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Original Article

The Effectiveness of Beltfish (Trichiurus lepturus) Protein Intake and Physical Activity on Muscle Mass Growth

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ABSTRACT

Malnutrition cases have increased every year. One of the causes is insufficient food intake, marked by a decrease in muscle mass. Preventing malnutrition can be done by increasing muscle mass through providing daily protein intake according to needs and engaging in physical activity. The fish species Trichiurus lepturus is a high-protein source from the sea. The protein content in this fish can help promote muscle mass growth. This study aims to determine the effectiveness of protein intake from Trichiurus lepturus combined with physical activity on increasing muscle mass. The research method is experimental, using test animals (Rattus norvegicus) given protein intake and physical activity for 28 days. The animals were divided into six groups: three control groups and three treatment groups receiving different protein levels. Muscle growth was assessed by observing the enlargement of the cruris muscle using HE (hematoxylin and eosin) staining under a 4x microscope magnification. The data obtained were analyzed using ANOVA with an Bonferroni test. The results showed a significant difference between all groups (p=0.000). The treatment groups receiving protein intake and physical activity had larger muscle sizes compared to the control groups (P≤0.05). The conclusion is that protein intake accompanied by physical activity causes changes in muscle mass. Additionally, the amount of protein intake is directly proportional to the increase in muscle size.

Keyword: Protein Intake, *Trichiurus lepturus,* Physical Activity, Muscle Mass, Malnutrition

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INTRODUCTION

Malnutrition is widely found in developing countries or those classified as low-income, especially in children, often referred to as stunting[1]. Worldwide, children under the age of five are more likely to experience nutritional problems, with estimates reaching nearly 22%, or almost 150 million children[2]. The prevalence of stunting reaches 40% in Africa and 54% in Asia among children under five[3]. Stunting is a multifactorial health problem related to poverty, food insecurity, poor access to hygienic conditions, and healthcare services[4]. Providing free supplementary food from the government is one solution to accelerate access for young children to receive nutritious food[3]. The nutrient essential for improving stunted children's condition is protein. This is because several studies have shown that protein levels in stunted children are lower compared to children who are not stunted[5]. Furthermore, stunting in children leads to decreased immunity, making them more vulnerable to infections that cause morbidity and mortality[6].

Assessment of malnutrition in children can be done by using muscle mass indicators. Several studies have shown a decrease in muscle mass in children suffering from malnutrition[7]. Increasing protein intake in children is necessary to improve muscle mass, which in turn positively affects their condition and quality of life[8]. The daily protein requirement ranges from 0.8 to 1.3 grams per kilogram of body weight[9]. Ensuring adequate protein intake in stunted children is challenging due to decreased appetite, thus requiring high-protein foods[10]. In addition, to accelerate muscle mass gain, physical activity needs to be gradually increased according to the child's condition[11].

Fish is a high-protein source that is easily accessible and prepared in communities. It also contains a variety of micronutrients such as omega-3, iodine, selenium, and vitamin D[12]. Layur fish (Trichiurus lepturus) is a local Indonesian food high in protein, making it a potential supplementary protein source for stunted children. The development of supplementary foods from protein sources like this can be a preventive solution for stunting[13]. However, further research is needed on the effectiveness of protein intake combined with physical activity from layur fish in increasing muscle mass.

DESIGN AND METHODS

This study is a randomized controlled trial (RCT) with a post-test group design using a Completely Randomized Design (CRD). It was conducted at the Faculty of Medicine, University of Surabaya, over 28 days from August to September 2025. The study sample consisted of experimental animals (Rattus norvegicus) weighing approximately 200 grams, with no abnormalities detected and healthy nutritional status. The care and maintenance of the experimental animals during the study adhered to the 3R principles (Replacement, Reduction, and Refinement).

There were 42 animals (Federer formula) divided into 6 groups, each consisting of 7 rats. These groups included a negative control group (no protein intake or physical activity), positive control group I (protein intake without physical activity),

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positive control group II (physical activity without protein intake), and treatment groups receiving three different doses of protein. The protein doses used corresponded to the daily protein intake requirements for humans—0.8; 1; and 1.3 g/kg body weight/day—converted using the Laurence & Bacharach table into 3 g, 4 g, and 5 g of protein extract per rat for each respective group. The positive control group I received a protein extract dose of 3 g.

