment or preference test is an assessment method to determine the level of public preference for the experimental results of stick laor. This assessment is carried out by testing where the panelists express their responses in the form of liking or disliking the properties or characteristics of the material being tested. Panelists were asked to express their opinions spontaneously, without comparing them with the standard sample or samples previously tested. Therefore, the tests are carried out sequentially, not presented together. For the Organoleptic Test the panelists used were untrained panelists. The preference test includes color, aroma, texture and taste which is used to determine the level of preference of the panelists on the sample. In this organoleptic test using a hedonic scale with four favorite criteria and given a score, namely very like (4), like (3), somewhat like (2), dislike (1).

The data obtained were water content, protein content, fat, carbohydrate, calcium, zinc, and fe products were tested for normality with the Shapiro-Wilk test and homogeneity test with Levene's Test. If the test results obtained data that are normally distributed and homogeneous, then a different test is carried out using the parametric statistical test of Analysis of Variance (Anova). Statistical tests were carried out with a 95% confidence level and the difference was significant if $p < 0.05$. If there is a significant difference, then it is continued with statistical test using Least Significance Difference (LSD).

RESULT

Nutritional Content of Laor Stick Formula

The stick laor formula is made from embal flour, laor flour, moringa leaf flour, eggs and spices. The stick formula was made with four treatments, namely sticks with the addition of

5% laor flour (F1), the addition of 10% laor flour (F2), the addition of 15% laor flour (F3) and fish sticks (F0). Laor stick formula is tested for nutritional content (protein, carbohydrates, fat, energy, Fe, Ca and Zn).

Table 1. Average Content of Macro Nutrients of Laor Sticks per 100 g

Formu la	Nutrients						
	Energy (kcal)	Protein (g)	carbohydrates (g)	Fat (g)	Fe (mg)	Kalsium (mg)	Zinc (mg)
F0	491.68 ± 0.33 ^a	8.89 ± 0.03 ^a	63.12 ± 0.07 ^a	22.52 ± 0.17 ^a	0.721 ± 0.0059	354.8 ± 5.67 ^a	0.201 ± 0.0010 ^a
F1	486.91 ± 3.77 ^a	8.76 ± 0.12 ^a	63.46 ± 0.24 ^b	22.30 ± 0.22 ^a	0.930 ± 0.0298	695.5 ± 4.94 ^{bc}	0.251 ± 0.0029 ^b
F2	491.79 ± 2.36 ^a	10.10 ± 0.19 ^b	61.68 ± 0.13 ^c	22.74 ± 0.20 ^a	0.957 ± 0.0029	600.1 ± 7.2 ^{bc}	0.275 ± 0.0003 ^c
F3	504.42 ± 2.89 ^b	10.75 ± 0.11 ^c	58.54 ± 0.06 ^d	25.39 ± 0.49 ^b	1.050 ± 0.0254	801.1 ± 9.88 ^{bc}	0.293 ± 0.0015 ^b
P	0.000	0.000	0.000	0.000	0.131	0.006	0.025

F0 (fish sticks) has an average energy content of 491.68 kcal, while F1 (5% laor sticks) is 486.91 kcal, F2 (10% laor sticks) is 491.79 kcal, F3 (15% laor sticks) of 504.42 kcal. Based on the Shapiro-wilk test and Levene's Test, the data is normally distributed and the variance is homogeneous. The results of the analysis of variance showed that there were differences in the energy content of the sticks. The Least Significance Difference (LSD) test showed that the F3 group was different from the F0, F1 and F2 groups.

The substitution of laor flour and Moringa leaves can increase the energy content of the sticks. The energy content of sticks laor exceeds the need for additional food for toddlers (337.5 - 350 kcal).

The highest protein content was in the F3 group (15% laor sticks) at 10.75 g, followed by the F2 group (10% sticks) at 10.10 g, the F0 group (fish sticks) at 8.89 g and the lowest was the F1 group (laor sticks 5%) of 8.76 g. Based on the Shapiro-wilk test and Levene's Test, the data is normally distributed and the variance is homogeneous. Statistical results with Anova showed that there were differences in the protein content of the sticks. The Least Significance Difference (LSD) test showed that the F2 group was different from F0, F1 and F3 and F3 was different from the F0, F1 and F2 groups.

Laor substitution on the sticks will increase the protein content, this is because the laor has a higher protein content than skipjack tuna. Sticks laor protein is high enough to exceed the needs of additional food for toddlers (> 5 g).

The carbohydrate content of F0 (fish sticks) is 63.12 g, F1 (5% laor sticks) is 63.46 g, F2 (10% laor sticks) is 61.68 g and F3 is 58.54 g. The average carbohydrate content of the four stick formulas met the requirements as food additives for 43 - 53.75 g. Based on the Shapiro-wilk test and Levene's Test, the data is normally distributed and the variance is homogeneous. Statistical results with Anova showed that there were differences in the carbohydrate content of the sticks. The Least Significance Difference (LSD) test showed that F0 was different from the F1, F2 and F3 groups. The F1 group is different from the F2, F3 and F0 groups. The F2 group is different from the F3 group.

