

## EFFECTS OF ETHANOL EXTRACT OF PADANG TARO LEAVES (*COLOCASIA GIGANTEA*) ON THE WEIGHT AND MUSCLE THICKNESS OF MALNOURISHED MODEL MICE

Adlina Karimina Nurul Husna<sup>1</sup>, Tri Widyawati<sup>1,2,3</sup>, Dwi Rita Anggraini<sup>1,4</sup>

1. Master of Biomedical Sciences Study Program, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia, adlina.karimina@unisayogya.ac.id

2. Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia, tri.widyawati@usu.ac.id

3. Master of Tropical Medicine Study Program, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia, tri.widyawati@usu.ac.id

4. Department of Anatomy, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia, dwirita@usu.ac.id.

5. Department of Pharmacology, Faculty of Medicine, Universitas 'Aisyiyah Yogyakarta, Yogyakarta, Indonesia, adlina.karimina@unisayogya.ac.id

Correspondence: Tri Widyawati (tri.widyawati@usu.ac.id))

### ABSTRACT

Malnutrition can cause impaired growth and muscle mass. Talas Padang (*Colocasia gigantea*) is one of the sources of natural ingredients with nutritional content. This study aims to analyze the effect of various doses of administration ethanol leaf extract of Talas Padang (ELETP) on the weight and thickness of skeletal muscle (soleus) in malnourished rat. Pure experimental research with Post-test Only Control Group design using 25 malnourished rat (Wistar strain). ELETP is obtained macerated using 96% ethanol. Treatment in 5 groups (n=5), K1: normal 56-day diet; K2: low-protein diet (LPD) 14 days + normal 42-day diet; K3, K4 and K5: 14-day LPD + normal diet and ELETP doses of 100, 200, 400/kgBB for 42 days. The soleus muscle is obtained by dissection in the posterior aspect of the mouse leg to measure weight and thickness. Muscle weight data were analyzed using Anova's one-way test and muscle thickness using the Wallis Kruskal test. The results showed average (gr) of muscle weight K3 ( $0.246 \pm 0.087$ ) > K5 ( $0.236 \pm 0.098$ ) > K4 ( $0.215 \pm 0.092$ ) > K1 ( $0.188 \pm 0.060$ ) > K2 ( $0.127 \pm 0.025$ ) and muscle thickness (mm) K4 (1 (1-1)) > K1, K3, K5 (1 (0,00-1)) > K2 (0,00 (0,00-1)). This study conclude that ELETP doses of 100mg/kgBB produce the largest muscle weight, while the largest muscle thickness is found at a dose of 200mg/kgBB.

**Keywords:** Colocasia gigantea, Malnutrition, Talas Padang.

### INTRODUCTION

Stunting is a sign of chronic malnutrition experienced over a long period of time<sup>1</sup>. According to UNICEF and WHO, in 2020 the global prevalence of stunting was 21.3%<sup>2</sup>. Stunting measurement is measured using *z-score* by measuring height-for-age, if it is more than 2 standard deviations or below the median Child Growth Standard of the World Health Organization (WHO) then it is classified as stunting<sup>3</sup>. When compared to map information on various continents, the prevalence of stunting in Indonesia is still higher than the prevalence in Southeast Asia, which is 24.7%. This is evidenced based on data from the 2019 Nutritional Status Study of Toddlers in Indonesia (SSGBI) that the prevalence of stunting in Indonesia is 27.67%<sup>4</sup>.

There are several causes of stunting through a molecular approach, namely, congenital (familial) conditions, endocrine diseases, chromosomes, chronic diseases, malnutrition and a history of previous breastfeeding. Broadly speaking, stunting is divided into two, namely based on family history and pathological conditions<sup>5</sup>. Malnutrition affects the global population at several stages of life. In public health epidemiology, this condition can occur in everyone, but the most vulnerable are the poor, children, adolescents, the elderly, those who suffer from diseases and have a vulnerable immune system as well as breastfeeding and pregnant women<sup>6</sup>.

