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Evaluation of Soybean Meal Substitution with *Padina australis* on Growth Performance and Feed Utilization Efficiency of Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

The increasing cost and sustainability concerns associated with conventional plant-protein ingredients have intensified the search for locally available functional feed materials for Nile tilapia. This study evaluated the effects of partially replacing soybean meal with the brown macroalga *Padina australis* meal on growth performance, feed utilization, and survival of Nile tilapia (*Oreochromis niloticus*). A 42-day feeding trial was conducted using a completely randomized design with four dietary treatments and three replicates. The treatments were TP-0 (30 g soybean meal; 100% soybean meal), TP-7.5 (7.5 g *P. australis* meal + 22.5 g soybean meal), TP-15 (15 g *P. australis* meal + 15 g soybean meal), and TP-22.5 (22.5 g *P. australis* meal + 7.5 g soybean meal). Fish with an initial body weight of 10.53 ± 0.34 g were stocked at six fish per aquarium and fed at 5% biomass day^{-1}. The measured variables included survival rate, weight gain, specific growth rate, feed intake, feed conversion ratio, and feed efficiency. Dietary treatment did not significantly affect any measured parameter ($P > 0.05$). Nevertheless, TP-7.5 produced the most favorable biological response, as indicated by the highest weight gain (6.21 ± 1.09 g), the highest specific growth rate (2.28 ± 0.21% day^{-1}), the lowest feed conversion ratio (3.75 ± 0.37), and the highest feed efficiency (26.81 ± 2.48%). These findings indicate that *P. australis* meal can be considered a low-inclusion functional ingredient in Nile tilapia diets, although higher inclusion levels require further nutritional optimization through balanced protein and energy formulation.

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is one of the most widely cultured freshwater aquaculture species because of its relatively rapid growth, broad environmental tolerance, and capacity to utilize plant-based diets more efficiently than many carnivorous fish species. However, the sustainability of tilapia production remains constrained by high feed costs, particularly due to reliance on conventional protein ingredients such as fish meal and soybean meal. Soybean meal is widely used as a major plant-protein source in tilapia feed formulation; nevertheless, its use is limited by price fluctuation, competition with human food demand, antinutritional factors, and potential amino acid imbalances when included at high levels (Magbanua & Ragaza, 2022; Viagem *et al*., 2024). Therefore, the identification of locally available alternative feed ingredients that provide both nutritional value and functional health benefits has become an important priority for the development of efficient and sustainable aquaculture (Wan *et al*., 2019; Øverland *et al*., 2019; Siddik *et al*., 2023).

Marine macroalgae are increasingly being investigated as aquafeed ingredients and functional additives because they contain complex carbohydrates, minerals, vitamins, pigments, fatty acids, polyphenols, and bioactive polysaccharides. Unlike terrestrial plant ingredients, macroalgae do not require agricultural land or freshwater for production, making them compatible with the principles of the blue economy and sustainable aquaculture (Wan *et al*., 2019; Ang *et al*., 2021). In fish diets, macroalgae can function as supplementary nutrient sources, natural prebiotics, antioxidants, immunostimulants, and modulators of gut health (Shannon & Abu-Ghannam, 2019; Siddik *et al*., 2023). Studies in tilapia have shown that macroalgal supplementation or macroalgal extracts may improve feed utilization, antioxidant responses, gut microbiota, haematological profiles, and resistance to stress or pathogens, although these responses are strongly influenced by algal species, ingredient form, processing method, and dietary inclusion level (Valente *et al*., 2016; Younis *et al*., 2018; Rohani *et al*., 2024; Carvalho *et al*., 2025).