The average fat content of the F0 (fish sticks) group was 22.52 g, while the F1 (5% laor sticks) was 22.30 g, F2 (10% laor sticks) was 22.74 g, F3 (15% laor sticks) of 25.39 g. The average fat content in the four stick formulas meets the requirements for additional food for toddlers, namely: 9 - 10

g. Based on the Shapiro-wilk test and Levene's Test, the data is normally distributed and the variance is homogeneous. The results of Anova showed that there were differences in the fat content of laor sticks. The Least Significance Difference (LSD) test showed that the F3 group was different from the F0, F1 and F2 groups.

The Fe content of F0 sticks (fish sticks) is 0.721 mg, while in F1 (5% laor sticks) it is 0.930 mg, F2 (10% laor sticks) is 0.957 mg, F3 (15% laor sticks) is 1.050 mg. Based on the Shapiro-wilk test and Levene's Test, the data is normally distributed and the variance is homogeneous. The Anova results showed that there was no difference in Fe content in laor sticks.

The Zn content in PI (fish sticks) was 0.201 mg, while F1 (5% laor sticks) was 0.251 mg, F2 (10% sticks) was 0.275 mg, F3 (15% laor sticks) was 0.293 mg. Based on the Shapiro-wilk test and Levene's Test, the data is not normally distributed and the variance is not homogeneous. The results of the Kruskal Wallis test showed that there were differences in the zinc content of the laor sticks. The Mann-Whitney difference test showed that the F0 group was different from the F1, F2 and F3 groups. Group F1 is different from F2 and F2 is different from F3.

The calcium content of F0 sticks (fish sticks) is 354.8 mg, while in F1 (5% laor sticks) it is 695.5 mg, F2 (10% laor sticks) is 600.1 mg, F3 (15% laor sticks) is 801.1 mg. Based on the Shapiro-wilk test and Levene's Test, the data is normally distributed and the variance is homogeneous. Anova results show that there are differences in calcium content in laor sticks. The Least Significance Difference (LSD) test showed that F0 was different from the F1, F2 and F3 groups.

Laor stick acceptability test

The acceptance of laor sticks was carried out by organoleptic tests in the form of hedonic tests by 30 moderately trained panelists, namely students from the Nutrition Department level II and III. Organoleptic testing of a food product is an assessment activity using the senses of sight, taste and smell. Through the results of organoleptic testing, the panelists (consumers) acceptability of the product will be known. The quality parameters tested were color, taste, aroma and texture. The assessment is carried out using a hedonic scale which shows the level of preference of the panelists to the product on a scale of 1 to 4. The results of the acceptance test can be seen in Table 2.

Table 2: Average Panelist's Likeness Level for Laor Sticks

Formula	Color	Taste	Aroma	Texture	Total
F0	3,50 ± 0,51 ^c	3,63 ± 0,49 ^{ab}	3,03 ± 0,49 ^a	3,33 ± 0,48	13,5 ± 1,22 ^{ab}
F1	3,23 ± 0,43 ^{ab}	3,07 ± 0,52 ^c	3,17 ± 0,38 ^a	3,07 ± 0,37	12,53 ± 0,97 ^c
F2	3,13 ± 0,35 ^a	3,67 ± 0,48 ^{ab}	3,63 ± 0,56 ^b	3,33 ± 0,55	13,77 ± 0,94 ^b
F3	3,17 ± 0,38 ^{ab}	3,43 ± 0,57 ^a	3,43 ± 0,50 ^b	3,23 ± 0,50	13,27 ± 0,78 ^a
P	0,003	0,000	0,000	0,068	0,000

Color plays an important role in determining the level of consumer preference for a product. The average value of the panelist's level of preference for the highest color attribute in the F0 group (fish sticks) was 3.50, including an assessment of like to very like. Friedman test results showed that the provision of laor flour (*Eunice viridis*) and Moringa leaves had an effect ($p < 0.05$) on the panelists' preference level on the color attribute of the sticks. The Wilcoxon test showed that the color in the F0 group was different from the F1, F2 and F3 groups. The color in the F1 group was not different from the F2 and F3 groups.

The average value of the panelists' preference for the highest taste attribute in the F2 group (10% stick laor) was 3.67, including an assessment between like to very like. Friedman test results showed that the provision of laor flour (*Eunice viridis*) had an effect ($p < 0.05$) on the panelists' preference level on the taste attribute of the sticks. The results of the Wilcoxon test showed that the taste in the F1 group was different from the F0, F2 and F3 groups.

The average value of the panelists' preference level for the highest aroma attribute in the F2 group (10% stick laor) was 3.63 including ratings between like to very like. Friedman test results showed that the provision of laor flour (*Eunice viridis*) had an effect ($p < 0.05$) on the panelists' preference level on the stick aroma attribute. The results of the Wilcoxon test showed that the aroma in the F1 group was not different from that in the F2 and F3 groups.

