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Physiological Effects of Sargassum Modified Cassava Composite Diet in Malnourished Mice

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ABSTRACT

Background: Malnutrition remains a significant public health concern, impairing both physical growth and physiological functions. Functional foods derived from local resources such as *Sargassum sp.* and modified cassava flour (MOCAF) may offer a potential strategy to improve nutritional status. This study aimed to examine the effect of a combination of *Sargassum sp.* from Jembrana coastal waters and MOCAF from Manihot esculenta Crantz on malnutrition in mice based on physical assessment.

Methods: A pre-experimental one group pre- and post-test design was conducted using 39 male Mus musculus (6-8 weeks old, 15–25 g). Mice were induced for malnutrition and then received orally a combination of *Sargassum sp.* and MOCAF for 14 days at ratios of 90:10 (P1), 85:15 (P2), and 80:20 (P3) at 3-5 g/day. Physical parameters were measured before and after interventions.

Results: Administration of *Sargassum sp.*–MOCAF combination could significantly improve body weight and APGAR (activity, pulse, grimace, appearance and respiration assessments) score in all groups. Body weight increased significantly in P1 (p=0.001), P2 (p=0.001), and P3 (p=0.001), with P2 showing the largest effect. APGAR score improved significantly in P1, P2, and P3 (all p<0.001) too, with P2 showing the highest improvement.

Conclusion: The *Sargassum sp.* and MOCAF combination effectively enhanced the physical condition of malnourished mice, with the 85:15 ratio (P2) to be the optimal. This combination showed promise as a functional food intervention against malnutrition, warranting further molecular and clinical studies.

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Introduction

Malnutrition remains a significant health problem in many developing countries, including Indonesia. According to data from the 2023 Indonesian Health Survey conducted by the Ministry of Health of the Republic of Indonesia, the prevalence of stunting among children under five years in 2023 reached 21.5% (1). Malnutrition affects not only physical growth but also impacts cognitive development and the immune system, leading to long-term consequences for the quality of human resources (2-4). The causes of malnutrition are highly complex; however, one of the main factors is an imbalanced nutritional intake (5-7). Therefore, fulfilling macronutrient and micronutrient requirements through the provision of nutrient-dense food interventions represents a strategic approach.

Jembrana Regency, located in Bali Province, is an area with an extensive coastline where fishing is the primary livelihood of the local community. The region is endowed with various natural resources, including *Sargassum sp.* (a type of brown seaweed), which has the potential to be developed as a functional food ingredient. *Sargassum sp.* is known to contain bioactive compounds such as sulfated polysaccharides, fucoidan, vitamins, and minerals that play important roles in improving nutritional status and immune function (8). In addition, the utilization of other local food sources with good nutritional value, such as Modified Cassava Flour (MOCAF), offers a relevant option in functional food formulation (9).

MOCAF is flour made from cassava (*Manihot esculenta* Crantz) that has been modified through fermentation using lactic acid bacteria. This process alters the characteristics of the flour, making it whiter in color, with a neutral taste and aroma, while enhancing its functional properties compared to conventional cassava flour (10). MOCAF is advantageous due to its wide availability, resistant starch content, and ability to improve gut microbiota. However, although MOCAF provides sufficient nutritional content, its protein level is relatively low, necessitating the addition of other nutrient-rich ingredients (11). Combining *Sargassum sp.* from the Jembrana coastal waters, which is rich in protein, with MOCAF, which provides complementary nutritional components, holds great potential in addressing malnutrition. Nevertheless, studies on the *in vivo* effectiveness of this combination, particularly from the perspective of physical parameters in animal models, remain limited.

