The potential of seluang fish (Rasbora spp.) to prevent stunting: The effect on the bone growth of Rattus norvegicus

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The potential of seluang fish (*Rasbora* spp.) to prevent stunting: The effect on the bone growth of *Rattus norvegicus*

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ABSTRACT: Stunting is a failure to achieve optimal growth. Seluang fish (*Rasbora* spp.) contains a high amount of protein and calcium needed for bone growth. The purpose of this study was to provide evidence that seluang fish can be a source of nutrition to prevent stunting. The study was conducted on *Rattus norvegicus* that were divided into three treatment groups: C (control), P1 (rats fed with a low-protein and low-fat meal), and P2 (rats fed with a seluang formula). The treatments were given for 8 weeks. Then, the rats were dissected, and blood and bone samples were obtained to measure bone calcium concentration, bone length, and serum IGF-1 level. Statistical analysis was carried out with ANOVA followed by Tukey's honestly significant difference test at the 95% confidence level. The analysis revealed that there were significant differences in bone length (2.92 vs 2.71 vs 2.94 cm, $p<0.01$), bone calcium level (1.68 vs 0.84 vs 1.34 mg/g, $p < 0.01$), and serum IGF-1 level (70.37 vs 91.37 vs 112.97 pg/mL, $p = 0.02$) among the C, P1, and P2 groups. In conclusion, the study indicates that seluang fish has the potential to prevent stunting.

Keywords: stunting, IGF-1, bone calcium, seluang fish (*Rasbora* spp.)

1 INTRODUCTION

There are many nutrition problems in Indonesia, including protein and energy deficiency, anemia, Vitamin A Deficiency (VAD), iodine deficiency, and overweight. One outcome of the deficiency of protein and energy is stunting. Stunting is a failure to achieve optimal growth, which is measured by the height-for-age index. The Indonesian Basic Health Research conducted in 2013 (Riskesdas 2013) reported that the prevalence of stunting in infants was 37.2% (an increase from 35.6% in 2010). The prevalence of severe stunting only slightly decreased from 18.5% in 2010 to 18.0% in 2013, while that of stunting increased to 19.2%. In addition, among the 33 provinces in Indonesia, 20 provinces had prevalence rates of stunting above the national average. South Kalimantan was ranked as the fifth highest province in the prevalence rate of stunting (about 40%) (Kemenkes RI, 2013). These data showed that the problem of malnutrition, especially stunting, in South Kalimantan has not yet been solved.

Stunting is a chronic malnutrition or growth failure and used as an indicator of long-term malnutrition among children. Children who have moderately poor or poor nutritional status and short or very short stature are at risk of having a lower Intelligence Quotient (IQ) by 10–15 points (Kementerian Bappenas, 2011). Many factors are associated with stunting in children, such as lack of energy and protein, chronic diseases, feeding mistake, and poverty. The prevalence of stunting increases with age. The increase occurs in the first two years of life when the growth of children reflects the standards of nutrition and health.

One of the factors that directly determines the nutritional status of infants and toddlers is inadequate nutrition that does not meet the amount and composition of the required nutrients, especially in the first 1,000 days of life. A crucial period in the development of metabolism and cognition in infants occurs during the first 1,000 days of life. This period can be divided into three phases: pregnancy (9 months), exclusive breastfeeding (6 months), and complementary feeding (18 months). Good nutrition during pre-pregnancy, pregnancy, lactation, and complementary feeding is the main factor that determines the nutritional status of infants, which can be anticipated early by using resources and local wisdom.