Texture is one of the properties of a material or product that can be felt by touching the skin or by tasting. Food textures include smooth, chewy, thick and others. The average value of the panelists' preference for the texture attribute was the highest in the F0 (fish sticks) and F2 (10% fish sticks) groups, which was 3.33 including ratings between like to very like. Friedman test results showed that the provision of laor flour (*Eunice viridis*) had no effect ($p > 0.05$) on the panelists' preference level on the texture attribute of the sticks.

The total score of panelists' acceptance of color, taste, aroma and taste was highest in the F2 group (10% laor sticks) which was 13.77, followed by the F0 group (fish sticks) which was 13.5, the F3 group (15% laor sticks) was 13.27 and the lowest was in the F1 group (5% laor stick) which was 12.53.

DISCUSSION

Laor with another name wawo worm, is usually consumed by residents in the Maluku Islands. Laor is one of the unique biota of Maluku waters which for generations has been in great demand by the inhabitants of this archipelago. In March or April, on a full night or a few days after, this biota undergoes swarming, which is an event when certain types of sea worms congregate in abundance around the surface of the water to mate. People usually catch laor using simple tools of 2 pieces of wood or bamboo and fine gauze, the community calls it kareng-kareng. This laor is phototaxis positive, namely towards the light so that in the process of catching people, people use torches to attract worms to their fishing gear. Furthermore, the caught laor is accommodated in a holding container and then cooked into lawar laor products or made

bakasang.^{30,31}

The results of the proximate analysis showed that the protein content of laor flour was 29.8%, fat was 3.17%, Fe was 3.149 ppm and Zn was 2.439 ppm. Latumahina reported the fresh laor protein content of 13.92% and 1.01% fat while Tapubolon et al. reported that fresh laor protein content was 13.73% and fat was 0.32%. By doing flour, it can increase the density of nutritional value in the sea.^{31,32}

So far, laor is still limited to being processed into lawar laor and petang laor, so efforts are needed to diversify the processed laor. Diversification of processed seafood aims to increase the added value of fresh seafood and also to overcome the perishable nature of the fish. Laor flour processing is a form of diversification of processed products and laor flour including intermediate processed products that can be added to other processed products.^{25,33}

The FAO/WHO/UNICEF organization emphasizes the use of local foods in formulating and follows the following principles high nutritional value; acceptability; low prices, and using local foods. The use of locally available food ingredients is an alternative choice for making formula foods, especially in developing countries, where nutritional problems are mostly caused by inadequate food intake, due to the inability of parents and families to get the right food (especially animal source foods). The selection and combination of appropriate household food ingredients can be used to formulate multi-mixtures that can be used as household-based food additives.^{34,35}

Laor Sticks Nutrition

Nutritional value is the value possessed by certain foods as seen from the nutrients contained in these foods per serving or per 100 g.

Energy

Energy in food is obtained from carbohydrates, proteins and fats in foodstuffs. By using the Atwater factor, the energy value of the food can be determined through calculations according to the composition of carbohydrates, fats and proteins as well as the physiologic energy value of the food.³⁶

The results of this study showed that the highest energy levels were group F3 (15% laor flour) with an average of 504.42 kcal, then F2 (10% laor flour) an average of 491.79 kcal and the lowest was the F1 group (laor 5 flour). %) an average of 486.91 kcal. The energy content in the laor sticks is mostly obtained from enbal and wheat flour. Enbal is a type of traditional food for the people of the Kei Islands (Southeast Maluku Regency and Tual City) which is made from bitter cassava/cassava (*Manihot esculenta* Crantz). Enbal is one of the food sources of energy because it contains high carbohydrates.

Laor sticks made from thickened flour, wheat flour, laor flour, Moringa leaf flour, butter and eggs have very high nutritional content in the form of protein, carbohydrates and fat so they are a good source of energy and can contribute energy for toddlers. Carbohydrates, protein, and fat are sources of energy that are needed by the body to do work. Optimal metabolism

of nutrients depends on the availability of other nutrients, namely vitamins and minerals. Laor sticks meet the requirements as additional food for underweight toddlers, which is a minimum of 400 kcal.

Protein

Protein is an important component of body tissues. Protein is an important nutrient for growth. Proteins consist of long chains of amino acids, which are linked to each other in peptide bonds. Amino acids consist of elements of carbon, hydrogen, oxygen and nitrogen, some amino acids in addition to containing elements of phosphorus, iron, iodine and cobalt.^{37,38}

Among many nutrients, protein content is one of the most important criteria for evaluating food quality. Protein is an essential constituent of food for a number of different reasons such as growth, replacement of metabolic losses and tissue damage. Protein is a source of energy, and contains essential amino acids needed for human health.^{22,39}

There are two factors that determine the nutritional value of a protein, namely: (1) its digestibility or digestibility value and (2) its essential amino acid content. Proteins that are easily digested (hydrolyzed) by digestive enzymes, and contain complete and balanced essential amino acids are proteins with high nutritional value.⁴⁰