One brown macroalga with potential for development as a local feed ingredient is *Padina australis*. This species has been reported to contain carbohydrates, protein, lipids, ash, amino acids, and essential minerals such as calcium, potassium, and iron (Salosso *et al*., 2020). In addition, *P. australis* contains bioactive compounds, including phenols, flavonoids, tannins, steroids, alkaloids, saponins, terpenoids, phlorotannins, and carotenoid pigments, which may exert antioxidant and antibacterial activities (Salosso *et al*., 2020; Arguelles & Sapin, 2022). In common carp, *P. australis* extract has been shown to improve growth performance, digestive enzyme activities, innate immune responses, intestinal gene expression, and resistance against *Aeromonas hydrophila* (Sheikhzadeh *et al*., 2022). Similar findings have been reported for other Padina species used in Nile tilapia diets, including P. pavonica and P. boergesenii, which have demonstrated potential to enhance growth, health status, immune responses, and resistance to bacterial infection (Maghawri *et al*., 2023; Cholaraj & Venkatachalam, 2023; Bakry *et al*., 2024).

Nevertheless, most studies on Padina in aquaculture have focused on extracts, polysaccharide fractions, or low-dose supplementation, whereas the use of whole *P. australis* meal as a partial substitute for soybean meal in Nile tilapia diets remains limited. This gap is important because the biological effects of extracts are not necessarily equivalent to those of whole macroalgal meal. Whole macroalgal meal contains both nutrient and non-nutrient fractions, including fibre, ash, structural polysaccharides, and phenolic compounds that may be beneficial at low levels but may reduce digestibility, energy density, palatability, or dietary nutrient balance at higher levels (Øverland *et al*., 2019; Wan *et al*., 2019; Ang *et al*., 2021). Thus, the ability of tilapia to utilize plant-based ingredients does not necessarily imply that increasing levels of macroalgal meal will consistently improve growth performance.

Based on these considerations, this study aimed to evaluate the effects of partially substituting soybean meal with *P. australis* meal in the diet on Nile tilapia performance, including survival rate, weight gain, specific growth rate, feed intake, feed conversion ratio, and feed efficiency. The study was expected to provide a scientific basis for defining the practical inclusion limit of *P. australis* meal in tilapia diets and to clarify whether this brown macroalga is more promising as a partial substitute ingredient or as a functional component in low- to moderate-inclusion aquafeed formulations.

METHODS

Experimental Period and Location

The study was conducted from January to February 2022. Fish rearing was carried out at the Laboratory of Aquaculture, Hatchery, and Fisheries Production, whereas proximate analysis of the experimental diets was performed at the Laboratory of Productivity and Aquatic Environment, Faculty of Fisheries and Marine Science, Halu Oleo University.

Preparation of *Padina australis* Meal

The *P. australis* used as a soybean meal substitute was collected from the coastal area of Baruta Doda Bahari Village, Central Buton. The samples were thoroughly washed with freshwater to remove mud, sand, epiphytes, and other adhering materials. The cleaned samples were then sun-dried until fully dry to reduce moisture content and prevent fungal growth and off-odour formation. The dried samples were ground using a blender and sieved to obtain a more uniform particle size. The resulting *P. australis* meal was stored in a dry container before incorporation into the experimental diet formulations.

Diet Formulation and Preparation

The experimental diets were prepared as formulated pellets. The feed ingredients consisted of fish meal, shrimp head meal, soybean meal, *P. australis* meal, corn meal, sago flour, fine rice bran, palm oil, and top mix. Dietary treatments were formulated by replacing part of the soybean meal with *P. australis* meal at different inclusion levels. The experimental diet formulations are presented in Table 1.

Table 1. Formulation of Experimental Diets for Nile Tilapia During the Feeding Trial

Feed ingredient (g)	TP-0	TP-7.5	TP-15	TP-22.5
Fish meal	30	30	30	30
Shrimp head meal	20	20	20	20
Soybean meal	**30**	**22.5**	**15**	**7.5**
P. australis meal	**0**	**7.5**	**15**	**22.5**
Corn meal	8	8	8	8
Sago flour	4	4	4	4
Fine rice bran	6	6	6	6
Palm oil	1	1	1	1
Top mix	1	1	1	1
Total	**100**	**100**	**100**	**100**

Note: Values indicate ingredient weight in g per 100 g diet.

