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# IN VIVO DIGESTIBILITY OF PROTEIN EXTRACTED FROM BRACKISH ALGAE *CHAETOMORPHA* SP.

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## ABSTRACT

***Background:*** *Chaetomorpha sp., a species of green brackish algae, has a high biomass distribution in extensive shrimp ponds in Mekong Delta.*
***Objective:*** *This study aims to evaluate the nutritional value of Chaetomorpha sp. algae protein concentrate by assessing its in vivo digestibility and comparing it to soy protein as a reference for food applications.*
***Materials and Methods****: Nutritive values such as PER (Protein Efficiency Ratio), NPR (Net Protein Ratio), and BV (Biological Value) were determined through in vivo trials on white mice. These mice were fed test diets containing Chaetomorpha sp. algae protein concentrate (APC), soy protein concentrate (SPC), and a regular diet provided by the Pasteur Institute in Ho Chi Minh City. The data were collected after 4 weeks of feeding the mice with the different diets.*
***Results****:The PER, NPR, and BV values of Chaetomorpha sp. APC were 2.12, 3.28, and 79.32, respectively. These values were comparable to those of SPC and much higher than those of regular rice bran-based diet.*
***Conclusion:*** *The protein concentrate derived from Chaetomorpha sp. algae has a high protein content (76.3% w/w db), possesses good nutritional value, and is a viable source of plant-based protein for food applications.*

***Keywords:*** *Chaetomorpha* sp.; *green brackish algae; in vivo digestibility; nutritive value; algae protein concentrate*

## INTRODUCTION

*Chaetomorpha* sp., a fast-growing species of green algae belonging to the *Cladophora* genus within the *Chlorophyta* division, can be found in both seawater and brackish water environments[1]. It can be easily collected and co-cultured with shrimp in brackish water shrimp ponds in the Mekong Delta. This algal species has a rapid growth rate of 5–12% per day. In some extensive shrimp ponds, it can reach an average biomass of 15 tons per hectare[2]. Algae are low-energy, low-lipid foods, but rich in carbohydrates, minerals, and proteins[3]. Algae proteins generally provide a more balanced essential amino acid profile than terrestrial plants. Species like *Chaetomorpha*, *Ulva*, and *Gracilaria* are rich in valine, threonine, leucine, isoleucine, and lysine - an amino acid often deficient in plant proteins[4]. *Chaetomorpha* sp. biomass is rich in protein (12-21% w/w db) with a balanced amino acid profile[2], making its protein potentially significant for human nutrition, particularly in regions with inadequate protein intake.

Protein quality is key for assessing a product's nutritional value, with indicators such as protein content, amino acid composition, and the essential-to-non-essential amino acid ratio[5,6]. In addition, *in vivo* evaluations are crucial for new protein sources, measuring digestion, absorption, and utilization of protein. Common indices include PER (Protein Efficiency Ratio), NPR (Net Protein Ratio), and BV (Biological Value). PER reflects weight gain relative to protein intake over 10 days, NPR accounts for weight loss on protein-free diets, and BV measures protein digestion, absorption, and conversion into body protein[7]. Some authors have studied the nutritional value of animal proteins (beef and meat products) and certain plant proteins (soybean, maize) using these indices[8,9]; or compared the relationship between *in vivo* digestibility (using indices such as PER and BV) with *in vitro* digestibility[10].

Laboratory animals are often fed cereal-based diets with unknown and unbalanced nutrient compositions, which may confound metabolic responses. Therefore, purified diets like AIN-93, with balanced defined nutrient profiles, are recommended[11]. Kinyi et al. (2023) indicated that Swiss albino mice fed AIN-93 for 15 weeks exhibited higher body weight gain, hemoglobin levels, and serum albumin levels compared to those on a regular diet, indicating improved protein utilization[12]. Several researchers have also investigated the nutritional characteristics of algae protein concentrate in *in vivo* studies. Wang et al. (2020) and Martínez et al. (2022) found that disrupting algae cell walls through thermal, high-pressure, or mechanical treatments enhanced protein extraction, yielding high-purity algae protein concentrate, improving protein efficiency ratio (PER) and biological value (BV) in mice fed the AIN-93 diet[6,13]. In addition, some studies also reported that the high polysaccharide and fiber content in algae protein concentrates from *Porphyra tenera*, *Undaria pinnatifida*, and *Laminaria japonica* reduced protein digestibility (PER, NPR, BV) in rats[14,15].