Several previous studies have explored the potential of *Sargassum sp.* as a nutritional supplement. It has been reported as one of the most promising seaweed species for development as a food source due to its relatively high protein content compared to other types of seaweed (12). The nutritional composition of *Sargassum sp.* includes carbohydrates (67.01%), moisture (6.66%), ash (17.75%), protein (8.19%), fat (0.39%), and essential minerals such as Fe (19.48 mg/L), Mg (16.58 mg/L), Ca (10.15 mg/L), and K (28.50 mg/L). In addition, it contains bioactive compounds including alkaloids, saponins, and steroids (13). Another study investigated the effect of *Sargassum sp.* supplementation on goat body weight gain. The experimental design included concentrate feed with different treatments as control (without *Sargassum sp.*), 10% *Sargassum sp.*, and 15% *Sargassum sp.* supplementation. The results showed that the average daily weight gain for the control group (P0) was 0.014 kg/head/day, for the 10% supplementation group (P1) was 0.052 kg/head/day, and for the 15% supplementation group (P2) was 0.066 kg/head/day (14).

Meanwhile, MOCAF has been recognized as a low-gluten and high-fiber food alternative. Its composition typically includes 9.25% moisture, 1.93% protein, 0.30% ash, 85.60% starch, 2.72% fat, and 0.21% fiber (15). In terms of micronutrients, 100 g of MOCAF provided approximately 1.2 g of protein and 15.8 mg of iron, highlighting its suitability for food diversification (16). Moreover, it has a relatively low glycemic index of 46 and a fiber content ranging between 1.9% and 3.4%, supporting its application as a functional food ingredient (17).

Previous studies have not specifically evaluated the combination of *Sargassum sp.* from the coastal waters of Jembrana with MOCAF in the context of malnutrition intervention, particularly based on physical assessment. Therefore, this study provided novelty in terms of the use of local ingredient combinations, the seaweed's geographic origin, the *in vivo* approach, and the physical indicators employed. Based on the aforementioned background, this study aimed to explore several key research questions. The primary question concerns whether the combination of *Sargassum sp.* from the coastal waters of Jembrana and MOCAF can improve physical growth parameters in animal models. Furthermore, the study sought to determine how different ratios of *Sargassum sp.* and MOCAF influenced body weight gain and other physical indicators.

Materials and Methods

The design of this study employed a pre-experimental method with a one-group pre- and post-test approach. This design was chosen to assess the effects of administering a combination of *Sargassum sp.* and MOCAF on the physical condition of malnourished mice before and after the interventions. The population in this study consisted of male mice (*Mus musculus*) induced to experience malnutrition. The sampling technique employed purposive sampling with the criteria of male mice aged 6-8 weeks, weighing 15-25 grams, and in a malnutrition-induced condition. A total of 39 mice were included as samples, determined based on considerations of the preliminary trial design and ethical guidelines for the use of experimental animals. This study was registered internally under the Laboratory Registration Number of BC039/7.8.2/LHU/IX/2025.

Primary data were collected from physical observations of the mice, including body weight measurements and APGAR assessments (activity, pulse, grimace, appearance, and respiration) before and after the treatments. The study began with the processing of raw materials. *Sargassum sp.* powder was obtained from fresh seaweed, which was washed thoroughly to remove sand and debris, then dried in an oven at 50°C for 24 hours. Once dried, the seaweed was ground and sieved using an 80-mesh sieve to produce fine *Sargassum sp.* powder. Meanwhile, MOCAF was prepared from cassava fermented with lactic acid bacteria, then dried and ground according to MOCAF quality standards.

Male mice were obtained and acclimated for 7 days to adjust to the laboratory environment. Malnutrition was induced in the mice through restriction of standard feed prior to the intervention. The experimental animals were divided into three treatment groups based on the MOCAF to *Sargassum sp.* ratio: P1 with a ratio of 90:10, P2 with 85:15, and P3 with 80:20. The combination of *Sargassum sp.* from the coastal waters of Jembrana and MOCAF was administered orally for 14 days, with a daily feed amount of 3-5 grams per mouse. Data were collected through body weight measurements and APGAR score assessment, which included activity, pulse, grimace, appearance, and respiration, conducted before and after the interventions to evaluate the effects of the combined food ingredients on the physical condition of the experimental animals.