Diet preparation was conducted by weighing all ingredients according to the formulation of each treatment. The ingredients were mixed gradually, starting from those with the smallest quantity or finest particle size and progressing to those with larger quantities, to ensure homogeneity. After all ingredients were thoroughly mixed, water was added at 25–30% until a cohesive dough was formed. The dough was pelleted using a grinder and then sun-dried until the pellets were no longer moist. The dried diets were stored in sealed containers and labelled according to treatment.

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Experimental Fish, Rearing Units, and Husbandry

The experimental fish were Nile tilapia with an average initial body weight of 10.53 ± 0.34 g. The fish were obtained from Cialam ponds, Konda, South Konawe. Only healthy, actively swimming fish without visible morphological abnormalities were selected. Upon arrival at the laboratory, the fish were acclimated for one week in fibre tanks. Before stocking into the experimental units, fish were fasted to empty the digestive tract and reduce handling stress during initial weighing.

The rearing units consisted of glass aquaria measuring 35 × 35 × 35 cm. The aquaria were washed, cleaned, dried, and filled with freshwater that had been previously settled in a fibre tank. Each aquarium was equipped with aeration connected to a blower to maintain dissolved oxygen levels. The feeding trial lasted 42 days, with six fish stocked in each aquarium. Fish were fed the experimental diets at 5% of biomass per day, divided into two feeding times at 08:00 and 17:00 Central Indonesia Time. Siphoning was conducted every morning to remove faeces and uneaten feed. Uneaten feed was collected, dried, and weighed to calculate feed intake.

Experimental Design

The study used a completely randomized design with four treatments and three replicates. The dietary treatments were TP-0: 30 g soybean meal per 100 g diet; TP-7.5: 7.5 g P. australis meal + 22.5 g soybean meal per 100 g diet; TP-15: 15 g P. australis meal + 15 g soybean meal per 100 g diet; and TP-22.5: 22.5 g P. australis meal + 7.5 g soybean meal per 100 g diet. The soybean meal inclusion level in the control diet was set at 30% of the total feed formulation, corresponding to 30 g per 100 g diet. This proportion is equivalent to the formulation reported by Ma et al. (2022). The substitution levels were then adapted from the modified soybean meal replacement design for tilapia diets described by Wattanakul et al. (2021), which applied replacement levels of 0%, 25%, 50%, 75%, and 100%.

Measured Parameters

The measured parameters included survival rate, mean weight gain, specific growth rate, feed intake, feed conversion ratio, and feed efficiency.

- Survival rate (SR) was estimated based on Kurnia *et al*. (2022):

$$SR = (\text{total number of fish at the end} / \text{total number of fish at the beginning}) \times 100\%$$

- Weight gain (WG) was estimated based on Kurnia *et al*. (2025a):

$$WG = Wt - W0$$

- Specific growth rate (SGR) was estimated based on Kurnia *et al*. (2025a):

$$SGR = [(\ln Wt - \ln W0) / t] \times 100\%$$

- Feed conversion ratio (FCR) was estimated based on Kurnia *et al*. (2025b):

$$FCR = F / WG$$

- Feed efficiency (FE) was estimated based on Kurnia *et al*. (2025b):

$$FE = (WG / F) \times 100\%$$

Where:

Wt = final fish body weight at the end of the experimental period (g)
W0 = initial fish body weight at the start of the experimental period (g)
F = feed intake
t = duration of the rearing period (days)

Statistical Analysis

Proximate composition of the experimental diets and water quality data were analysed descriptively. Data on survival rate, weight gain, specific growth rate, feed intake, feed conversion ratio, and feed efficiency were analysed using analysis of variance (ANOVA) at a 95% confidence level.

RESULTS

Survival rate remained relatively high across all dietary treatments. The highest survival rates were recorded in TP-15 and TP-22.5, both at 94.44 ± 9.62%, followed by TP-0 at 88.89 ± 9.62%, whereas the lowest value was observed in TP-7.5 at 83.33 ± 16.67% (Figure 1). Analysis of variance indicated that dietary treatment did not significantly affect the survival rate of Nile tilapia (P > 0.05).