Thus, various factors can influence the *in vivo* protein digestibility in mice, including the nutrient composition of regular and AIN-93 diets, as well as the fiber, carbohydrate, and protein content of algae protein concentrates used as the sole protein source in the AIN-93 diet. Although some studies have investigated the *in vivo* digestibility of proteins from various algae species, research on *Chaetomorpha* sp. has primarily focused on obtaining protein concentrates from this algae, achieving over 70% purity through various extraction and purification methods[16,17]. The nutritional value and factors affecting its digestibility remain unstudied. Thus, this study aims to assess the nutritional value of *Chaetomorpha* sp. algae protein by evaluating its *in vivo* digestibility in mice fed an AIN-93 diet and comparing it to soy protein, a widely used protein source, for potential food applications.

## MATERIALS AND METHODS

### Algae protein Concentrate (APC)

*Chaetomorpha* sp. algae, primarily *Chaetomorpha aerea*, were harvested from extensive shrimp ponds in Bac Lieu Province, Vietnam. The algae were transported to the laboratory on the same day, cleaned to remove impurities, and dried at 50–60°C to achieve a moisture content of 9–10%. Algae protein concentrate was prepared based on the method described on the study of Bach et al. (2019)[16]. Dried algae biomass from *Chaetomorpha* sp. was ground through an 80 mesh screen to obtain a fine powder; then lipids, wax, and chlorophyll were removed by diethyl ether extraction. The biomass was then extracted by using 1 N NaOH pH 10.0 solution (biomass to NaOH solution ratio 1:20 w/v), at 50ºC for 1 h. The slurry was then centrifuged at 10,000 g for 30 min at 4°C to separate algae biomass residue. The protein in the extract was precipitated with 70% saturated $(NH_4)_2SO_4$ solution at 10°C. After centrifugation, the protein precipitate was dissolved in water and any remaining salt was removed by dialysis through a 1000 kDa cellulose membrane. Finally, the protein solution was freeze-dried to obtain APC powder and stored at -4°C.

**Soy protein concentrate (SPC)**: was purchased from Cargill Co. Ltd (Ho Chi Minh city, Vietnam).

**Chemicals:** Enzymes used in the Total Dietary Fiber Assay (TDF-100A), including heat-stable α-amylase (A3306) and protease (P3910) from *Bacillus licheniformis* and amyloglucosidase (A9913) from *Aspergillus niger*, were purchased from Sigma Aldrich (USA). Petroleum ether, $H_2SO_4$, $Na_2SO_4$, anhydrous $CuSO_4$, $K_2SO_4$, $(NH_4)_2SO_4$, used in crude protein and fat analysis, were supplied by Supelco (Merck, Germany). Other chemicals, such as NaOH, KOH, HCl, ethanol, acetone, etc., were obtained from Cemaco and Xilong Scientific (China).

### In vivo protein digestibility assay

APC from *Chaetomorpha* sp. and SPC were used as the sole protein sources to prepare feeds for experimental mice, with the nutritional composition designed according to the AIN-93 formula[11].

The experiment was carried out according to Kinyi et al. (2023) and Singha et. al (2020), with some modifications[12,18]. Adult male Swiss albino mice, scientifically known as *Mus musculus*, were provided by the Pasteur Institute in Ho Chi Minh City. The mice were approximately 5-6 weeks old, weigh between 16-20g. They were healthy and free from illness. The mice were divided into 4 groups, each consisting of 5 individuals. Each group was fed with one of 4 different diets:

- Group 1: Mice were fed with AIN-93 diet modified to contain zero protein (protein-free diet).
- Group 2: Mice were fed with protein-based regular diet derived from Pasteur Institute of Ho Chi Minh City (made from rice bran).
- Group 3: Mice were fed with protein-based AIN-93 diet derived from APC.
- Group 4: Mice were fed with protein-based AIN-93 diet derived from SPC.

**Table 1**. **AIN-93 protein-based diet and regular diet derived from Pasteur Institute**

| Composition (% w/w db) | Regular diet | AIN-93 diet |
|---|---|---|
| Carbohydrate (starch and sugar) | 58.8 | 72.0 |
| Fiber | 1.1 | 5.0 |
| Protein | 20.4 | 10.0 |
| Lipid | 19.7 | 9.0 |
| Ash/Salt | - | 4.0 |

The mouse cages, made of glass, measured 15 cm × 30 cm and were equipped with stainless steel hanging troughs attached to the cage walls for food and water. They were placed in standard housing conditions with a 12h/12h light/dark cycle and a constant temperature of 24 ±1°C throughout the experiment. The experimental procedure was conducted according to the National Research Council (US) guidelines for the Care and Use of Laboratory Animals (2011)[19].