Dipasquale et al., stated that acute malnutrition is malnutrition due to insufficient energy or protein intake. Proteins derived from food are necessary for a source of amino acids for the synthesis of body proteins and other compounds that have various functional roles. Energy is

essential for all biochemical and physiological functions in

EFFECTS OF ETHANOL EXTRACT OF PADANG TARO LEAVES (*COLOCASIA GIGANTEA*)... Adlina Karimina Nurul  
Husna1, Tri Widyawati1,2,3, Dwi Rita Anggraini1,4

Activated Protein Kinase). The role of AMPK as an energy sensor is very important in the grid which shows highly volatile energy cycling<sup>8</sup>. AMPK is heavily involved in controlling skeletal muscle metabolism through the regulation of many targets. Due to its effects on anabolic and catabolic cellular processes, AMPK plays an important role in controlling the development of skeletal muscle growth and regulates muscle mass and its regeneration, AMPK  $\alpha$ 1 is important in stimulating anabolism and regulating muscle cell dynamics during regeneration, while AMPK  $\alpha$ 2 regulates muscle degradation during atrophy<sup>9</sup>.

Malnutrition can be one of the causes of muscle atrophy or a condition of loss of skeletal muscle mass. Muscle atrophy causes muscle weakness and can lead to disability<sup>10</sup>. It is known that muscle atrophy results from decreased protein synthesis and accelerated proteolysis. In accordance with the pro-atrophic role of TNF $\alpha$ , it has been found that the cytokine decreases protein synthesis and tends to increase proteolysis in L6 myotubes<sup>11</sup>. There was evidence that the weight and *cross-sectional area* (CSA) of plantaris muscle fibers in the malnourished group were lower than in the control group. However, the weight and CSA of soleus muscle fibers did not change much in malnourished conditions. This suggests that malnutrition induces a loss of plantaris muscle mass but not the soleus muscle<sup>12</sup>. Meanwhile, according to Silva et al., the soleus muscle is proven to experience weight loss after giving a low-protein diet<sup>13</sup>.

There are many factors that affect the increase in muscle mass in each individual, one of which is the dietary factor. Consuming foods that contain high protein and reducing the consumption of foods that contain high fat will greatly affect the development of muscle mass of a person<sup>14</sup>. Taro leaves are one of the nutrient-rich plants because they contain a lot of various nutrients including carbohydrates and proteins. One of the species that is often consumed as a source of nutritious food is the *species Colocasia gigantea* or in Indonesia known as Taro Padang. Traditionally, *Colocasia* leaves show great potential for food as well as drug development<sup>15</sup>. In general, *Colocasia* leaves have good nutritional content, such as protein, carbohydrates, fats, Calcium, phosphorus, iron, and vitamins A, B, and C. The nutritional content of *Colocasia leaves* is 86.94% water content, 16.48% crude protein, 17.24% crude fiber, potassium 1.45%, phosphorus 0.4%, fat 4.3%, BETN 30.46% and gross energy 3966 kcal/kg<sup>16</sup>.

Until now, no one has studied the effect of *Colocasia gigantea* leaves on muscle conditions in malnutrition, especially on the soleus muscle. According to the USDA Agricultural Research Service, every 100 grams of *Colocasia leaves* contain 85.7 grams of water, 42 kcal of energy, 4.98 grams of protein, 0.74 grams of total fat, 6.7

grams of carbohydrates, 3.7 grams of fiber, 107 grams of

0.167gr, lysine 0.246gr, tryptophan 0.048gr, phenylalanine 0.195gr and histidine 0.114gr. Therefore, with the potential of *Colocasia gigantea leaves* as a source of protein that can help improve nutrition in malnourished conditions, researchers are interested in finding out how the effect of administering ethanol extract of *Colocasia gigantea* leaves on the muscle weight and thickness of malnourished model mice.

## MATERIALS AND METHODS

This study is purely experimental using an experimental animal model with a *Post-test Only Control Group research design* that has received permission from the USU Ethics Commission No.0150/KEPH-FMIPA/2023. The population of 6-8 week-old male white mice of the Wistar strain who were induced into malnourished model mice was 25 based on Federer's formula which was randomly divided into 5 groups. The sample of this study was the right and left soleus muscles measured on average weight and thickness from malnourished model mice. Inclusion criteria: intact soleus muscle and no deformity. Exclusion criteria: apparent soleus muscles with deformity. Provision of ELETTP with Padang Taro leaf simplicia powder that has been macerated with 96% ethanol solvents. The K1 group was a group of normal rats who were given a normal diet for 56 days, K2 as a positive control was given DRP for 14 days then a normal diet for 42 days, K3 was given DRP for 14 days then a normal diet and ELETTP at a dose of 100mg/kgBB orally once a day for 42 days, K4 was given DRP for 14 days then a normal diet and ELETTP at a dose of 200mg/kgBB orally once a day for 42 days and K5 was given DRP for 14 days later, normal diet and ELETTP dose of 400mg/kgBB orally once a day for 42 days.