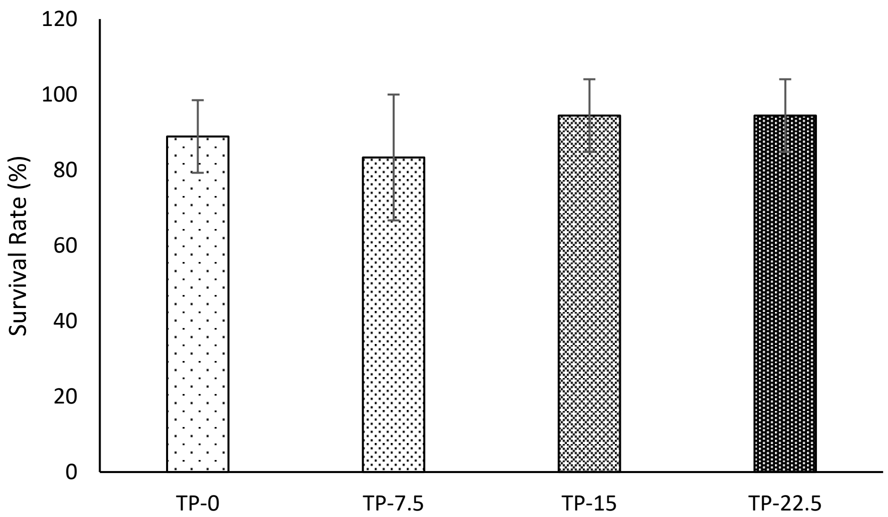

Figure 1. Survival Rate of Nile Tilapia Fed Diets in Which Soybean Meal was Partially Substituted with *P. australis* Meal For 42 Days.

Weight gain varied among treatments. The highest value was recorded in TP-7.5 at 6.21 ± 1.09 g, followed by TP-15 at 5.60 ± 2.49 g and TP-22.5 at 4.81 ± 0.35 g. The lowest weight gain was observed in TP-0 at 3.37 ± 0.75 g (Figure 2). Although TP-7.5 yielded the highest numerical value, ANOVA indicated that the differences among treatments were not significant (P > 0.05).

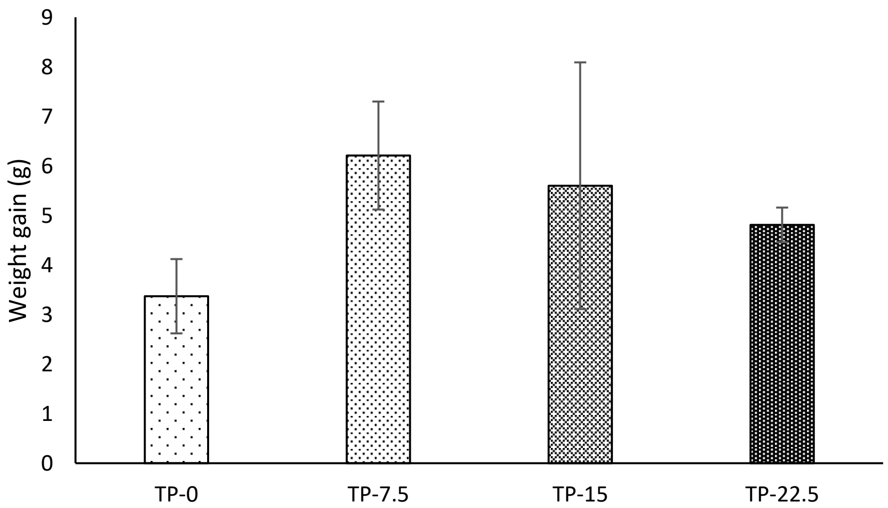

Figure 2. Weight Gain of Nile Tilapia Fed Diets Containing Different Levels of *P. australis* Meal as a Partial Substitute for Soybean Meal