Experimental mice were provided with approximately 2 g of food per day, shaped into pellets and placed in hanging troughs attached to the cage walls. Additionally, clean drinking water supplemented with 0.15 ml of a vitamin mixture was provided in 500 ml bottles with spouts. After 24 h, the leftover was collected to determine the amount of food consumed during the day. Each week, as well as at intervals of 10 and 20 days, mice were weighed. Faeces and urine of mice were collected daily and analyzed for total nitrogen content. Furthermore, blood samples were taken to measure glucose, triglyceride, HDL-cholesterol, and LDL- cholesterol levels to assess the mice's health status at the time of blood sampling.

The data obtained from experimental mice were used to calculate following parameters:

**PER** (Protein Efficiency Ratio): the ratio of grams of protein digested per grams of body weight gained

$$\mathbf{PER} = \frac{W}{P}$$

W: Weight gain of experimental mice after 20 days of experiment (g)
P: Protein weight consumed during 20 days (g)

**NPR** (Net Protein Ratio): $NPR = \frac{T + G}{P}$

T: The average weight gain of the group using protein diet after 10 days (g); G: The average weight loss of the control group using a protein-free diet after 10 days (g); P: Protein weight consumed during 10 days (g)

**BV** (Biological Value): $BV = \frac{I - (F+U)}{I - F} \times 100$

I: The total amount of nitrogen consumed during 20 days (mg); F: Total amount of nitrogen excreted in the faeces during 20 days (mg); U: Total amount of nitrogen excreted in the urine during 20 days (mg)

### Analytical methods

Moisture, protein, fat and ash contents of APC and SPC were determined according to the methods of AOAC 950.46 2006[20], 992.15[21], 960.39[22] and 920.153[23] respectively. Carbohydrate content was estimated by subtracting the percentages of protein, lipid, ash, and moisture content. Total dietary fiber (TDF) was analyzed according to AOAC 991.43[24].

The total nitrogen content in feces and urine of mice was determined using the Kjeldahl method (AOAC 978.02)[25]. The mice's blood glucose, triglyceride, HDL-cholesterol, and LDL-cholesterol levels (biochemical analysis) were conducted using the Hitachi 7020 automated analyzer at Hospital 115 (Ho Chi Minh city, Vietnam).

Each analysis was done in triplicate, and data were reported as means ± standard deviation.

### Statistical Analysis

The data were analyzed by Analysis of Variance (ANOVA). Least Significant Differences (LSD) were calculated at $p < 0.05$ to compare treatment means using the Statgraphics Centurion XV (StatPoint Inc., USA) software for Windows 8.

## RESULTS

### Weight loss in mice fed a protein-free diet

The weight of the group of mice fed a protein-free diet (group 1) during the experiment is presented in Table 2. Results show that, after 10 days on the protein-free diet, the experimental mice exhibited a significant decrease in weight, averaging around 6 g per individual, representing over 30% of their initial weight. Additionally, the mice exhibited sluggish behavior, or lazy activity, and their fur appeared less glossy than initially.

**Table 2. Mice weights of group 1 after 10 days on a protein-free diet**

| Mice individual | Weight (g) | | |
|---|---|---|---|
| | Initial | After 10 days of feeding | Difference |
| 1 | 17.28 | 11.28 | 6.00 |
| 2 | 19.32 | 12.73 | 6.59 |
| 3 | 18.88 | 12.52 | 6.36 |
| 4 | 18.10 | 12.75 | 5.35 |
| 5 | 18.20 | 12.30 | 5.90 |
| Mean | 18.36 ± 0.78 | 12.32 ± 0.61 | 6.04 ± 0.47 |

**Weight gain in mice fed a protein-based diet**

The nutritional composition of the regular diet provided by the Pasteur Institute and recommended by AIN-93 is presented in Table 1. The weights of the mice groups when fed diets supplemented with APC, SPC (AIN-93 formula), and regular diet (Pasteur Institute formula) are shown in Table 3.