In this study, the protein content of the sticks was 8.76 – 10.75 g and the highest protein content was the F3 group with an average of 10.75 g and the lowest was the F1 group with an average of 8.76 g. The substitution of laor and Moringa leaves on the sticks added to the protein content, the greater the concentration of laor the higher the protein content. This is due to the high protein content in laor flour, which is 29.8%, besides that, laor contains complete amino acids. Fish and seafood (laor) are sources of complete and high-quality animal protein due to their high range of essential amino acids, high digestibility and absorption, because fish contains unsaturated fatty acids where the fatty acid bonds have double bonds that are easily released during the digestive process. The protein fiber is shorter and the proportion of connective protein (collagen) is much lower than that of livestock. Fish and laor are also rich sources of minerals.²⁵

Laor sticks meet the quality requirements for additional food for toddlers, namely at least 4-5 g, so they are suitable for consumption by toddlers, but when compared with the results of Arfiyanti's research on the formula of snakehead fish cookies as additional food for pregnant women in the second trimester, the protein content is 26.7g higher than the instant papada formula.⁴¹ Fish meat protein is unstable and has properties that can change with changing environmental. Fish protein content in both wet and dry bases can change depending on the type of species and processing method.⁴²

The results of amino acid testing of laor flour produced 10 essential amino acids and 8 non-essential amino acids. The essential amino acids contained in laor flour are histidine 0.98%, threonine 2.40%, arginine 19.39%, thryptophan and methionine 1.35%, valine 2.09%, phenylalanine 1.58%, isoleucine 1.84%, leucine 3.07%, lysine 5.45%. Non-essential amino acids contained in laor flour are aspartic 3.96%, glutamic 5.57%, asparagine 0.14%, serine 2.43%, glutamine <0.05, glycine 3.39%, alanine 3.08 %, tyrosine 4.50%. The content of essential amino acids in laor flour is higher than that of eggs and milk.

Carbohydrates

Carbohydrates play an important role because they are the main source of energy from the human diet. The main role of

carbohydrates in the body is to provide glucose for body cells, which is then converted into energy. Carbohydrates also give food a sweet taste, especially mono and disaccharides.⁴³ The main source of carbohydrates in stik laor is from the carbohydrate content of enbal flour and wheat flour. The highest carbohydrate content was the F1 group (5% laor) with an average of 63.46 g and the lowest was the F3 group with an average of 58.54 g. The average carbohydrate content in the four stick formulas meets the requirements as additional food for toddlers, which is 43-44g. Although the number of calories produced by 1 gram of carbohydrates is only 4 kcal, when compared to protein and fat, carbohydrates are a cheap source of calories. Carbohydrates are broken down into simple sugars such as glucose, which pass easily across the placenta and provide energy to support the growth of the baby during pregnancy.⁴⁴

Fat

Fat is an important part of nutrition obtained from food. Fat is a source of energy, essential fatty acids, and fat-soluble vitamins (A, D, E, and K). In addition, dietary fat has an important role in promoting good health and improving the sensory quality of food.^{45,46}

The highest fat content was the F3 group (laor 15%) with an average of 25.39 g and the lowest was the F1 group with an average of 22.30 g. In 100 g of laor flour contains 3.17 g of fat, so there is a tendency to increase the fat content with the greater the proportion of laor.

The content of unsaturated fatty acids in laor is palmitoleic acid (C16: 1, n-7) : 16 mg, oleic acid (C18: 1 n-9) : 56 mg, linoleic acid (C18: 2, n-6) : 15 mg, alpha-linolenic acid (C18: 3, n-3) : 14 mg, 11-eichosanoic acid (C20: 1, n-9) : 14 mg, arachidonic acid (C20: 4, n-6) : 164 mg , EPA (C20:5, n-3) : 58 mg, DHA (C22:6, n-3) : 21 mg.⁴⁷

During pregnancy the need for omega-3 fatty acids increases more than normal to support fetal growth, especially the brain and eyes, therefore it is important to supply omega-3 fatty acids to the fetus in sufficient quantities during pregnancy. To optimize pregnancy outcomes and fetal health, it is recommended for pregnant women to consume at least 200 mg of DHA per day.⁴⁸

According to the Regulation of the Minister of Health of the Republic of Indonesia number 51 of 2016 concerning additional food standards for underweight toddlers, the nutritional composition in 100 g of product, the minimum content of total fat is 10 - 18 g. The fat content of laor sticks is higher than the standard additional food for thin toddlers.

Iron

Iron has many functions in the human body including assisting muscles in storing and using oxygen, playing a role in protein and enzyme function, and supporting metabolic support. Iron in the diet can be in two different forms, namely heme or nonheme. Foods of plant origin contain iron only in the nonheme form, while foods of animal origin contain iron in both heme and nonheme forms.^{49,50}

In this study, the highest iron level was in the F3 group with an average of 1.050 mg and the lowest was in the F0 group with an average of 0.721 mg. The substitution of laor and moringa leaves on sticks increases the iron content, the greater the concentration of laor and moringa leaves the higher the iron content, this is because the iron content in laor and moringa leaves is quite high. In 100 g of laor flour contains 3.149 mg of iron.