The specific growth rate of Nile tilapia ranged from 1.28 to 2.28% day^{-1}. The highest value was recorded in TP-7.5 at 2.28 ± 0.21% day^{-1}, followed by TP-15 at 1.96 ± 0.77% day^{-1} and TP-22.5 at 1.80 ± 0.11% day^{-1}. The TP-0 treatment produced the lowest value at 1.28 ± 0.25% day^{-1} (Figure 3). ANOVA showed that dietary treatment did not significantly affect specific growth rate (P > 0.05).

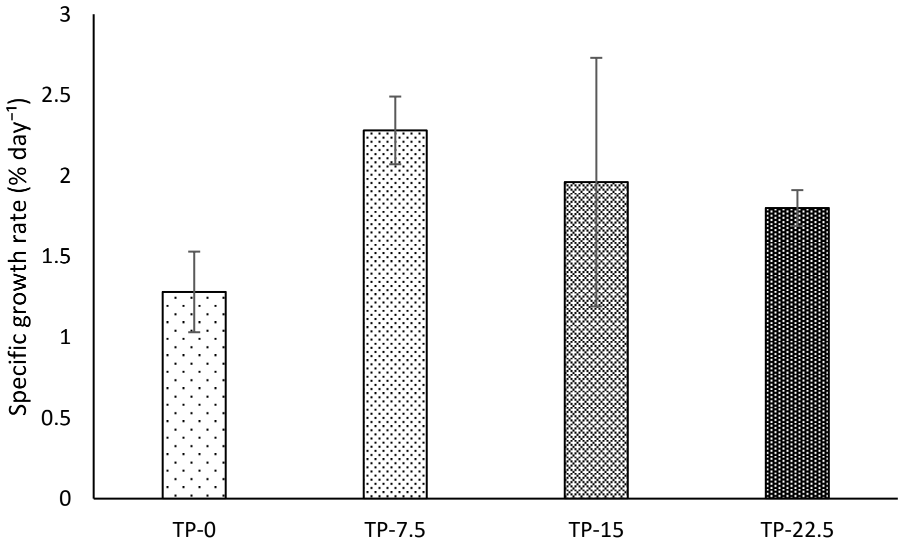

Figure 3. Specific Growth Rate of Nile Tilapia After 42 Days of Feeding with Experimental Diets Containing Different Substitution Levels of Soybean Meal with *P. australis* Meal

The highest feed intake was observed in TP-15 at 129.01 ± 1.90 g, followed by TP-22.5 at 127.45 ± 4.31 g and TP-0 at 126.48 ± 5.20 g (Figure 4). The lowest feed intake occurred in TP-7.5 at 121.82 ± 7.02 g. Analysis of variance showed that dietary treatment did not significantly affect feed intake (P > 0.05).