**Table 3. The weight of mice after 4 weeks on a protein-based diet**

| Time | Weight (g) | | |
|---|---|---|---|
| | Regular diet (Group 2) | AIN-93, APC (Group 3) | AIN-93, SPC (Group 4) |
| Initial | $18.8 \pm 0.62^{dA}$ | $18.9 \pm 0.41^{eA}$ | $18.8 \pm 0.66^{eA}$ |
| After 1 week | $20.0 \pm 0.67^{cB}$ | $21.7 \pm 0.94^{dA}$ | $21.2 \pm 0.76^{dA}$ |
| After 2 weeks | $22.2 \pm 0.60^{bC}$ | $23.8 \pm 0.40^{cB}$ | $24.7 \pm 0.30^{cA}$ |
| After 3 weeks | $26.1 \pm 0.53^{aB}$ | $28.0 \pm 0.66^{bA}$ | $28.0 \pm 0.58^{bA}$ |
| After 4 weeks | $26.0 \pm 0.84^{aB}$ | $29.8 \pm 0.58^{aA}$ | $30.1 \pm 1.17^{aA}$ |

*Values with the same letter in the same raw (uppercase) and column (lowercase) are not statistically significant (p > 0.05).*

Results in Table 3 show that, when fed with AIN-93 diet, mice body weight significantly increased by 2-3 g weekly (10-15% of initial weight). After 4 weeks, there was a noticeable difference in body weight gain among the three mice groups. The group fed an AIN-93 diet containing SPC and *Chaetomorpha* sp. APC exhibited an additional weight gain of 60-63%, which was significantly higher than that of the group, fed a regular diet (54% increase).

Table 4 presents the biochemical composition of APC and SPC, used in the AIN-93 formula diet for groups 3 and 4 of mice, respectively. It can be seen that, APC derived from *Chaetomorpha* sp. and SPC had similar chemical compositions, both containing over 70% protein and low levels of carbohydrates, fiber, and ash. When comparing two groups of mice fed the AIN-93 diet with two different protein sources, SPC and APC, no statistically significant difference (p > 0.05) was observed in the weight gain of mice fed the APC-based diet compared to those fed the SPC-based diet after 4 weeks.

**Table 4. Biochemical composition of the APC and SPC**

| Composition (g/100g) | APC | SPC |
|---|---|---|
| Moisture | $10.4 \pm 0.2^{a}$ | $10.2 \pm 0.09^{a}$ |
| Carbohydrate | $5.3 \pm 0.2^{b}$ | $7.4 \pm 0.18^{a}$ |
| Protein | $76.3 \pm 0.41^{a}$ | $73.2 \pm 0.98^{b}$ |
| Lipid | $4.8 \pm 0.13^{a}$ | $4.7 \pm 0.07^{a}$ |
| Ash | $3.2 \pm 0.08^{b}$ | $4.5 \pm 0.05^{a}$ |

*Values with the same letter in the same raw are not statistically significant (p>0.05).*

**Protein Efficiency Ratio (PER), Net Protein Ratio (NPR), and Biological Value (BV)**

The PER, NPR and BV were determined based on the method proposed by Sarwar and colleagues, following the AOAC standard, with the mice's growth period of 4 weeks[26]. PER, NPR, and BV indices obtained from different feeding diets are shown in Table 5.

Regarding the protein sources used in the AIN-93 diet, APC and SPC did not show any statistically significant differences in PER and NPR values (p > 0.05). However, the BV of APC from *Chaetomorpha* sp. was 79.32, significantly higher than the value of 70.25 ± 2.40 for SPC. When comparing the diets, mice fed the regular diet (group 2) had PER, NPR, and BV values of 0.85 ± 0.07, 1.35 ± 0.87, and 54.21 ± 5.38, respectively, which were significantly lower than those of mice fed the AIN-93 diet (groups 3 and 4).

**Table 5. PER, NPR and BV indices of different protein sources**

| Protein source | Group of mice | PER | NPR | BV |
|---|---|---|---|---|
| APC (AIN-93 diet) | Group 3 | $2.12 \pm 0.26^{a}$ | $3.28 \pm 0.41^{a}$ | $79.32 \pm 2.47^{a}$ |
| SPC (AIN-93 diet) | Group 4 | $2.28 \pm 0.12^{a}$ | $3.46 \pm 0.12^{a}$ | $70.25 \pm 2.40^{b}$ |
| Regular diet | Group 2 | $0.85 \pm 0.07^{b}$ | $1.35 \pm 0.87^{b}$ | $54.21 \pm 5.38^{c}$ |

*Values with the same letter in the same column are not statistically significant (α=0.05).*

**The mice's blood biochemical parameters**

The results of biochemical analysis of the mice's blood are presented in Figure 1. Results show that mice fed an AIN-93 diet containing SPC had the lowest blood glucose and triglyceride levels. Compared to the SPC-fed group, those fed an AIN-93 diet with APC from *Chaetomorpha* sp. exhibited slightly higher blood glucose and triglyceride levels, though the differences were not significant. Supplementing the diet with *Chaetomorpha* sp. APC reduced LDL-cholesterol and increased HDL-cholesterol levels compared to the SPC-based diet. When comparing the diets, the mice group fed regular diet had blood glucose level 3-4 times higher, and blood triglyceride level was also significantly higher than those of the AIN-93 APC and AIN-93 SPC-fed group.