South Kalimantan is one of the provinces in Indonesia that had an increase in the number of short toddlers (stunting) in 2013 (43%) compared with 2010. This rate remained above the target of the Medium-Term National Development Plan (RPJMN) in 2014 (32%). National Development Planning Agency (Badan Perencanaan Pembangunan Nasional, Bappenas) found that South Kalimantan is included in stratum 3 group that had the prevalence rate of stunting children under five years old of >32% and the proportion of the population with food insecurity of ≤14.47%, while 39.3% of the population had energy consumption rate below the minimum and 28.0% had protein consumption rate below the minimum (Kemenkes RI, 2010). It is an irony, because South Kalimantan has abundant food resources. One source of food in South Kalimantan is the freshwater (rivers) that contains various types of fish. According to the data from the Department of Fisheries and Marine in South Kalimantan in 2010 (Dinas Perikanan dan Kelautan, 2010), the level of fish consumption by South Kalimantan population was 36.84 kg/person/year. This estimate is higher than the national fish consumption (33.89 kg/person/year), but it is still below that of Malaysia (55.4 kg/person/year) and Singapore (37.9 kg/person/year).

Fish is a good source of protein and calcium, especially fish with edible flesh and bones. Seluang fish (*Rasbora* spp.) is a river fish that is widely consumed by the population in South Kalimantan and included as a type of fish endemic to Borneo and Sumatra. Nutrient content per 100 g of type of fish amounts to 361 kcal, 10 g protein, 3.2 g fat, 80 mg calcium, 224 mg phosphorus, and 4.7 mg iron (Komunikasi edukasi dan Jaringan Usaha, 2013). However, the contents of essential amino acids and essential fatty acids are still unknown. The content of these nutrients may differ between the regions of the origin of the fish. Therefore, we conducted a research on the potential of seluang fish with an effort to address the nutritional problems prevailing in Indonesia. This study aimed to determine whether seluang fish in South Kalimantan can be a source of nutrition to prevent stunting by using a rat model (*Rattus norvegicus*).

2 MATERIALS AND METHODS

This study used female white rats (*Rattus norvegicus*) as models. This study was approved by the Animal Care and Experimentation Committee (Ethical Committee), Faculty oh Medicine Lambung Mangkurat University).

White rats were maintained for 1 week before treatment, to provide an equal physical and psychological condition. During maintenance, white rats were given distilled water and the same food as needed (*ad libitum*).

Fresh seluang fish (*Rasbora* spp.) was minced and made into raw fish porridge. The porridge was steamed with hot steam for 1 hour, and then dried in an oven until the moisture content reached 8%. Seluang fish was grinded again to break down the clots or bone particles. After it became dry grain, the seluang fish meal was made into pellets and used as rat feed.

Rats were classified into three treatment groups: Control (C) group, where mice were fed with a standard diet; treatment group 1 (P1), where mice were fed with a low-protein and low-fat meal; and treatment group 2 (P2), where mice were fed with a seluang fish meal. Feeding was started at 4 weeks of age and given *ad libitum* until 12 weeks. The P1 group was fed with pellets of *nasi karak* (dry spoiled rice), equivalent to a 4% low-protein feed (Illiandri, 2010). The P2 group was fed with the seluang fish meal, containing 25% standard feed mixed with 75% fish meal, and then and made into pellets.

Blood samples were obtained from the heart, and then the samples were centrifuged at 3000 rpm for 10 minutes to obtain serum. Serum was re-centrifuged at 6000 rpm for 10 minutes to obtain the supernatant. The supernatant was then used to measure the levels of IGF-1. Serum IGF-1 levels were measured using ELISA, according to the manufacturer's instructions. Absorbance was measured using an ELISA reader at a wavelength of 450 nm.

Long bones (femurs) were also obtained to determine calcium levels in the bones. Left femur bones were crushed and homogenized, and calcium levels were measured by using the titrimetric method. Scales and caliper were used to measure the right femur bone length. It was measured from the femoral head to the distal of the femur.

The normal distribution of the data was tested using the Shapiro–Wilk normality test. Despite data transformation, some data were still not normally distributed and not homogeneous. For non-normally distributed data, the differences between the groups were analyzed by the Kruskal–Wallis test, followed by the Mann–Whitney test. For normally distributed data, one-way ANOVA was used to assess the differences, followed by Tukey's Honestly Significant Difference (HSD) test. All analyses were conducted at the significance level of 5%.

3 RESULTS

After 8 weeks of treatment, the rats were killed, and the blood and bone samples were collected. The results of the measurement of body weight, long bones, bone calcium levels, and serum IGF-1 levels are respectively shown in Figures 1 to 4.