Physical activity was administered over a 28 day period using a treadmill at a speed of 20 meters/minute, which was gradually increased based on duration and inclination. The protocol was as follows: the first week involved 5 minutes with no incline; the second week involved 7.5 minutes with a 3 degree incline; the third week involved 10 minutes with a 7 degree incline; and the second week involved 10 minutes with a 10 degree incline[14].

After the treatment was completed, the test animals would be terminated for the collection of *crural* muscle tissue and the preparation of slides with HE (hematoxylin and eosin) staining. Subsequently, the increase in muscle mass was determined by measuring the diameter of the muscle tissue on the slide in 4 fields of view (FOV), where 1 FOV was measured 10 times. The assessment of muscle mass increase was done using the average of all field of view calculations in 1 slide. Muscle tissue assessment used an Olympus BX53 microscope with 4× magnification connected using the calibrated Olympus cellSens Standard application.

The data obtained will be analyzed using normality and homogeneity tests.Furthermore, a one-way ANOVA test will be performed to determine the difference among all research groups, and the Bonferroni test will be performed to show the existence of significant differences between groups or treatments.

RESULT AND DISCUSSION

The study results show the average muscle tissue size for each group. Table 1 shows the highest average was found in group 6 at 130.74±2.44, while the lowest average was in group III at 123.80±3.40. All obtained data passed the normality test ($p>0.05$), indicating the data were normally distributed. Next, the homogeneity test was conducted ($p=0.635$), concluding that the data were homogeneous ($p<0.05$). The differences in results for each group were then analyzed using the ANOVA test ($p=0.000$), followed by the Bonferroni test. The significant differences in measurements across all groups indicate histological changes in muscle tissue size with each treatment, illustrating that protein intake and physical activity can improve skeletal muscle mass[15].

Based on Table 2, there was no difference between groups I and II, indicating that sufficient protein intake without accompanying physical activity does not lead to an increase in muscle mass. Several studies have shown that protein intake plays a role in growth, repairing damaged cells, and hormone synthesis. However, without physical activity stimulation, the anabolic process to increase skeletal muscle mass (hypertrophy) and muscle strength will not be triggered. Although the mean values were higher in the group receiving protein intake, there was no significant difference in muscle tissue size between the two groups[16].

Furthermore, no difference was found between groups I and III, indicating that physical activity without adequate protein intake will not increase skeletal muscle mass. Insufficient protein intake to meet daily requirements results in an imbalance between protein synthesis and breakdown, negatively impacting skeletal muscle mass, function, immunity, and hormone synthesis[17]. In addition, inadequate protein intake causes muscle mass to be catabolized as a reserve of amino acids for endogenous protein synthesis in tissues and organs during physiological processes. Under these conditions, muscle wasting occurs, indicating that protein intake needs have not been met[18,19]. This is reflected by the lower mean value in group III compared to group I, although the difference was not yet significant.

The groups receiving protein intake and physical activity (Groups IV, V, VI) showed gradual increases in muscle tissue size (Figure 1). The protein intake given to the treatment groups stimulates muscle protein synthesis (MPS) through an anabolic process. However, this process is determined by the balance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB), where a greater rate of protein synthesis compared to muscle protein breakdown will trigger muscle mass formation[20,21]. An increase in protein synthesis can be stimulated by physical activity, especially resistance exercise (RE), leading to muscle hypertrophy[22]. Protein intake will activate the mTORC1 pathway, which is the central regulator of muscle protein synthesis (MPS). Activation of mTOR coordinates the synthesis of and biomolecules such as lipids, proteins, and nucleotides[23]. While mTOR is normally regulated under physiological conditions, loss of control can lead to uncontrolled cell growth associated with cancer and tumors[24]. The result of activating muscle protein synthesis (MPS) is hypertrophy of skeletal muscle mass[25].

Table 1. Means of muscle tissue size between all groups

Groups		Mean±SD	Normality	Homogeneity	Anova Test
I	A group only given daily intake	123.95±2.77	0.338	0.635	0.000
II	A group given daily intake and 3 ml protein intake	126.27±1.59	0.495		
III	A group given daily intake and physical activity	123.80±3.40	0.358		
IV	A group given daily intake, 3 ml protein intake and physical activity	127.40±2.88	0.648		
V	A group given daily intake, 4 ml protein intake and physical activity	129.20±2.28	0.103		
VI	A group given daily intake, 5 ml protein intake and physical activity	130.74±2.44	0.657		

Table 2. The results of Bonferroni on muscle tissue size between all groups.