Iron is a micro mineral that is important in the formation of hemoglobin. In infancy, iron deficiency can cause cognitive and

physical impairment and increase the risk of death. This is because iron plays a role in circulating oxygen to all body tissues. If oxygenation to bone tissue is reduced, then the bones will not grow optimally so the risk for experiencing growth disorders is higher. Iron deficiency can also have an impact on the occurrence of iron nutritional anemia and can negatively affect brain function, resulting in decreased children's learning achievement.^{51,52}

Zinc

Zinc plays a role in cell division, cell growth, wound healing, and the breakdown of carbohydrates. Foods of animal origin contain zinc with high bioavailability.^{13,53} In this study, the highest zinc level was in the F3 group with an average of 0.293 mg and the lowest was the F0 group with an average of 0.201 mg. Laor substitution on the sticks increases the zinc content, the greater the concentration of laor the higher the zinc content, this is due to the zinc content in the seafood being higher than skipjack tuna. In 100 g of laor flour contains 2.439 mg zinc levels, while in skipjack tuna it is 1.829 mg. The calcium content in the two sticks did not meet the standard for additional food for toddlers 6 – 59 months in the thin category.

Calcium

Calcium is a very important mineral for the human body. The main function of calcium is to fill the density (density) of bones. Calcium also plays a role in the formation of teeth. Calcium is needed for blood clotting, nerve transmission, muscle stimulation, acid-base stability (pH) of blood, and maintaining water balance. Calcium also plays an important role in enzyme reactions, blood pressure, and preventing colon cancer, so calcium is very important in life and health. The results of the analysis of the calcium content of the sticks produced in this study showed that F0 (fish sticks) was 354.8 mg, while in F1 (5% laor sticks) it was 695.5 mg, F2 (10% laor sticks) was 600.1 mg, F3 (15% laor stick) of 801.1 mg. The calcium content in the four sticks met the standard standard for additional food for toddlers 6-59 months in the lean category, namely 225-450 mg. In the four samples of sticks with the addition of laor flour and Moringa leaves can be used as additional food for toddlers 6 - 59 months in the underweight category to meet the nutritional needs of toddlers.

Laor stick acceptability

Organoleptic test is a scientific method used to generate, measure, analyze, and interpret responses to perceived products through the senses of sight, smell, touch, taste, and hearing. Organoleptic tests were carried out on four parameters, namely color, aroma, taste, and texture because whether or not consumers like a product are influenced by color, smell, taste, and oral stimulation.

The results of the organoleptic test show that the panelists (consumers) accept the product. The assessment is carried out using a hedonic scale that shows the level of panelists' preference for the product on a scale of 1 to 4.

Color

In food products, consumers often judge the initial quality of the product by its color and appearance. The appearance and color of these products are the main indicators of perceived quality. Color is a perception in the brain that results from detecting light after it interacts with an object. The perceived color of an object is influenced by the physical and chemical composition of the object, the spectral composition of the light source that illuminates the object, and the spectral sensitivity of the consumer's eye.⁵⁴

The average value of the panelists' preference for color

attributes is the highest in the F0 group, which is 3.50, including an assessment between like to very like, then the F1 group is 3.23 (likes), the F3 group is 3.17 (likes) and the lowest is the F2 group is 3.13 (likes). Giving laor flour and Moringa leaves affects the color of the sticks because Laor flour and Moringa leaves are green.

Taste

Taste is an important parameter in evaluating the acceptability of a food product. A food product that is attractive and has a high energy content but tastes bad may not be acceptable.⁵⁵ Taste involves more of the five senses of the tongue. Taste sensing can be divided into four main tastes, namely salty, sour, sweet and bitter.

The average value of the panelists' level of preference for the highest taste attribute in the F2 group is 3.67, including the rating between like to very like and the lowest rating in the F1 group is 3.07 like category. Friedman test results showed that the provision of laor flour (*Eunice viridis*) had a significant effect ($p < 0.05$) on the panelists' preference level on the taste attribute of the sticks. The addition of laor flour adds a savory taste to the sticks.

Fishery products have a unique taste associated with the extra active components. The active components of this flavor include several free amino acids such as glutamate, glycine, alanine, arginine, and nucleotides such as inosine 5'-monophosphate (IMP), adenosine 5'-monophosphate (AMP), and guanosine 5'-monophosphate (GMP). Evaluated creatinine and lactate in dried skipjack tuna as flavor-activating components. Taste is very specific and varies with species, differences in concentrations of flavor-producing components in different parts of the body, chemical structure and active components, seasonal habitats and species habitats.

Aroma

Aroma is an integral part of the taste and general acceptance of a food before it is put into the mouth. Therefore, this parameter is very important when testing the acceptability of formula foods. Aroma is generally considered to consist of volatile components that are felt in the nose, either through the nostrils (orthonasally) and from inside the mouth.⁵⁵

The average value of the panelists' preference level for the aroma attribute was the highest in the F2 group, which was 3.63, including the rating between like to very like, while the lowest was in the F0 group, which was 3.03 in the like category. The proportion of laor has an effect on the aroma of the sticks in the formulas F1, F2 and F3, the laor flour added is 5%, 10% and 15%, respectively. Fresh laor which has an aroma like squid, after the drying process gives rise to a fragrant aroma that is liked by the panelists. Friedman test results showed that the provision of laor flour (*Eunice viridis*) had a significant effect ($p < 0.05$) on the panelists' preference level on the stick aroma attribute.