As shown in Figure 1, there is a difference in the average body weight among the three groups, in which the average weight gain in the P1 group was smaller than that in the other groups. The Kruskal–Wallis test showed a significant difference in body weight among the groups ($p < 0.01$). Subsequently, the Mann–Whitney test showed that the body weight of the P1 group was significantly different from that of the C group ($p = 0.01$) and the P2 group ($p = 0.02$), but no significant difference

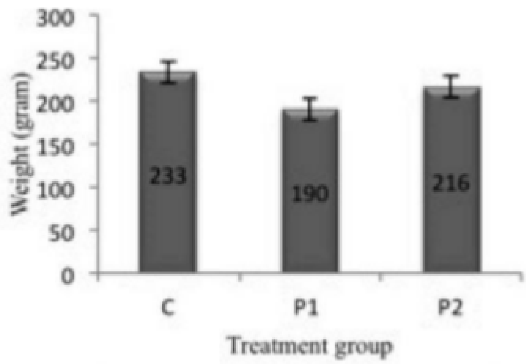

Figure 1. Average body weight after 8 weeks of treatment ($p < 0.05$). C = control group, P1 = low-protein and low-fat diet, P2 = seluang formula diet.

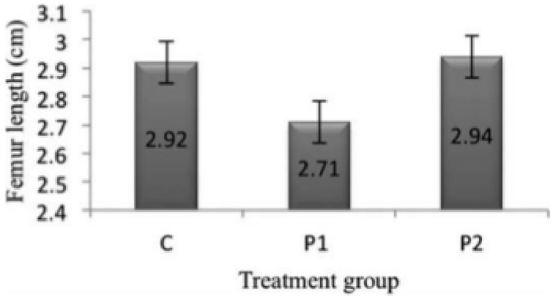

Figure 2. Average femur length after 8 weeks of treatment ($p < 0.05$). C = control group, P1 = low-protein and low-fat diet, P2 = seluang formula diet.

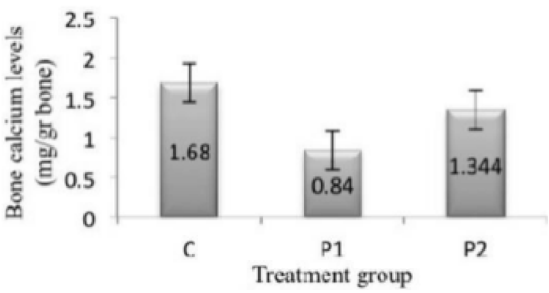

Figure 3. Average bone calcium levels after 8 weeks of treatment ($p < 0.05$). C = control group, P1 = low-protein and low-fat diet, P2 = seluang formula diet.

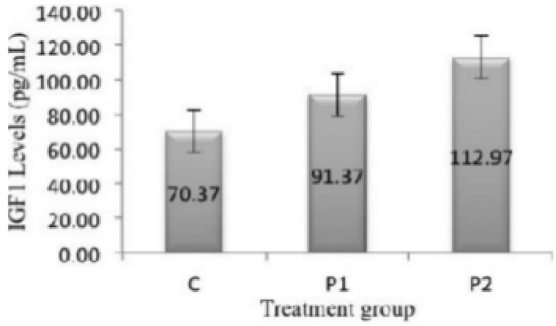

Figure 4. Average IGF-1 levels after 8 weeks of treatment ($p < 0.05$). C = control group, P1 = low-protein and low-fat diet, P2 = seluang formula diet.

was found between the C group and the P2 group ($p = 0.42$). These results indicate that the seluang formula has the same amount of energy as the standard feed. Thus, seluang feeding did not lead to weight loss. The low-protein and low-fat diet contained less energy than the standard feed.