Groups	I	II	III	IV	V	VI
I	-	-	-	-	-	-
II	0.108	-	-	-	-	-
III	0.911	0.086	-	-	-	-
IV	0.019	0.426	0.015	-	-	-
V	0.001	0.044	0.000	0.207	-	-
VI	0.000	0.003	0.000	0.023	0.278	-

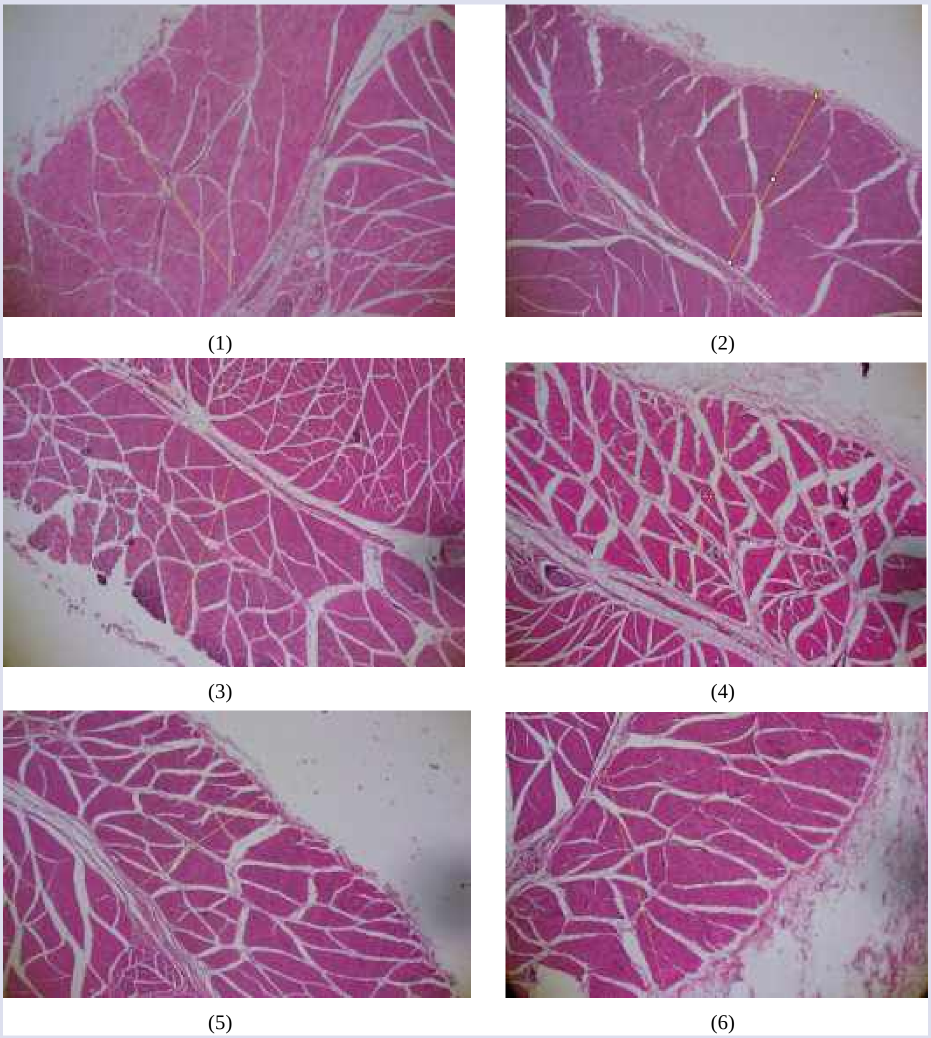

Figure 1. Preparat Jaringan otot cruris pewarnaan Hematoxylin Eosin dengan pembesaran mikroskop 4x. (1) Group only given daily intake; (2) Group given daily intake and 3 ml protein intake; (3) Group given daily intake and physical activity; (4) Group given daily intake, 3 ml protein intake and physical activity; (5) Group given daily intake, 4 ml protein intake and physical activity; (6) Group given daily intake, 5 ml protein intake and physical activity

CONCLUSION

Providing protein intake cannot maximally increase muscle mass without accompanying physical activity. Moreover, the amount of protein consumed also affects the rate of muscle mass gain when combined with appropriate physical activity.

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