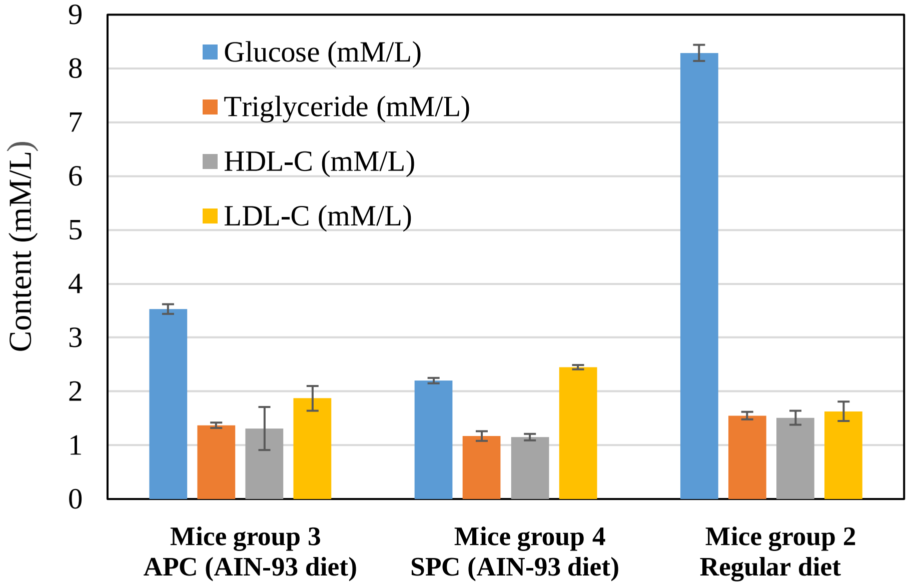


**Figure 1. The mice's blood glucose, triglyceride, HDL-cholesterol, and LDL-cholesterol levels**

## DISSCUSION

### Changes of body weight in mice fed protein-free and protein-based diets

Group 1 of mice, fed a protein-free diet, was used to estimate weight loss due to endogenous metabolic protein loss, a key factor in calculating the Net Protein Ratio (NPR). The significant weight loss observed in this group after 10 days confirmed the essential role of dietary protein in sustaining life and provided data for NPR calculation.

When comparing the two diets provided for mice in the *in vivo* experiment, as shown in Table 1, the diet supplied by the Pasteur Institute contained twice the amount of protein and fat as the recommended AIN-93 diet[11]. In contrast, the AIN-93 diet formulated with APC and SPC contained less protein and fat but more carbohydrates, minerals, and fiber. According to Wang et al. (2020), the composition of the diet affects outcomes related to protein utilization, weight gain, and blood parameters in mice[6]. A diet formulated according to the AIN-93, containing all essential nutrients, supported good weight gain in groups 3 and 4 of mice. In contrast, group 2, fed a regular diet, showed poorer weight gain, likely due to an imbalanced nutrient ratio - high in protein but deficient in energy sources from carbohydrates, as well as fiber and minerals.

The protein origin plays a role in body-building and efficiency of protein utilization[7]. According to the findings of Urbano et al. (2002) and Bleakley et al. (2017), the low fiber and carbohydrate content in protein preparations can positively affect the digestion and absorption of protein and vice versa[14,15]. SPC is a widely used, high-value plant protein source, while APC from *Chaetomorpha* sp. is rich in essential amino acids with a high essential-to-non-essential amino acid ratio[2]. The high protein content and low levels of carbohydrates and fiber in both SPC and APC probably contributed to efficient protein digestion and utilization for weight gain in mice. The similarly good weight gain observed in groups 3 and 4 indicates that APC from *Chaetomorpha* sp. is an effective plant protein source, comparable to SPC in promoting weight gain in *in vivo* experiments on mice.

**Protein Efficiency Ratio (PER), Net Protein Ratio (NPR)**

PER is a metric used to measure protein quality. It compares the body weight gain ratio of mice over at least 10 days when fed a protein-based diet to the amount of protein consumed[26]. Regarding the protein sources used in the AIN-93 diet, the similar composition of SPC and APC may explain their equivalent digestibility and absorption, leading to the same PER values. Plant proteins typically have lower PER values compared to animal proteins. A PER below 1.5 approximately describes a protein of low or poor quality, a value between 1.5 and 2.0 indicates intermediate quality, and above 2.0 indicates good to high quality[9] .