Termination was carried out by giving concentrated chloroform by inhalation, then the soleus muscle sampling was carried out by dissection on the posterior aspect of the rat limb and then identifying the gastrocnemius muscle adjacent to the achilles tendo and soleus muscle, then the soleus muscle was taken and immediately weighed using an analytical scale and then the muscle thickness was measured with a caliper. All data were analyzed to determine the difference in the average weight and thickness values of each treatment group with the Anova one-way test which had previously been tested for normality using the Shapiro Wilk test because the number of samples was <50 and the homogeneity test using the Levene test. In the results of the prerequisite tests that do not meet normality and homogeneity, the Kruskal-Wallis test is carried out.

## RESULT

### EFFECTS OF ETHANOL EXTRACT OF PADANG TARO LEAVES (COLOCASIA GIGANTEA)...

analytical scale and then after obtaining the two weights, the average weight is calculated in grams. In table 4.1, it can be seen that the average muscle weight of soleus muscle from each group, the average muscle weight in the low-protein diet group that was not given *Colocasia gigantea* leaf ethanol extract (K2) had the smallest average muscle weight ( $0.127 \pm 0.025$ ), while the average muscle weight was the largest in the K3 group, namely the low-protein diet group given *Colocasia gigantea* leaf ethanol extract with a dose of 100mg/kgBB ( $0.246 \pm 0.087$ ).

In the Shapiro-Wilk *normality test*, it was known that the *p-value* data in each group was greater than 0.05, thus, the data in each group was normally distributed. After that, a homogeneity test was carried out *Levene's Test* and a *p-value* of 0.466 was obtained which was greater than 0.05. This means that the data between groups has a homogeneous data variance. Because the prerequisite test has been fulfilled and then the *Anova one-way* test has been carried out, the treatment group that has the highest average is the K3 group. Meanwhile, for a *p-value* of 0.153 which is greater than 0.05, there was no significant difference in the average weight of the soleus muscle of each treatment group (Table 1).

**Table 1.** Effect of ELETP on Soleus Muscle Weight Information:

Group	N	Soleus Muscle Weight (gr) (Mean $\pm$ SD)	p-value
K <sub>1</sub>	5	0,188 $\pm$ 0,060	0,153
K <sub>2</sub>	5	0,127 $\pm$ 0,025	
K <sub>3</sub>	5	0,246 $\pm$ 0,087	
K <sub>4</sub>	5	0,215 $\pm$ 0,092	
K <sub>5</sub>	5	0,236 $\pm$ 0,098	

*One way-Anova* test with a significant value of  $p < 0.05$

K1: normal; K2 : positive control; K3: ELETP dose 100mg/kgBB; K4: ELETP dose 200mg/kgBB; K5: ELETP dose 400mg/kgBB.

#### Effect of ELETP on Soleus Muscle Thickness

The results of macroscopic measurements of the thickness of the soleus muscle in each group have been obtained. The thickness of the soleus muscle is measured at the distance between the anterior to posterior midpoint The soleus muscles use a shovel. The average thickness of the soleus muscle in each group is, K1 group: 0.99mm, K2 group: 0.66mm, K3 group: 0.87mm, K4 group: 1mm and K5 group: 0.95mm. The K2 group has the smallest soleus muscle thickness, while the largest group K4 has. After

conducting the prerequisite test, it turned out that the data did not meet the assumptions of normality and homogeneity. Thus, a hypothesis test to find out the difference in average muscle thickness cannot be carried out using the *Anova one-way* test, but the *Kruskal-Wallis* test with a *p-value* of 0.233, greater than 0.05. This means that there was no significant difference in the mean thickness of the soleus muscle in each treatment group (Table 3.2).

**Table 2** Effect of ELETP on Soleus Muscle Thickness

Group	N	Soleus Muscle Thickness (mm) [Median (min-max)]	p-value
K <sub>1</sub>	5	1 (0,00-1)	0,233
K <sub>2</sub>	5	0,00 (0,00-1)	
K <sub>3</sub>	5	1 (0,00-1)	
K <sub>4</sub>	5	1 (1-1)	
K <sub>5</sub>	5	1 (0,00-1)	

Information:

*Kruskal-Wallis test* with a significant value of  $p < 0.05$

K1: normal; K2 : positive control; K3: ELETP dose 100mg/kgBB; K4: ELETP dose 200mg/kgBB; K5: ELETP dose 400mg/kgBB.