This study was conducted in accordance with ethical guidelines for research involving experimental animals. The research was carried out following the principles of the Declaration of Helsinki and reported according to the ARRIVE (Animal Research: Reporting of *in vivo* experiments) guidelines, ensuring transparency, reproducibility, and humane treatment of all animals involved. All procedures were designed with a focus on animal welfare and implemented following the 3R principles of Replacement, Reduction, and Refinement. Replacement refers to efforts to substitute the use of animals with alternative methods, such as cell cultures or computer models, whenever possible. Reduction involves using the minimum number of animals necessary without compromising the validity of the research results, while Refinement encompasses the improvement of methods to minimize pain, stress, and discomfort in animals, including the use of anesthesia and post-procedure care.

In addition to the 3R principles, the study also considered other ethical aspects, such as scientific justification for animal use, establishment of humane endpoints to prevent unnecessary suffering, and involvement of competent and trained personnel in the handling of laboratory animals. Strict monitoring was conducted throughout the study to detect signs of discomfort in the animals, and all procedures were transparently documented as a form of scientific accountability. In compliance with regulations, this study received ethical approval from the Health Research Ethics Committee of Triatma Mulya University (No. 023/EA/UNTRIM-KEPK/VII/2025), ensuring that all stages of the research met ethical standards and fully considered animal welfare.

This study involved two main types of variables. The dependent variable was the physical condition of the mice, evaluated through two parameters of body weight (BW) measurements and APGAR score assessment, which includes activity, pulse, grimace, appearance, and respiration. The APGAR score in this study encompassed these five key aspects, providing a comprehensive overview of the physiological condition of the mice. The independent variable was the administration of a combination of *Sargassum sp.* from the coastal waters of Jembrana and Modified Cassava Flour (MOCAF). The combination was administered orally for 14 days, with dosage variations based on the ratio of MOCAF to *Sargassum sp.*: 90:10 (P1), 85:15 (P2), and 80:20 (P3), with a daily feed amount of 3–5 grams per mouse.

All statistical analyses in this study were conducted using SPSS software (version 27, Chicago, IL, USA). Data were analyzed using the paired t-test for normally distributed data and the Wilcoxon signed-rank test for non-normally distributed data. The test was used to examine significant differences in the physical condition of the mice before and after the interventions. A p value of less than 0.05 ($p<0.05$) was considered statistically significant.

Results

The Shapiro-Wilk normality test showed that for body weight measurements, the significance values were $p=0.007$ for P1, $p=0.001$ for P2, and $p=0.406$ for P3. These results indicated that the data for groups P1 and P2 were not normally distributed, whereas group P3 was normally distributed. Because not all groups followed a normal distribution, subsequent analysis for the BW variable was conducted using the Wilcoxon Signed-Rank Test (Table 1). For APGAR score, the significance values were $p=0.386$ for P1, $p=0.070$ for P2, and $p=0.230$ for P3, indicating normal distribution in all three groups. Therefore, subsequent analysis of APGAR score was conducted using the Paired t-test (Table 1).

The Wilcoxon Signed-Rank Test was used to assess changes in mice body weight before and after the intervention across all groups (Table 1). The results showed a significant increase in BW in all groups.

Table 1: Summary of normality test, body weight (BW), and APGAR score in mice (N=39).

Group	BW Shapiro-Wilk (p)	BW Distribution	APGAR Shapiro-Wilk (p)	APGAR Distribution	BW (Wilcoxon Signed-Rank Test) Z	BW *p* value	APGAR (Paired t-test) t	APGAR *p* value
P1 (90:10)	0.007	Non-normal	0.386	Normal	-3.220	0.001	-12.707	0.000
P2 (85:15)	0.001	Non-normal	0.070	Normal	-3.304	0.001	-24.009	0.000
P3 (80:20)	0.406	Normal	0.230	Normal	-3.189	0.001	-12.239	0.000

BW=Body Weight; APGAR=Activity, Pulse, Grimace, Appearance, and Respiration assessments

The Wilcoxon signed-rank test showed a significant increase in body weight BW in all groups, including P1 (Z=−3.220, p=0.001), P2 (Z=−3.304, p=0.001), and P3 (Z=−3.189, p=0.001). Among the groups, P2 exhibited the largest absolute Z value, indicating the strongest statistical evidence for an increase in BW.