The femur length of rats in each treatment group is shown in Figure 2. It shows that the bone length in the P1 group is shorter than that in the other groups. The average femur length in the P2 group is not significantly different from that in the C group. The ANOVA test showed that there were significant differences in the average femur lengths between the treatment groups ($p < 0.01$). A *post hoc* analysis with Tukey's HSD test revealed that there were significant differences between the P1 group and the C ($p = 0.02$) and P2 ($p = 0.01$) groups. The average femur length of rats in the P2 group was not significantly different from that in the C group ($p = 0.96$). This indicated that the P1 group had nutrient deficiency, especially calcium for bone growth, whereas the P2 group had a sufficient intake of nutrients, especially calcium, so that the average bone length in the P2 group was not significantly different from that in the C group.

Bone growth depends on nutritional factors. Intake of calcium and protein will lead to denser and stronger bones. In this study, the average bone calcium levels after administration of treatment for 8 weeks are shown in Figure 3. The average bone calcium levels in the P1 group were lower than that in the other groups. The Kruskal–Wallis test showed that there were significant differences between the treatment groups ($p < 0.01$). The Mann–Whitney test showed that the bone calcium levels between the C, P1, and P2 groups were significantly different ($p < 0.05$). The P1 group had the lowest bone calcium levels. This is due to the low calcium content present in the feed. Meanwhile, the P2 group had bone calcium levels lower than those fed with the standard feed. This is because the standard feed consisted of various mixtures of feed materials, while seluang feed content was purely derived from seluang fish.

IGF-1 is a growth factor that has a similar structure to that of insulin. The average level of IGF-1 measured in each group is shown in Figure 4.

The ANOVA test showed significant differences among the treatment groups ($p = 0.02$). A subsequent analysis with Tukey's HSD test showed that the IGF-1 level in the C group was not significantly different from that in the P1 group ($p = 0.30$), but was significantly different from that of the P2 group ($p = 0.01$). Meanwhile, the IGF-1 level in the P1 group was not significantly different from that in the P2 group ($p = 0.29$). Nevertheless, there was a tendency that the IGF-1 level in the P2 group was the highest among the three groups. This suggested that the intake of nutrients from the seluang meal may increase IGF-1 synthesis, which plays a role in bone growth.

4 DISCUSSION

Stunting is a failure to achieve optimal growth, which is measured by the height-for-age index. The most effective intervention for stunting is a combination of handling the infection and nutritional programs. Multisectoral cooperation is also needed, namely agriculture sector for food procurement, health sector by giving supplements to pregnant women and children up to the age of 5 years, accompanied by the evaluation of the physical growth of children, and education sector by providing information to the communities. All of these can be carried out properly if they are supported by a good infrastructure (Remans, 2011).

We conducted a study to determine the potential of the existing food, seluang fish, as a source of nutrients to prevent stunting in South Kalimantan. Fish is a good source of protein and calcium, especially fish with its whole flesh and bones that are edible. Seluang fish are well known as a river fish and widely consumed by the population in South Kalimantan, which is included as the fish endemic to Borneo and Sumatra. The study by Noor suggested that each 100 g of seluang fish from Barito River contains 18 g protein, 20 g fat, 52 g water, and 10 g ash (Noor, 1993). The specific nutrient content analyzed by Yunanto *et al*. in seluang fish from South Kalimantan are presented in Table 1 (Yunanto, 2014).

Based on this analysis, it can be observed that the nutrients in seluang fish are complete, including essential fatty acids and essential amino acids. Calcium in seluang fish from South Kalimantan is about 1.8 g/100 g or 180 mg/100 g dried seluang fish. It can be used as a source of calcium for bone growth, especially in childhood. The calcium content is higher than that present in the same fish species in other areas. Calcium is one of the essential nutrients that is needed for various body functions. Calcium functions as a nutrient essential

Table 1. Nutrients in seluang fish from South Kalimantan, per 100 grams.