In marine fish and seafood, the production of trimethylamine from trimethylamine oxide by microorganisms causes an increase in fishy taste. The chemical changes of volatile carbonyls during storage and processing contribute to the various flavors of fish. Taste is related to fish species associated with the occurrence of specific compounds, many of which are accumulated from the environment through the food chain.⁵⁶

The smell of fresh fish is common in the first few days after catching. After that, oxidation products and microbial metabolites dominate the fish aroma. The compounds associated with the aroma of fresh fish are mostly 6-, 8-, and 9-carbon aldehydes, ketones and alcohols derived from the

characteristic unsaturated fatty acids of fish with lipoxigenase activity.⁵⁷

Texture

Food texture is important for consumer acceptance. However, unlike color and taste, texture is often used by consumers not as an indicator of food safety, but as an indicator of food quality. In some foods, perceived texture is the most important sensory attribute of the product. Many surface characteristics of food products not only affect the perceived appearance of the product but also affect the perception of texture.⁵⁴

Texture is one of the properties of materials or products that can be felt by touching the skin or by tasting. Food textures include smooth, chewy, thick and others. The average value of the panelists' preference for the highest texture attributes in the F0 and F2 groups is 3.33, including an assessment between like to like very much. Friedman test results showed that the provision of laor flour (*Eunice viridis*) had no significant effect ($p > 0.05$) on the panelists' preference level on the texture attribute of the sticks.

CONCLUSION

Based on the results of the study, it can be concluded:

1. Stick formula using laor (*Eunice viridis*) 15% (F3) has higher energy, protein, fat, calcium, iron and zinc content than other stick formula.
2. Stick formula using laor (*Eunice viridis*) 10% (F2) has the best acceptability in terms of color, taste, aroma and texture
3. Giving laor sticks (10% laor flour) increased energy, protein and calcium intake significantly

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CONFLICT OF INTEREST

The author declared that don't have conflict of interest

ETHICAL CLEARANCE

The study was conducted after obtaining approval from Health research ethics committee Maluku Health Polytechnic No.LB.02.01/6.2/3408/2021.