The paired t-test demonstrated a significant increase in APGAR scores from before to after the intervention in all groups (Table 1). Significant improvements were observed in P1 (t=−12.707, p<0.001), P2 (t=−24.009, p<0.001), and P3 (t=−12.239, p<0.001). Among the groups, P2 exhibited the largest absolute t value, indicating the strongest statistical evidence for an improvement in APGAR scores.

Discussion

The role of food ingredients malnutrition and nutritional diseases has been emphasized before (18, 19). In this relation, administration of supplements and herbals has demonstrated prominent influences (20, 21). This study demonstrated that administration of a combination of MOCAF and *Sargassum sp.* for 14 days had a positive effect on increasing BW and APGAR score in mice. Analysis using the Wilcoxon Signed-Rank test for BW showed significant differences before and after interventions in all treatment groups, with the most pronounced effect observed in group P2 (85:15 ratio). Similarly, the Paired t-test for APGAR score indicated a significant increase in all groups, with the highest improvement seen in group P2 too. These results suggest that a ratio of 85% MOCAF to 15% *Sargassum sp.* represented an optimal composition for providing physiological benefits in mice.

These findings are consistent with evidence from the literature regarding the bioactive potential of *Sargassum sp.* Some studies have illustrated that *Sargassum sp.* contains pigments such as chlorophyll, carotenoids, and phycobiliproteins, which exhibit antioxidant, anti-inflammatory, antibacterial, and anticancer activities (22, 23). Such antioxidant activity may help reduce oxidative stress and support physiological functions, which could contribute to the increased vitality observed in mice. In addition to pigments, polysaccharides and oligosaccharides from *Sargassum sp.* have demonstrated significant antioxidant activity. Low-molecular-weight oligosaccharide fractions have been reported to reduce reactive oxygen species (ROS) production and enhance the activity of antioxidant enzymes (24, 25). This mechanism is in line with the findings of the present study, where the intervention improved the physiological status of mice.

From a nutritional perspective, *Sargassum sp.* has also been identified as an alternative protein source containing essential amino acids, phenolics, and phlorotannins, which play a role in energy metabolism and nutritional improvement (26, 27). Several studies have even shown that supplementation with *Sargassum sp.* can improve metabolic conditions, including obesity (28-31). This supports the notion that the bioactive compounds in *Sargassum sp.* can provide tangible physiological benefits. Other findings have shown that *Sargassum sp.* extract can improve the hematological profile of Tilapia fish (*Oreochromis niloticus*), highlighting its role in enhancing immunity and overall health in test organisms (32). In poultry, dietary supplementation with *Sargassum sp.* powder has been shown to enhance immune responses, suppress *Salmonella* growth, and improve gut microflora (33, 34). These results reinforce the view that *Sargassum sp.* can serve as a functional food ingredient that supports health through multiple mechanisms.

Regarding MOCAF, several studies have demonstrated its role as a gluten-free carbohydrate source that can be combined with other ingredients to enhance nutritional value (35, 36). A study on gluten-free pancakes made from MOCAF blended with local tuber flours showed that an optimal ratio of 70% MOCAF and 30% additional flour resulted in favorable texture, color brightness, and panelist preference (37). This indicates that MOCAF can serve as a flexible matrix for incorporating bioactive ingredients such as *Sargassum sp.* Moreover, studies on MOCAF-based analog rice have emphasized that adding micronutrient-rich ingredients, such as snakehead fish or cassava leaves, increases protein, calcium, polyphenol content, and antioxidant activity (38, 39). This mechanism is comparable to the MOCAF–*Sargassum sp.* formulation, which not only provides energy from carbohydrates but also contributes beneficial bioactive compounds that support metabolism and physiological status.