#### DISCUSSION

Body muscle mass can be defined as total muscle weight<sup>17</sup>. In malnutrition, there is Increased oxidative stress that decreases mitochondrial metabolic capacity thereby lowering anabolism and affects the decrease in muscle mass, so it can be considered a source of nutritious food from natural ingredients needed to help improve muscle mass in malnutrition. In this study, the average muscle weight in the low-protein diet group that was not given *Colocasia gigantea leaf ethanol extract* showed the smallest average muscle weight, while the average muscle weight was the largest in the low-protein diet group given *Colocasia gigantea leaf ethanol extract* at a dose of 100mg/kgBB. This can be associated with calorie restriction conditions on a low-protein diet, where reduced calories can reduce weight and skeletal muscle weight (gastrocnemius), as stated by Torrens-Mas et al., that it occurs in both sexes, but skeletal muscle weight is lower in female rats compared to male rats<sup>18</sup>.

Less than normal muscle mass falls into the criteria for diagnosing malnutrition<sup>19</sup>. However, muscle assessment is rarely performed in screening and nutritional assessment, due to the limited anthropometric and biochemical tools for the evaluation of muscle mass and function in malnourished patients<sup>20</sup>. According to Prado et al., nutritional interventions are very important in spurring anabolism,

reducing catabolism and supporting the improvement of subnormal muscle conditions and malnutrition. In the assessment of muscle mass, an examination can be carried out to assess muscle mass in the process of improving nutrition.

When body composition tests are not available, physical examinations of muscles and anthropometry (upper middle arm circumference and calves) can be performed. In the calf muscle examination, it can represent muscle mass, including the gastrocnemius and soleus muscles<sup>21</sup>.

Malnutrition of protein energy, increased catabolism and inadequate nutrition lead to the loss of lean body mass with shrinking muscles accompanied by weak immune conditions<sup>22</sup>. In this study, the thickness of the soleus muscles in the group given a low-protein diet without being given *Colocasia gigantea leaf* ethanol extract had the smallest average thickness, while the group given a dose of 200mg/kgBB showed an average muscle thickness that exceeded that of the normal diet group, in addition, The greatest muscle thickness is also found at a dose of 200mg/kgBB. Based on this, malnutrition is related to decreased muscle function and mass as well as a decrease in health status. Protein supplementation is known to prevent muscle mass loss<sup>23</sup>. Protein supplementation can increase body weight, lean muscle mass, bone area, mineral content and body density<sup>24</sup>. Leucine supplementation diets have been shown to increase protein synthesis in muscles. Long-term supplementation can increase growth hormone resistance in mice with protein energy malnutrition. Leucine can spur skeletal muscle protein synthesis by regulating downstream anabolic signaling transduction<sup>25</sup>.

Leucine is a branched-chain amino acid, which has been known recently as a therapeutic agent for the treatment of various conditions related to muscle wasting due to its ability to accelerate protein synthesis and reduce protein breakdown in the muscles. Leucine supplementation is also safe and effective for increasing muscle protein synthesis as well as reducing the loss of lean mass under catabolic conditions<sup>26</sup>. L-leucine and its derivatives can regulate protein synthesis by direct or indirect activation of the mTORC1 kinase, which further improves muscle protein balance<sup>27</sup>. The mTOR (*mechanistic Target of Rapamycin*) pathway regulates homeostasis by directly influencing protein synthesis, transcription, autophagy, metabolism, and biogenesis and organelle maintenance<sup>28</sup>.

Research by Pertille et al., showed that skeletal muscle regeneration after malnutrition conditions showed a higher number of connective tissue and areas of inflammation in the MRI (*Malnourished Recovered and Injured group*). In addition, the CSA area of the *regenerated myofiber* decreased on MRI and there was a slow change in the process of anterior tibial muscle regeneration and slow muscle repair even after nutrient recovery<sup>29</sup>. From this, additional protein supplements can be given to improve nutrition, in addition to being given adequate nutrition to improve malnutrition conditions due to

the provision of a low protein diet. In this study, we tried to

Adlina Karimina Nurul Husna<sup>1</sup>, Tri Widyawati<sup>1,2,3</sup>, Dwi Rita Anggraini<sup>1,4</sup>

(*Colocasia gigantea*). As mentioned by the USDA Agricultural Research Service, every 100 grams of *Colocasia* leaves contain 9 essential amino acids, of which leucine has the highest content, which is 0.392 grams. Although the level of essential amino acids in the ELETP has not been examined in this study, it can still be seen in giving a dose of 100 mg/kgBB that shows the greatest muscle weight and in a dose of 200mg/kgBB shows the greatest muscle thickness.