REFERENCES

1. Drozdowski LA, Clandinin T, Thomson ABR. Ontogeny, growth and development of the small intestine: Understanding pediatric gastroenterology. *World J Gastroenterol WJG*. 2010; 16(7):787.
2. Charlesworth R. *Understanding child development*. Cengage learning; 2016.
3. Carpenter H. *Secret gardens: a study of the golden age of children's literature*. Faber & Faber; 2012.
4. Goodway JD, Ozmun JC, Gallahue DL. *Understanding motor development: Infants, children, adolescents, adults*. Jones & Bartlett Learning; 2019.
5. Rasyidah UM. Pengaruh pemberian zink dan lisin pada kadar zink serum dan pertumbuhan linier (TB/U) Balita stunting usia 2-4 tahun. Universitas Airlangga; 2013.
6. Sari M, De Pee S, Bloem MW, Sun K, Thorne-Lyman AL, Moench-Pfanner R, et al. Higher household expenditure on animal-source and nongrain foods lowers the risk of stunting among children 0-59 months old in Indonesia: implications of rising food prices. *J Nutr*. 2010; 140(1):195S-200S. <https://doi.org/10.3945/jn.109.110858>
7. Lestari HEP, Villasari A, Kartika K. Historical Relationship of Premature Labor and Low Born Weight with Nutrition Status of Children Under Three Years Old. 2020;
8. Riskesdas RI. Riset kesehatan dasar tahun 2013. Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan RI. 2013;
9. Kemenkes RI. Hasil utama riskesdas 2018. Jakarta Kemenkes RI. 2018;
10. Millward DJ. Nutrition, infection and stunting: the roles of deficiencies of individual nutrients and foods, and of inflammation, as determinants of reduced linear growth of children. *Nutr Res Rev*. 2017; 30(1):50-72. <https://doi.org/10.1017/S0954422416000238>
11. Mehta NM, Corkins MR, Lyman B, Malone A, Goday PS, Carney L, et al. Defining pediatric malnutrition: a paradigm shift toward etiology-related definitions. *J Parenter Enter Nutr*. 2013; 37(4):460-81. <https://doi.org/10.1177/0148607113479972>
12. Suhaimi A. *Pangan, gizi, dan kesehatan*. Deepublish; 2019.
13. Hafeez B, Khanif YM, Saleem M. Role of zinc in plant nutrition-a review. *Am J Exp Agric*. 2013; 3(2):374. <https://doi.org/10.9734/AJEA/2013/2746>
14. Mandal K, Lu H. Zinc deficiency in children. *IJSIT*. 2017; 6(1):9-19.
15. Ernawati F, Prihatini M, Yuriestia A. Gambaran Konsumsi Protein Nabati Dan Hewani Pada Anak Balita Stunting Dan Gizi Kurang Di Indonesia (the Profile of Vegetable-Animal Protein Consumption of Stunting and Underweight Children Under Five Years Old in Indonesia). *Nutr Food Res*. 2016; 39(2):95-102. <https://doi.org/10.22435/pgm.v39i2.6973>
16. Hamza RT, Hamed AI, Sallam MT. Effect of zinc supplementation on growth Hormone Insulin growth factor axis in short Egyptian children with zinc deficiency. *Ital J Pediatr*. 2012; 38(1):1-7. <https://doi.org/10.1186/1824-7288-38-21>
17. Maggio M, De Vita F, Lauretani F, Buttò V, Bondi G, Cattabiani C, et al. IGF-1, the cross road of the nutritional, inflammatory and hormonal pathways to frailty. *Nutrients*. 2013; 5(10):4184-205. <https://doi.org/10.3390/nu5104184>
18. Roohani N, Hurrell R, Kelishadi R, Schulin R. Zinc and its importance for human health: An integrative review. *J Res Med Sci Off J Isfahan Univ Med Sci*. 2013; 18(2):144.
19. Hojyo S, Fukada T. Roles of zinc signaling in the immune system. *J Immunol Res*. 2016; 2016. <https://doi.org/10.1155/2016/6762343>
20. Weigent DA. Lymphocyte GH-axis hormones in immunity. *Cell Immunol*. 2013; 285(1-2):118-32. <https://doi.org/10.1016/j.cellimm.2013.10.003>
21. Pramojanee SN, Phimpilai M, Chattipakorn N, Chattipakorn SC. Possible roles of insulin signaling in osteoblasts. *Endocr Res*. 2014; 39(4):144-51. <https://doi.org/10.3109/07435800.2013.879168>
22. Suprayitno E, Sulistiyati TD. *Metabolisme protein*. Universitas Brawijaya Press; 2017.
23. Rotua M. Original Patterns, Macro Nutrition and Vitamin-A Intake in Wasting Toddlers in Sukarami Health Center Palembang City. In: *First International Conference on Health, Social Sciences and Technology (ICOHSSST 2020)*. Atlantis Press; 2021. p. 46-9. <https://doi.org/10.2991/assehr.k.210415.011>
24. Lopez MJ, Mohiuddin SS. *Biochemistry, essential amino acids*. 2020;
25. Ristanti EY, Soetjipto ACA. Nutrition contents and sensory characteristics of the instant papada enrich with laor (polychaeta) as supplementary food for pregnant woman. *Indian J Public Health Res Dev*. 2019; 10(6):434. <https://doi.org/10.5958/0976-5506.2019.01308.1>
26. Riry J, Lawalata VN, Tapotubun EJ, Far-Far RA. Mutu organoleptik produk enbal fortifikasi (makanan tradisional Kepulauan Kei) ditinjau dari daya terima konsumen. *J Masy Pengolah Has Perikan Indones*. 2013; 16.