Nutrients —	Content (%, w/w)	Nutrients	Content (%, w/w)
Ca	1.6	Histidine	1.82
Fe	19.9 ppm	Arginine	3.05
P	1.67	Threonine	2.15
Zn	122.7 ppm	Alanine	2.83
	11.77	Proline	1.92
Linoleic acid	7.33	Valine	2.41
DHA	1.04	Methionine	0.48
Aspartate	3.71	Isoleucine	2.07
Glutamate	4.98	Leucine	3.62
Serine	1.94	Phenylalanin	2.37
Glycine	3.06	Lysine	4.81
Histidine	1.82	Cysteine	0.32
Tyrosine	1.62		

for growth, contributing to the development of optimal motor function. The bones and teeth contribute up to 99% of the total calcium body, and the rest is found in the blood. When blood calcium level decreases, it will be replaced by bone calcium to maintain the calcium level in the blood (Suptijah et al, 2012; Logesh et al, 2012).

This was proven in this study. The bone lengths of the group given seluang fish were longer than that of the group fed with a low-protein and low-fat meal; however, it was not significantly different from the group fed with the standard feed. The bones of seluang fish are edible, so the fish can meet the calcium requirement. Consumption of calcium with vitamin D from sunlight may enhance the growth of the long bones (arms and legs) and optimize the bone health. Bone health is characterized by a larger bone cell size, density, and better bone remodeling (Prentice et al, 2006).

In this study, we also measured calcium levels in the bones. In people with calcium intake deficiency, the bone density is reduced. This occurs through the regulation of blood calcium levels. When calcium intake is low, the blood calcium level decreases, and there is an immediate response by the pituitary by stimulating the parathyroid glands to produce Parathyroid Hormone (PTH). Parathyroid hormone stimulates the formation of cytokines, namely Interleukin-1 (IL-1), Interleukin-6 (IL-6), and Tumor Necrosis Factor (TNF), in the bones. Cytokines activate osteoclasts to stimulate the absorption of calcium in the bones and release into the blood. Their absorption of calcium from the bones will lead to a decrease in bone density, a condition called osteoporosis (Siki, 2009).

This study showed that the group with low-protein and low-fat intake had the lowest bone calcium content, while there was not much difference in bone calcium content between the group fed the standard feed and the group fed with the seluang meal. Bone density is determined by a dynamic balance between bone formation and resorption processes. When the linear growth and the highest volume of bone mass have been reached, the process of remodeling aims to maintain bone mass (Ganong, 2008; Kurniawan, 2012). Bone tissue growth and development are influenced by genetic factors, nutrition, and hormonal functions that affect the rate of bone growth, shape, and size (Ganong, 2008).

During the growth period, there is a specialized separation area at the end of each long bone (epiphyseal) from the shaft of the bone by a plate of cartilage that actively proliferates, the epiphyseal plate. With the new bone placed at the ends of the bone's shaft by these plates, the length of the bone increases. The width of the epiphyseal plates equals to the speed of bone growth. The widening of the bone is influenced by a number of hormones, but most prominently by the pituitary growth hormone and Insulin-like Growth Factor 1 (IGF-1). Bone growth alignment may occur during the epiphyseal bone separation from the shaft, but it is stalled after the epiphyseal growth fusion with the shaft (epiphyseal closure). Epiphyseal growth plate closure begins in a regular order, and the final epiphyseal closure occurs after puberty. The process of bone development begins with the formation of bone, which is an increase in the number of the basic cell substance. At the same time, the size of the cell increases, as a polyhedral shape, and then they are interconnected through a number of processes of adjacent cells. At this stage, these cells are known as osteoblasts. They will prepare the surface layer of the bone. Bones become thicker by the addition of a matrix layer produced by osteoblast activity (Ganong, 2008).

In this study, we also measured the IGF-1 levels in each treatment group. IGF-1 levels in the group fed with seluang fish were higher than that in the other groups. This suggested that the protein in seluang fish is able to increase the synthesis of IGF-1 that plays a role in the bone formation and growth. A study conducted by Wan Nazaimon *et al*. (1997) reported that children who were moderately or severely malnourished had lower IGF-1 levels compared with those administered with normal nutrition. The study also found a correlation of IGF-1 levels with the body height and weight in the period before puberty. Malnutrition is known to cause interference rearrangement on the axis of GH/IGF-1, causing an increase in GH levels and a decrease in IGF-1 levels. The study reported a positive and a very significant correlation between the IGF-1 level and its receptor IGFBP3, indicating that both IGF-1 and IGFBP3 simultaneously affect malnutrition. In addition, it has been reported that in terms of malnutrition and growth failure, there was a relationship between the increasing age and IGF-1 levels (Wan Nazaimon *et al*, 1997).