Furthermore, combining MOCAF with functional food sources has been shown to increase antioxidant activity and β-carotene content in processed products, such as MOCAF–pumpkin cookies (40). This finding is relevant as it demonstrates that blending MOCAF with phytonutrient-rich ingredients, including *Sargassum sp.* can yield food formulations that are not only nutritionally valuable but also play a role in oxidative protection. The combination of MOCAF and *Sargassum sp.* in this study likely produced synergistic effects. MOCAF provides energy through its high starch content, while *Sargassum sp.* contributes protein, minerals, and bioactive compounds that enhance metabolism and physiological function. The 85:15 ratio in group P2 appears to represent an optimal balance, where sufficient energy from MOCAF is complemented by the nutritional and bioactive contributions of *Sargassum sp.*, resulting in significant improvements in body weight and physiological condition, as reflected in APGAR scores.

These findings not only reinforce previous research but also offer new opportunities for the development of functional food products based on a combination of MOCAF and *Sargassum sp.* Considering the wide availability of *Sargassum sp.* in Indonesian waters and MOCAF as a local cassava-based product, utilizing these ingredients could support food security while adding value to local functional food products. This study provided important preliminary evidence on the beneficial effects of combining MOCAF and *Sargassum sp.* on physiological parameters in experimental animals. Nevertheless, some limitations should be acknowledged to place the findings in a broader scientific context. The current research did not include mechanistic analyses at the molecular or cellular level. Parameters such as gene expression related to energy metabolism, oxidative stress regulation, or antioxidant defense pathways were not investigated, which restricts the understanding of how the observed physiological improvements were mediated.

The duration of the experiment was relatively short, focusing only on a 14-day intervention period. This timeframe, while sufficient to demonstrate initial physiological responses, may not fully capture potential long-term effects of supplementation, such as chronic toxicity, cumulative metabolic changes, or sustained improvements in physiological status. Similarly, the use of a single animal model also limits the generalizability of the results to other species, including humans. The study design did not explore different levels of supplementation to assess dose–response relationships. Information on whether higher or lower concentrations of MOCAF and *Sargassum sp.* could lead to more pronounced or adverse effects remains unknown. Furthermore, no hematological, biochemical, or histopathological parameters were assessed, which could have provided a more comprehensive picture of the systemic and organ-specific impacts of the intervention.

Future research is therefore recommended to address these gaps through longer-term studies involving detailed biochemical, hematological, and molecular analyses. Such investigations should include the evaluation of metabolic biomarkers, antioxidant enzyme activities, and the profiling of gene expression related to stress and energy regulation. Additional work is also needed to establish the optimal formulation and dosage, ensuring both efficacy and safety. Ultimately, to support translational application, clinical trials in humans are essential. These should focus on safety, tolerability, and acceptability as well as measurable health benefits when MOCAF and *Sargassum sp.* are used as dietary supplements or functional food ingredients. By expanding the scope of research in these directions, the scientific foundation for developing MOCAF and *Sargassum sp.* combinations as innovative nutritional products will be considerably strengthened.

Conclusion

The administration of a combination of MOCAF and *Sargassum sp.* for 14 days significantly improved body weight and APGAR score in malnourished mice. The 85:15 ratio of MOCAF to *Sargassum sp.* was identified as the most effective composition, suggesting an optimal balance between energy provision and nutritional/bioactive support. These results highlight the potential of combining locally available ingredients, such as MOCAF and *Sargassum sp.*, for the development of functional foods aimed at addressing malnutrition. Further studies, including mechanistic analyses, long-term safety assessments, and human trials, are warranted to confirm these findings and support their application in functional food development.

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Authors' Contribution

IKAP: Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND: Final approval of the version to be published; AND: Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved, ELS: Drafting the work or reviewing it critically for important intellectual content; AND: Final approval of the version to be published. All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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