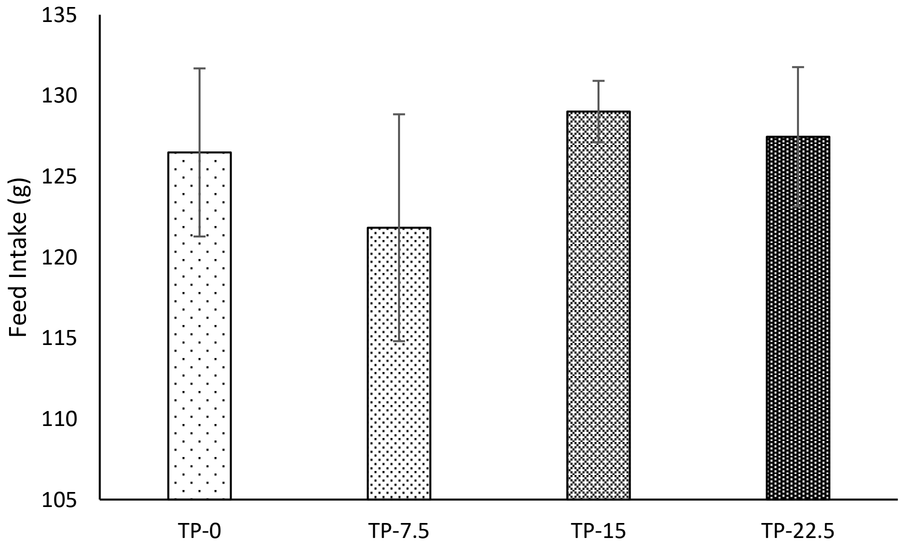

Figure 4. Feed Intake of Nile Tilapia Fed Diets with Partial Substitution of Soybean Meal by *P. australis* Meal

The highest feed conversion ratio was recorded in TP-0 at 6.49 ± 1.65, whereas the lowest value was observed in TP-7.5 at 3.75 ± 0.37. The TP-15 and TP-22.5 treatments produced FCR values of 4.71 ± 2.65 and 4.61 ± 0.42, respectively (Figure 5). ANOVA indicated that dietary treatment did not significantly affect FCR (P > 0.05). The opposite trend was observed for feed efficiency, with the highest value recorded in TP-7.5 at 26.81 ± 2.48%, followed by TP-15 at 25.28 ± 10.82%, TP-22.5 at 21.80 ± 2.06%, and TP-0 at 16.11 ± 4.18% (Figure 6). Feed efficiency also did not differ significantly among treatments (P > 0.05).

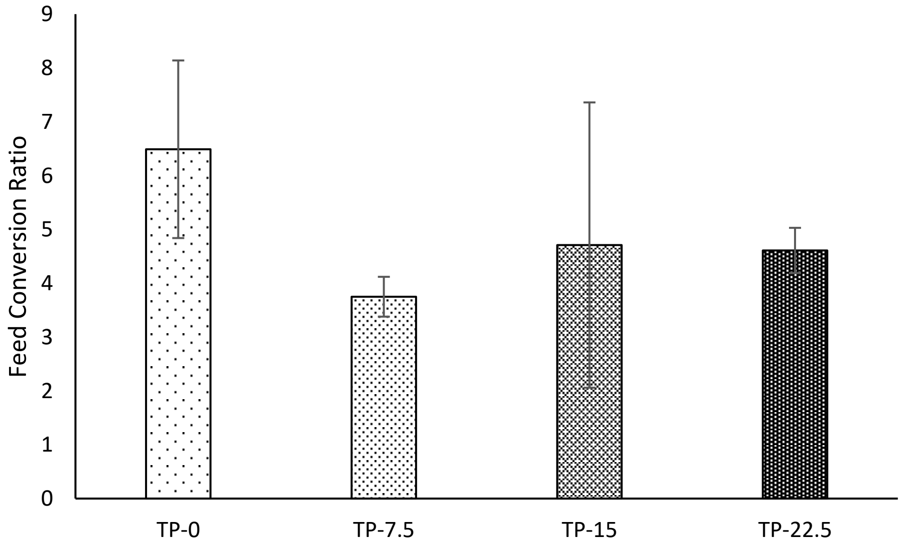

Figure 5. Feed Conversion Ratio of Nile Tilapia Fed Experimental Diets Containing Different Levels of *P. australis* Meal