The results in Table 5 indicate that SPC had a PER of 2.28, similar to the PER values reported by Sarwar, 1997 (2.69)[26] and Abiose et al., 2015 (2.30)[8]. *Chaetomorpha* sp. APC had a PER of 2.12, which was lower than that of animal proteins reported by Sarwar et al. (1997), such as casein and egg (3.85), fish (3.5), and lactalbumin (4.29)[26]. Compared to the PER of some plant proteins, such as rice (2.2) and potato (2.6)[26], the PER of APC was similar. However, it was much higher than the value of 0.95 for wheat flour protein[9] and 0.87 for black bean protein[26]. When comparing the diets, the regular feed for mice contained 20.4% protein, 2.0 times more than the AIN-93 formula. Despite consuming nearly double the protein, mice on the regular diet showed lower weight gain. The imbalance in nutrition, with high fat and low carbohydrates, minerals, and fiber, is likely the reason for the very low PER of 0.85 for the protein in the regular diet.

The PER index, which relies solely on the additional body weight gain of experimental mice, doesn't provide a comprehensive reflection of protein retention in the body. To address this, the Net Protein Ratio (NPR) is used. NPR indicates that protein is not only necessary for body growth but also essential for sustaining life. NPR is calculated similarly to PER but also takes into account the weight loss observed when mice are fed a protein-free diet[7]. Consequently, NPR values are consistently higher than PER, with the extent of difference varying depending on the type of protein and the composition of the diets. According to Abdel-Aziz et al. (1997), the difference between these two indices was 28% for lean beef, while it was lower for wheat[27]. For regular diet, AIN-93 diets with SPC and APC from *Chaetomorpha* sp., the differences between PER and NPR were 37%, 34%, and 35%, respectively. The largest difference between these indices occurred with the regular diet. A low-carbohydrate diet in regular feed may not provide sufficient energy, leading to protein being utilized for maintaining life. This could explain the inefficient use of protein for the body's protein biosynthesis.

**Biological Value (BV)**

Biological Value (BV) measures protein efficiency in growth and maintenance by assessing the percentage of absorbed nitrogen retained in the body. It depends on the adequacy of essential amino acids in food protein compared to body protein. When there is a significant difference in amino acid compositions, protein cannot efficiently convert to tissue protein. As a result, the nitrogen from amino acids is excreted as urea in urine, leading to a lower BV[7] .

In term of protein sources used in the AIN-93 diet, the BV of APC from *Chaetomorpha* sp. was 79.32, higher than that of SPC. This means that approximately 79% of the introduced protein was utilized for tissue protein or life-sustaining functions, aiding in body development, while the remaining 21% was not absorbed and was excreted from the body. APC from *Chaetomorpha* sp. had a higher protein content (76.3%) compared to SPC (73.2%), and lower content of carbohydrates and ash (Table 4). The non-protein components in SPC might negatively impact protein digestion, lowering BV. Additionally, protein of *Chaetomorpha* sp. APC contained up to 42% essential amino acids, ensuring a balanced amino acid profile compared to other plant proteins[2]. This contributed to improved protein utilization efficiency and increased BV. The BV value is often high in animal proteins, as indicated by numerous studies. Lima E Silva et al. (2014) reported a BV of 87.82 for casein[7]. BV values vary widely among plant proteins; rice protein's BV ranged from 65 to 75, while carrot protein ranged from 77 to 82. Grain protein from new varieties of amaranths had a BV ranging from 44.53 to 62.8[28] and the BV of roots and leaves of Anchote (*Coccnia abyssinica*) were 26.76 and 47.09, respectively[29]. Thus, APC from *Chaetomorpha* sp. had a BV lower than casein but similar to carrot protein, and notably higher than that of rice and amaranth seed protein.

Compared to the AIN-93 diet, the regular diet had a significantly lower BV. Using protein from rice bran, the main ingredient in producing the regular diet for mice, may lead to a deficiency in certain essential amino acids[30], resulting in poor protein utilization efficiency.