27. Leasa WB, Amanah S, Fatchiya A. Kapasitas Pengolah Ubi Kayu "Enbal" dan Pengaruhnya terhadap Keberlanjutan Usaha di Maluku Tenggara. *J Penyul*. 2018; 14(1):11-26. <https://doi.org/10.25015/penyuluhan.v14i1.17843>
28. Affandi NN. Kelor Tanaman Ajaib Untuk Kehidupan Yang Lebih Sehat. Deepublish; 2019.
29. Afifah AN. Efektivitas biskuit kombinasi ganyong daun kelor dan aktivitas fisik terhadap tekanan darah tikus sprague dawley menopause. Universitas Muhammadiyah Semarang; 2019.
30. Pamungkas J. Pengamatan Jenis Cacing Laor (Annelida, Polychaeta) di Perairan Latahalat, Pulau Ambon, dan Aspek Reproduksi. *Triton*. 2009; 5(2):1-7.
31. Latumahina MCA. Pengolahan dan komposisi gizi cacing polychaeta di Pulau Ambon. In: *Prosiding Seminar Nasional: Pengembangan Pulau-Pulau Kecil*. 2011.
32. Tapotubun EJ. Kandungan gizi dan masa simpan makanan tradisional enbal asal Kepulauan Kei dengan penambahantepung ikan layang. tesis]. Bogor: Sekolah Pascasarjana Institut Pertanian Bogor; 2012.
33. Elizabeth R. Revitalisasi Implementasi Pemberdayaan Kelembagaan Pertanian Berkesinambungan Mendukung Pencapaian Daya Saing Produk Olahan. *UNES J Sci Res*. 2019; 4(1):52-68.
34. Lestari W, Rezeki SHI, Siregar DM, Manggabarani S. Faktor yang berhubungan dengan kejadian stunting pada anak sekolah dasar negeri 014610 Sei Renggas Kecamatan Kisaran Barat Kabupaten Asahan. *J Dunia Gizi*. 2018; 1(1):59-64. <https://doi.org/10.33085/jdg.v1i1.2926>
35. Briend A, Akomo P, Bahwere P, De Pee S, Dibari F, Golden MH, et al. Developing food supplements for moderately malnourished children: lessons learned from ready-to-use therapeutic foods. *Food Nutr Bull*. 2015; 36(1_suppl1):S53-8. <https://doi.org/10.1177/15648265150361S109>
36. Almatsier S. *Daur Kehidupan dan Gizi. Gizi Seimbang dalam Daur Kehidup*. 2013; 1.
37. Wu G, Bazer FW, Dai Z, Li D, Wang J, Wu Z. Amino acid nutrition in animals: protein synthesis and beyond. *Annu Rev Anim Biosci*. 2014; 2(1):387-417. <https://doi.org/10.1146/annurev-animal-022513-114113>
38. Moesijanti Soekarti S. *Gizi seimbang dalam daur kehidupan*. Gramedia Pustaka Utama; 2013.
39. Day L. Proteins from land plants-potential resources for human nutrition and food security. *Trends Food Sci Technol*. 2013; 32(1):25-42. <https://doi.org/10.1016/j.tifs.2013.05.005>
40. Muchtadi ID. *Nutrifikasi Protein (Bagian 1)*. Jakarta Univ Terbuka. 2013;
41. Afriyanti A. Cookies Ikan Gabus Sebagai Makanan Tambahan Untuk Ibu Hamil Trimester II. *Pros SEMIRATA 2013*. 2013;1(1).
42. Gambling L, McArdle H. Mineral requirements of the mother and conceptus. In: Symonds M, Ramsay M, editors. *Maternal-Fetal Nutrition during Pregnancy and Lactation*. New York: Cambridge University Press; 2010.
43. Bloomfield F, Harding J. Fetal nutrition. In: Thureen P, Hay Jr W, editors. *Neonatal Nutrition and Metabolism*. 2nd ed. New York: Cambridge University Press; 2006. <https://doi.org/10.1017/CBO9780511544712.002>
44. Brown L, Regnault T, Rozance P, Barry J, Hay Jr. W. Pregnancy and Feto-Placental Growth : Macronutrients. In: Symonds M, Ramsay M, editors. *Maternal-Fetal Nutrition during Pregnancy and Lactation*. New York: Cambridge University Press; 2010.
45. Akoh CC. *Food lipids: chemistry, nutrition, and biotechnology*. CRC press; 2017. <https://doi.org/10.1201/9781315151854>
46. Topkafa M. Evaluation of chemical properties of cold pressed onion, okra, rosehip, safflower and carrot seed oils: triglyceride, fatty acid and tocol compositions. *Anal methods*. 2016; 8(21):4220-5. <https://doi.org/10.1039/C6AY00709K>
47. Muhamad, Asrar; Ety Yuni R. Effect of giving instant papeda with laor powder (*Eunice viridis*) on the increase in weight pregnant rats and birth weight. *Sapporo Med J*. 2021; 55(06).
48. Ristanti E, Soetjipto, Adi A. Nutrition contents and sensory characteristics of the instant papeda enrich with laor (polychaeta) as supplementary food for pregnant woman. *Indian J Public Heal Res Dev*. 2019; 10(6):416-20. <https://doi.org/10.5958/0976-5506.2019.01308.1>
49. Buratti P, Gammella E, Rybinska I, Cairo G, Recalcatti S. Recent advances in iron metabolism: relevance for health, exercise, and performance. *Med Sci Sport Exerc*. 2015; 47(8):1596-604. <https://doi.org/10.1249/MSS.0000000000000593>
50. Lesjak M, KS Srail S. Role of dietary flavonoids in iron homeostasis. *Pharmaceuticals*. 2019; 12(3):119. <https://doi.org/10.3390/ph12030119>
51. Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. *J Res Med Sci Off J Isfahan Univ Med Sci*. 2014; 19(2):164.
52. Hotz K, Augsburg H, Walczyk T. Isotopic signatures of iron in body tissues as a potential biomarker for iron metabolism. *J Anal At Spectrom*. 2011; 26(7):1347-53. <https://doi.org/10.1039/c0ja00195c>
53. Suman PBM. A review on role of zinc deficiency in humans and its rectification through Horti-agro food supplements. 2020;
54. Lawless HT, Heimann H. *Sensory Evaluation of Food Principles and Practices*. Second edi. Science. New York: Springer; 2010. 79-82, 227-230, 303-308, 325-328. p.
55. Muhimbula HS, Issa-Zacharia A, Kinabo J. Formulation and sensory evaluation of complementary foods from local, cheap and readily available cereals and legumes in Iringa, Tanzania. *African J Food Sci*. 2011; 5(1):26-31.
56. Lindsay R. Fish flavors. *Food Rev Int*. 1990; 6(4). <https://doi.org/10.1080/87559129009540886>
57. Hognadóttir Á. *Flavor Perception and Volatile Compounds in Fish*. 1999.