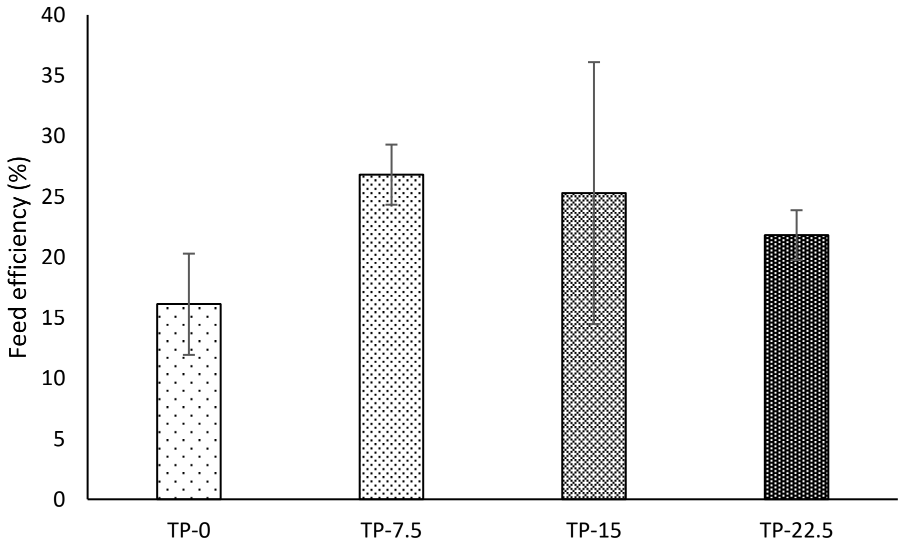

Figure 6. Feed Efficiency of Nile Tilapia Fed Diets in Which Soybean Meal was Partially Substituted with *P. australis* Meal

DISCUSSION

The results of this study showed that partial substitution of soybean meal with *P. australis* meal did not produce statistically significant differences in survival rate, weight gain, specific growth rate, feed intake, feed conversion ratio, or feed efficiency of Nile tilapia. Nevertheless, the biological response pattern among treatments provides important nutritional and physiological insights. The TP-7.5 treatment produced the highest weight gain, the highest specific growth rate, the lowest feed conversion ratio, and the highest feed efficiency. Although these differences were not significant, the trend suggests that low-level replacement of soybean meal with *P. australis* meal may improve feed utilization. This response is consistent with the concept that macroalgae are more effective when used as functional ingredients at low to moderate inclusion levels, because their contribution is not limited to protein supply but also includes polysaccharides, minerals, pigments, and bioactive metabolites that may support digestive function and the physiological status of fish (Wan *et al*., 2019; Siddik *et al*., 2023; Viagem *et al*., 2024).

Nile tilapia is an omnivorous species with herbivorous tendencies and is therefore more adaptable to plant-derived feed ingredients than carnivorous fish. This capacity is associated with digestive tract flexibility, carbohydrase enzyme activity, and the ability to utilize limited amounts of fibrous ingredients (Magbanua & Ragaza, 2022; Viagem *et al*., 2024). In this context, the use of *P. australis* as a partial soybean meal substitute has a strong biological rationale. *P. australis* has been reported to contain carbohydrates, protein, minerals, amino acids, phenols, tannins, flavonoids, steroids, alkaloids, saponins, and terpenoids (Salosso *et al*., 2020; Arguelles & Sapin, 2022). These compounds may function as antioxidants, antibacterial agents, and immune-response modulators. In common carp, *P. australis* extract improved growth, digestive enzyme activity, innate immune responses, intestinal gene expression, and resistance to *A. hydrophila* (Sheikhzadeh *et al*., 2022). Therefore, the improved growth and feed efficiency observed in TP-7.5 were likely not attributable solely to macronutrient contribution, but also to the functional effects of *P. australis* bioactive components on digestion and gut health.

Interestingly, increasing the inclusion of *P. australis* from TP-7.5 to TP-15 and TP-22.5 did not further improve growth. Weight gain decreased from 6.21 g in TP-7.5 to 5.60 g in TP-15 and 4.81 g in TP-22.5. A similar pattern was observed for specific growth rate and feed efficiency. These findings indicate the presence of an optimum inclusion threshold for *P. australis* meal. At low inclusion levels, macroalgae may exert prebiotic and immunonutritional effects; conversely, at higher levels, fibre, ash, structural polysaccharides, and certain metabolites such as tannins or saponins may limit nutrient digestibility, reduce dietary energy availability, or disturb dietary amino acid balance (Øverland *et al*., 2019; Wan *et al*., 2019; Shannon & Abu-Ghannam, 2019). This explanation is relevant because soybean meal generally contains a higher protein level than *P. australis* meal. If substitution is conducted on an ingredient-weight basis without isonitrogenous and isoenergetic adjustment, increasing the proportion of *P. australis* may reduce dietary protein density. Consequently, fish may continue consuming the diet, but biomass gain does not increase proportionally.