**The mice's blood biochemical parameters**

The mice's blood glucose, triglyceride, HDL-cholesterol, and LDL-cholesterol levels can partially reflect the health status of the experimental mice at the time of blood sampling. Soybean is a nutritious protein source with health benefits[30], as indicated by low blood glucose and triglyceride levels observed in mice when SPC was included in the AIN-93 diet. These levels also remained low when mice were fed the AIN-93 diet containing APC. Additionally, lower LDL-cholesterol and higher HDL-cholesterol levels in APC-fed group 3 compared to other groups of mice suggest that the AIN-93 diet with SPC from *Chaetomorpha* sp. not only supported weight gain and protein synthesis but also improved cholesterol balance. Other studies also found that algae protein-based diets can regulate fat metabolism and reduce LDL cholesterol[4].

Regarding the diets, the group of mice fed a regular diet had significantly higher blood glucose and triglyceride levels than those fed an AIN-93 diet. This indicates that despite using a diet rich in protein and low in carbohydrates, high fat content and imbalance in nutrient ratios, lack of fiber, and minerals can lead to health issues[15]. Thus, diets containing SPC and APC from *Chaetomorpha* sp. may help maintain health and prevent the risk of common chronic diseases like cardiovascular diseases, diabetes, and obesity.

## CONCLUSION

The APC from *Chaetomorpha* sp., with a protein content of 76.3%, when used in the AIN-93 diet, demonstrated good *in vivo* digestibility with corresponding PER, NPR, and BV values of 2.12, 3.28, and 79.32, respectively. These values were comparable to those of soy protein concentrate. The AIN-93 diet using APC and SPC also resulted in significantly higher PER, NPR, and BV values compared to the regular diet using rice bran's protein. With such nutritional value, APC shows significant potential for applications in human diets. Future research should focus on investigating the functional properties and sensory characteristics of APC from *Chaetomorpha* sp., as well as its potential utilization in specific food products.

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## REFERENCES

1. Naselli-Flores L, Barone R. Green Algae, Encyclopedia of Inland Waters. Elsevier Inc.; 2009.166-173. DOI: 10.1016/B978-012370626-3.00134-4
2. Hoang KA, Le THA, Bach NM, Nguyen MH. Chemical composition of *Chaetomorpha* sp. brackish algae in the Mekong Delta. Journal of Science and Technology. 2014; 52(5A):247-254.
3. Xu J, Liao W, Liu Y, Guo Y, Jiang S. and Zhao C. An overview on the nutritional and bioactive components of green seaweeds. Food Production, Processing and Nutrition. 2023; 5(1):18. DOI:10.1186/s43014-023-00132-5
4. Wu JY, Tso R, Teo HS, and Haldar S. The utility of algae as sources of high value nutritional ingredients, particularly for alternative/complementary proteins to improve human health. Frontier in Nutrition. 2023; 10.3389. DOI 10.3389/fnut.2023.1277343
5. Karabulut G, Purkiewicz A, Goksen G. Recent developments and challenges in algal protein and peptide extraction strategies, functional and technological properties, bioaccessibility, and commercial applications, Comprehensive Review in Food Science and Food Safety. 2024; 23(3):e13372. DOI: 10.1111/1541-4337.13372
6. Wang Y, Tibbetts SM, Berrue F, McGinn PJ, MacQuarrie SP, Puttaswamy A, Patelakis S, Schmidt D, Melanson R. and MacKenzie SE. Rat Study to Evaluate the Protein Quality of Three Green Microalgal Species and the Impact of Mechanical Cell Wall Disruption. Foods. 2020; 9:1531. DOI:10.3390/foods9111531
7. Lima E Silva MCB, Hiane PA, Braga Neto JA, Macedo MLA. Proteins of Bacuri almonds - Nutrional value and in vivo digestibility. Food Science and Technology. 2014; 34:55-61. DOI: 10.1590/S0101-20612014000100008
8. Abiose SH, Ikujenlola AV, Abioderin FI. Nutritional quality assessment of complementary foods produced from fermented and malted quality protein maize fortified with soybean flour. Polish Journal of Food and Nutrition Sciences. 2015; 65(1):49-56. DOI: 10.1515/pjfns-2015-0004
9. Asemi Z, Taghizade M, Sarahroodi S, Tehran HA. Biological evaluation of protein quality of