IGF-1 is one of the peptide hormones or growth hormones that is synthesized in the liver and other tissues, locally acting as a paracrine or autocrine hormone (Puche & Castilla-Cortazar, 2012). These hormones affect the growth and differentiation of cells, including bone cells. Nutritional status affects the serum concentration of IGF-1. The lack of energy and protein causes a resistance to growth hormone. This condition is associated with the failure of a growth hormone signaling receptor, which decreases the synthesis of IGF-1 in the liver. Moreover, the growth hormone and IGF-1 are anabolic hormones, while malnutrition causes catabolism. In a state of catabolism, IGF-1 concentration is low, which will inhibit the growth hormone (Roith, 1997).

During bone growth, IGF-1 is required for osteoblast maturation and function. Through the PI3K pathway, IGF-1 reduces the apoptosis of osteoblasts and triggers osteoblastogenesis through the stabilization of B-catenin, increasing Wnt activity. This effect is associated with complete mitogenic activation, causing an increase in the number of osteoblasts, and increases osteoblastic function and bone formation. IGF-1 induces the synthesis of Receptor Activator of Nuclear Factor κ B Ligand (RANK-L) and improves the function of osteoclasts. IGF-1 also induces the expression of Vascular Endothelial Growth Factor (VEGF) in skeletal cells, and VEGF angiogenesis processes integrate with endochondral formation of bone and osteoblastic differentiation and function (Ahmed & Farquharson, 2010).

IGF-1 synthesis in the liver and other tissues needs some essential amino acids. Seluang fish has complete essential amino acids and thus can meet the requirements of essential amino acids, which is about 12% of the total energy needs. This was evident in the group of rats fed with a low-protein and low-fat diet that had lower levels of IGF-1, short long bones, and low calcium levels. Meanwhile, in the group fed with seluang fish, there were high IGF-1 levels, long bones, and high calcium levels. Thus, it is apparent that there is a correlation between the levels of IGF-1, long bones, and calcium levels.

Physical growth disturbance is associated with the bone health; therefore, the nutrition provided should include nutrients for bones. If bone health is not optimal, there is a risk of suffering from stunted growth, among other things. Nutrients consumed should be started from the intrauterine period, so pregnant women should consume vitamin D, protein, fat, Ca, P, Mg, K, vitamin C, Cu, Zn, and folic acid. After birth, nutrition through breastfeeding and complementary micronutrients after receiving solid foods should be provided. These nutrients can increase the length of the bone, which is reflected in the body height and bone health, and can reduce the risk of fracture. The function of these nutrients is affected by the amount consumed, absorption, and excretion by the body (Prentice *et al*, 2013). Growth and body size in childhood are correlated with bone mass in the late adolescence phase. Fur- thermore, the duration of exclusive breastfeeding and bone turnover in the first 6 months is positively correlated with the mass of the spine at the age of 17 years (Molgaard *et al*, 2011). Thus, the entire community, especially pregnant women, should be encouraged to eat fish. Seluang fish has been shown to have the potential to prevent the inci- dence of stunting, so it can be used as a functional food ingredient from South Kalimantan.

5 CONCLUSION

Rats fed with seluang fish had significantly longer femur lengths compared with those fed with a low-protein and low-fat diet. Bone calcium lev- els in the group fed with seluang fish were higher than those in the group fed with a low-protein and low-fat diet, but were lower than those in the group fed with the standard feed. The group fed with seluang fish had higher IGF-1 levels than the group fed with the standard feed and the group fed with a low-protein and low-fat diet. Thus, it indi- cates that seluang fish has the potential to prevent stunting.

Future research on the potential of seluang fish is necessary. The health sector can collaborate with other sectors such as the Ministry of Maritime Affairs and Fisheries and the Ministry of Trade to promote the potential of seluang fish, so that it can be used as a functional food ingredient from South Kalimantan.

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