Feed intake data support this interpretation. The highest feed intake occurred in TP-15, followed by TP-22.5, whereas TP-7.5 had the lowest feed intake. However, TP-7.5 produced the best growth and feed efficiency. This indicates that fish in the TP-7.5 treatment converted feed into biomass more efficiently than fish in the other treatments. Conversely, the higher feed intake in TP-15 and TP-22.5 was not accompanied by improved growth. This phenomenon may indicate that diets with higher substitution levels remained palatable, but nutrient utilization efficiency began to decline. A study on Nile tilapia fed *Gracilaria arcuata* also showed that macroalgal utilization is feasible within a certain inclusion range, whereas excessive inclusion may reduce growth due to changes in digestibility and dietary nutrient composition (Younis *et al*., 2018). Similar findings have been reported for *Padina pavonica* and *Padina boergesenii* in Nile tilapia, where positive responses were more consistent when macroalgae were used as supplements or functional extracts rather than as major protein replacements (Maghawri *et al*., 2023; Cholaraj & Venkatachalam, 2023; Bakry *et al*., 2024).

Fish survival ranged from 83.33 to 94.44% and did not differ significantly among treatments. Biologically, the high survival observed across all treatments indicates that *P. australis* meal at up to 22.5 g in the formulation did not exert acute toxicity in Nile tilapia. This is important because some macroalgae contain bioactive compounds that are beneficial at low doses but may become limiting factors at excessive inclusion levels (Siddik *et al*., 2023). Phenolic compounds, phlorotannins, and fucoxanthin in brown algae may reduce oxidative stress and support immunity, whereas polysaccharides and alginates may interact with the gut microbiota and strengthen mucosal immune responses (Valente *et al*., 2016; Thépot *et al*., 2021; Rohani *et al*., 2024).

The FCR values in all treatments were relatively high compared with the expected performance of commercial tilapia diets; nevertheless, comparisons among treatments remain informative. The lowest FCR in TP-7.5 indicates that replacing 25% of the soybean meal component with *P. australis* meal was the most efficient level in this study. The higher FCR values in TP-15 and TP-22.5 indicate that increasing *P. australis* inclusion began to reduce feed conversion efficiency. This may be associated with the high mineral and ash content of *P. australis*, as reported by Salosso *et al*. (2020). Essential minerals such as calcium, potassium, and iron are beneficial for metabolism; however, excessive ash content may reduce dietary energy density and increase the proportion of feed that is not metabolized. In addition, the cell wall of brown macroalgae contains complex polysaccharides that may be partly indigestible to fish unless supported by pretreatment processes such as fermentation, extraction, or enzymatic processing (Øverland *et al*., 2019; Ang *et al*., 2021; Carvalho *et al*., 2025).

Overall, the findings of this study support the use of *P. australis* meal as a partial functional feed ingredient rather than as a dominant substitute for soybean meal. The TP-7.5 treatment provided the most favourable biological signal by improving growth, reducing FCR, and producing the highest feed efficiency, although these responses were not statistically significant.

CONCLUSION

Partial substitution of soybean meal with *Padina australis* meal in Nile tilapia diets did not significantly affect survival rate, weight gain, specific growth rate, feed intake, feed conversion ratio, or feed efficiency. Nevertheless, the TP-7.5 treatment showed the most favourable biological response, characterized by the highest weight gain and specific growth rate, the lowest feed conversion ratio, and the highest feed efficiency. Based on these findings, *P. australis* meal appears more promising as a low-inclusion functional feed ingredient, with TP-7.5 representing the most prospective treatment for further development in Nile tilapia diet formulation.

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