two homemade cereal/legume mixtures and a commercial weaning food. Electronic Physician. 2010; 2:85-94.
10. Tavano OL, Neves VA, and da Silva Júnior SI. In vitro versus in vivo protein digestibility techniques for calculating PDCAAS (protein digestibility-corrected amino acid score) applied to chickpea fractions. Food Research International. 2016; 89(1):756-763. DOI:10.1016/j.foodres.2016.10.005
11. Reeves PG, Rossow KL, and Lindlauf J. Development and testing of the AIN-93 purified diets for rodents: results on growth, kidney calcification and bone mineralization in rats and mice. The Journal of nutrition. 1993; 123(11):1923-1931. DOI: 10.1093/jn/123.11.1923.
12. Kinyi HW, Kato CD, Tusubira D, Kiwanuka GN. Comparison of the Nutritional Status of Swiss Albino Mice Fed on Either a Purified or Cereal-Based Diet for 15 weeks, Biochemistry Research International. 2023; 2023:e9121174. DOI:10.1155/2023/9121174
13. Martínez R, García-Beltrán A, Kapravelou G, Mesas C, Cabeza L, Perazzoli G, Guarnizo P, Rodríguez-López A, Vallejo RA, Galisteo M, Aranda P, Prados J, López-Jurado M, Melguizo C and Porres JM. In Vivo Nutritional Assessment of the Microalga Nannochloropsis gaditana and Evaluation of the Antioxidant and Antiproliferative Capacity of Its Functional Extracts. Marine Drug. 2022; 20:318. DOI:10.3390/md20050318
14. Urbano MG, Goñi I. Bioavailability of nutrients in rats fed on edible seaweeds, nori (Porphyra tenera) and wakame (Undaria pinnatifida), as a source of dietary fibre. Food Chem. 2002; 76:281–286. DOI:10.1016/S0308-8146(01)00273-4
15. Bleakley S. and Hayes M. Algal Proteins: Extraction, Application, and Challenges Concerning Production. Foods. 2017; 6(5):33. DOI:10.3390/foods6050033
16. Bach NM, Huynh HM, Hoang KA, Ngo KS. Optimization of protein extraction from green alage Chaetomorpha sp. biomass using surface response methodology. Science & Technology Development Journal: Natural Sciences. 2019; 3(3):136-143. DOI:10.32508/stdjns.v3i2.864
17. Geada P, Moreira C, Silva M, Nunes R, Madureira L, Rocha MRC, Pereira NR, Vicente AA, Teixeira JA. Algal proteins: Production strategies and nutritional and functional properties. Bioresource Technology. 2021; 332:125125. DOI:10.1016/j.biortech.2021.125125
18. Singha HA, Sengupta M, Bawari M. Neurobehavioral responses in Swiss albino mice induced by an aqueous leaf extract from a medicinal plant named Heliotropium incanum Ruiz & Pav. Bioinformation. 2020;16(9): 679-687. DOI: 10.6026/97320630016679
19. National Research Council. Guide for the Care and Use of Laboratory Animals, 8th Edition, National Academies Press (US). 2011. DOI: 10.17226/12910
20. AOAC Official Method 950.46 2006: Moisture in Meat
21. AOAC Official Method 992.15: Crude Protein in Meat and Meat Products
22. AOAC Official 960.39: Fat (Crude) or Ether Extract in Meat
23. AOAC Official Method 920.153: Ash of Meat
24. AOAC Official Method 991.43: Total, Soluble, and Insoluble Dietary Fiber in Foods: Enzymatic-Gravimetric Method, MES-TRIS Buffer
25. AOAC Official Methods 978.02: Determination of Kjeldahl nitrogen in fertilizers
26. Sarwar G. The protein digestibility - corrected amino acid score method overestimates quality of proteins containing antinutritional factors and of poorly digestible proteins supplemented with limiting amino acids in rats. The Journal of nutrition. 1997; 127(5):758-764. DOI:10.1093/jn/127.5.758
27. Abdel-Aziz S, Hussein L, Esmail S, el-Awadi N. In vivo rat assay for true protein digestibility and protein quality of beef and meat products extended with soy protein. International journal of food sciences and nutrition. 1997; 48(1):51-56. DOI:10.3109/09637489709006963.
28. Agular EG, Albarracin GDJ, Unates MA, Piola HD, Camina JM, Escudero NL. Evaluation of the nutritional quality of the grain protein of new amaranths varieties. Plant Foods for Human Nutrition. 2015; 70(1):21-26. DOI: 10.1007/s11130-014-0456-3.
29. Ayalew Y, Retta N, Desse G, Mohammed A, Mellesse A. Amino acid profile and protein quality in tuber and leaf of Cocnia abyssinia (Lam.) (Cogn.) accessions of Ethopia. Food Science and Nutrition. 2016; 5(3):722-729. DOI: 10.1002/fsn3.452.
30. Zeng Y, Chen E, Zhang X, Li D, Wang Q and Sun Y. Nutritional Value and Physicochemical Characteristics of Alternative Protein for Meat and Dairy - A Review. Foods. 2022: 11(21):3326. DOI: 10.3390/foods11213326.