## CONCLUSION

Based on the results and discussion of this study, it was concluded that there was no significant difference in the average muscle weight and soleus muscle thickness in each treatment group. However, ELETP at a dose of 100mg/kgBB produced the most muscle weight, while the one that showed the greatest muscle thickness was ELETP at a dose of 200mg/kgBB. The suggestion for the next study is to conduct a study with an intervention duration of more than 42 days, which is approximately 63 days to see the effect of the test material on the soleus muscle in malnourished model mice.

## ACKNOWLEDGMENTS

Thanks to Supportive Grant: Ministry of Research and Technology and Higher Education Republic of Indonesia, Research and Community Service, Universitas Sumatera Utara Grant (No.9417/UN5.1.R/PPM/2022)

## BIBLIOGRAPHY

1. Vonaesch P, Tondeur L, Breurec S, Bata P, Nguyen LBL, et al. (2017) Factors associated with stunting in healthy children aged 5 years and less living in Bangui (RCA). PLOS ONE 12(8):e0182363. <https://doi.org/10.1371/journal.pone.0182363>
2. WHO (World Health Organization). (2020). Levels and trends in child malnutrition: Key findings of the 2020 Edition of the Joint Child Malnutrition Estimates. Geneva, 24(4), 1–16.
3. WHO (World Health Organization). (2018). Reducing stunting in children: equity considerations for achieving the Global Nutrition Targets 2025.
4. Sudikno II, Setyawati B, Wiryawan Y, Puspitasari DS, Widodo Y, Ahmadi F, Amaliah N. (2019). Laporan Akhir Penelitian Studi Status Gizi Balita Di Indonesia Tahun 2019. Pusat Litbang Upaya Kesehatan Masyarakat, Badan Penelitian dan Pengembangan Kesehatan, Kementerian Kesehatan RI.
5. Flora R. (2021). *Stunting dalam kajian molekuler*. Cetakan pertama, Universitas Sriwijaya, Palembang.
6. Dukhi. (2020). Global prevalence of malnutrition:



- effects and treatment. *Nutrients*, 12(8), 1–9. <https://doi.org/10.3390/nu12082413>
8. Kjobsted, R., Hingst, J. R., Fentz, J., Foretz, M., Sanz, M. N., Pehmøller, C., ... & Lantier, L. (2018). AMPK in skeletal muscle function and metabolism. *The FASEB journal*, 32(4), 1741.
9. Thomson, D. M. (2018). The role of AMPK in the regulation of skeletal muscle size, hypertrophy, and regeneration. *International Journal of Molecular Sciences*, 19(10). <https://doi.org/10.3390/ijms19103125>
10. Pierik, V. D., Meskers, C. G., Van Ancum, J. M., Numans, S. T., Verlaan, S., Scheerman, K., ... & Maier, A. B. (2017). High risk of malnutrition is associated with low muscle mass in older hospitalized patients—a prospective cohort study. *BMC geriatrics*, 17(1), 1–8.
11. De Larichaudy, J., Zufferli, A., Serra, F., Isidori, A. M., Naro, F., Dessalle, K., ... & Némóz, G. (2012). TNF- $\alpha$  and tumor-induced skeletal muscle atrophy involves sphingolipid metabolism. *Skeletal muscle*, 2(1), 1–19.
12. Hirabayashi, T., Nakanishi, R., Tanaka, M., Nisa, B. U., Maeshige, N., Kondo, H., & Fujino, H. (2021). Reduced metabolic capacity in fast and slow skeletal muscle via oxidative stress and the energy- sensing of AMPK/SIRT1 in malnutrition. *Physiological Reports*, 9(5), e14763.
13. Silva, F. H. S. D., Dos Santos, M. P., Pereira, M. P., Buzelle, S. L., Allebrandt Neto, E. W., Gai, B. M., ... & Kawashita, N. H. (2019). The antioxidant system in the soleus muscle of growing rats is stimulated by the administration of a low-protein/high-carbohydrate diet. *Archives of Physiology and Biochemistry*, 125(3), 276–283.
14. Rabi, G., Soethama, R., Silakarma, D., Ayu, I., & Wiryanthini, D. (2012). Pengaruh latihan beban terhadap peningkatan massa otot pectorals mayor dan biceps pada remaja dan dewasa. *Maj Ilm Fisioter Indones*, 2, 52–7.
15. Gupta, K., Kumar, A., Tomer, V., Kumar, V., & Saini, M. (2019). Potential of Colocasia leaves in human nutrition: Review on nutritional and phytochemical properties. *Journal of Food Biochemistry*, 43(7), 1–16. <https://doi.org/10.1111/jfbc.12878>
16. Suwitari, N. K. E., Suariani, L., & Yudiastari, N. M. (2022, June). The Effect of The Use of Taro Leaf Flour on The Digestiveness of Native Chicken Rate. In *WICSTH 2021: Proceedings of the 1st Warmadewa International Conference on Science, Technology and Humanity, WICSTH 2021, 7-8 September 2021, Denpasar, Bali, Indonesia* (p. 56). European Alliance for Innovation.
17. Prihatingrum, R., Sumekar, T. A., & Hardian, H. (2016). Pengaruh Latihan Zumba Terhadap Massa Otot Tubuh Pada Wanita Usia Muda. *Jurnal Kedokteran Diponegoro (Diponegoro Medical Journal)*, 5(2), 115–121.
18. Torrens-Mas, M., Navas-Enamorado, C., Wahl, D., Sanchez-Polo, A., Picca, A., Oliver, J., Roca, P., & Gonzalez-Freire, M. (2022). Sex Specific Differences in sponse to Calorie Restriction in Skeletal Muscle of Young Rats. *Nutrients*, 14(21), 4535. <https://doi.org/10.3390/nu14214535>.
19. Landi, F., Camprubi-Robles, M., Bear, D. E., Cederholm, T., Malafarina, V., Welch, A. A., & Cruz-Jentoft, A. J. (2019). Muscle loss: The new malnutrition challenge in clinical practice. *Clinical Nutrition*, 38(5), 2113–2120.
20. García Almeida, J. M., Bascuñana Ambrós, H., Terrados Cepeda, N., Sanz Barriuso, R., López Pedrosa, J. M., Campos Gorgona, N., & Luis Román, D. D. (2018). The role of muscle in disease-related malnutrition. Decalogue of good practices. *Progress in Nutrition*, 20.
21. Prado, C. M., Landi, F., Chew, S. T., Atherton, P. J., Molinger, J., Ruck, T., & Gonzalez, M. C. (2022). Advances in muscle health and nutrition: A toolkit for healthcare professionals. *Clinical Nutrition*.
22. Wong JJ, Ong JS, Ong C, Allen JC, Gandhi M, Fan L, Taylor R, Lim JK, Poh PF, Chiou FK, Lee JH. Protein supplementation versus standard feeds in underweight critically ill children: a pilot dual-centre randomised controlled trial protocol. *BMJ open*. 2022 Jan 1;12(1):e047907.
23. Dormal V, Pachikian B, Debock E, Buchet M, Copine S, Deldicque L. Evaluation of a Dietary Supplementation Combining Protein and a Pomegranate Extract in Older People: A Safety Study. *Nutrients*. 2022 Dec 6;14(23):5182.
24. Yang KP, Wong CP, Khanna SK, Bray TM. Supplementation of ocean-based advance protein powder (APP) for restoration of body growth, bone development and immune functions in protein malnourished mice: Implications for preventing child malnutrition. *Ecology of Food and Nutrition*. 2020 Sep 2;59(5):552–74.
25. Gao X, Tian F, Wang X, Zhao J, Wan X, Zhang L, Wu C, Li N, Li J. Leucine supplementation improves acquired growth hormone resistance in rats with protein-energy malnutrition. *PLoS One*. 2015 Apr 24;10(4):e0125023.
26. Wamiti J, Kogi-Makau W, Onyango FE, Ngala S. Leucine supplementation in the management of protein energy malnutrition: A review. *East African Medical Journal*. 2017 Apr 18;94(1):20–4.
27. Soares JD, Howell SL, Teixeira FJ, Pimentel GD. Dietary amino acids and immunonutrition supplementation in cancer-induced skeletal muscle mass depletion: A mini-review. *Current pharmaceutical design*. 2020 Mar 1;26(9):970–8.
28. Lipton JO, Sahin M. The neurology of mTOR. *Neuron*. 2014 Oct 22;84(2):275–91.
29. Pertille A, Moura KF, Matsumura CY, Ferretti R, Ramos DM, Petrini AC, Oliveira PC, Silva CA. Evaluation of skeletal muscle regeneration in experimental model after malnutrition. *Brazilian Journal of Biology*. 2016 Jul 4;77:83–91.

