











Including seaweed meal in the diet for juvenile of dusky grouper *Epinephelus marginatus* (Lowe, 1834)

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Abstract

This study aimed to evaluate the effects of including flour from the seaweed, *Schizochytrium* sp. in the feed provided to dusk grouper and evaluate its effects on the zootechnical performance of the species kept in a recirculation system. Dusky juveniles acquired from a commercial laboratory of marine fish fingerlings were used in the experiment grouper. The experimental units consisted of 12 circular polyethylene tanks, each with a capacity of 1,000 L. Each unit consisted of 10 fish, and the duration of the experiment was 55 days. The experimental diets were formulated according to the nutritional requirements of marine species. through the extrusion process. The experiment had three treatments and four replications, as follows: treatment 1, control diet © without seaweed meal; treatment 2, diet (D2) enriched with 2% seaweed meal; and treatment 3, diet (D4) enriched with 4% seaweed meal. After analyzing the data, it was observed that the control treatment had the highest average values for zootechnical performance variables, followed by the treatments with 2% and 4% seaweed meal inclusion. There was no significant difference between the control treatment and the 2% inclusion treatment. However, the 4% inclusion had a negative impact on fish performance. Survival rates were similar across all treatments at the end of the experiment.

Keywords: nutrition, marine species, sustainability, marine fish farming

Resumo - Inclusão de farinha de algas marinhas na dieta de juvenis garoupa *Epinephelus marginatus* (Lowe, 1834)

Este trabalho teve por objetivo avaliar o efeito da inclusão da farinha da alga *Schizochytrium* sp. na ração para juvenis de garoupa verdadeira, avaliando seu desempenho zootécnico, em sistema de recirculação. Foram utilizados juvenis adquiridos de um laboratório comercial. As unidades experimentais consistiram em 12 tanques de polietileno em formato circular com capacidade de 1.000 litros cada. A unidade foi composta por 10 peixes e a duração do experimento foi de 55 dias. As dietas experimentais foram formuladas de acordo os requisitos nutricionais para espécies marinhas e fabricadas através do processo de extrusão. O experimento contou com 3 tratamentos e 4 repetições, sendo: tratamento 1, ração controle (C) sem adição de farinha de alga; tratamento 2, ração (R2) enriquecida com 2% de farinha de alga e tratamento 3, ração (R4) enriquecida com 4% de farinha de alga. Após análise dos dados, foi verificado que o tratamento controle apresentou os maiores valores médios para as variáveis de desempenho zootécnico, seguido pelo tratamento com 2 e 4% de inclusão de farinha de alga respectivamente. Entretanto, não houve diferença significativa entre o tratamento controle e o tratamento com inclusão de 2%. A inclusão de 4% afetou negativamente o desempenho dos peixes. Ao final do experimento, não foi observada diferença significativa na sobrevivência entre os tratamentos.

Palavras-chave: nutrição, espécies marinhas, sustentabilidade, piscicultura marinha

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Resumen - Inclusión de harina de algas en la dieta de los juveniles de mero *Epinephelus marginatus* (Lowe, 1834)

El objetivo del trabajo fue evaluar el efecto de la inclusión de harina del alga *Schizochytrium* sp. en la ración suministrada durante la alimentación de juveniles de merluza azul y evaluar sus efectos en el rendimiento zootécnico de la especie criada en un sistema de recirculación. En el experimento se utilizaron juveniles de merluza azul adquiridos en un laboratorio comercial de alevines de peces marinos. Las unidades experimentales consistieron en doce tanques de polietileno de forma circular con una capacidad de 1.000 litros cada uno. Cada unidad estaba compuesta por 10 peces y la duración del experimento fue de 55 días. Las dietas experimentales se formularon de acuerdo con los requisitos nutricionales para especies marinas y se fabricaron mediante el proceso de extrusión. El experimento contó con 3 tratamientos y 4 repeticiones, a saber: tratamiento 1, ración de control (C) sin adición de harina de algas; tratamiento 2, ración (R2) enriquecida con un 2 % de harina de algas y tratamiento 3, ración (R4) enriquecida con un 4 % de harina de algas. Tras el análisis de los datos, se comprobó que el tratamiento de control presentó los valores medios más altos para las variables de rendimiento zootécnico, seguido por el tratamiento con un 2 % y un 4 % de inclusión de harina de algas, respectivamente. Sin embargo, no hubo diferencias significativas entre el tratamiento de control y el tratamiento con un 2 % de inclusión. La inclusión del 4 % afectó negativamente al rendimiento de los peces. Al final del experimento, no se observaron diferencias significativas en la supervivencia entre los tratamientos.

Palabras clave: nutrición, especies marinas, sostenibilidad, piscicultura marina

Introduction

The global aquaculture industry is expanding across different sectors, playing a crucial role in providing high-quality food and meeting the growing demand for fish. This demand cannot be fully met by wild-caught fish alone (FAO, 2024). As aquaculture production increases, new marine fish species with potential for cultivation, such as *Balistes capriscus* (Mattos et al., 2025), *Gramma brasiliensis* (Araújo-Silva et al., 2021), *Mugil Liza* (Manhães et al., 2024), and dusky grouper, *Epinephelus marginatus* (Aride et al., 2021), are being explored in aquaculture systems.

Dusky grouper (*E. marginatus*) in particular, shows promise for aquaculture due to its large size and high-quality meat. However, there is a lack of research on its development in a production setting, and further studies are necessary to establish an effective protocol for large-scale production (Aride et al., 2021).

One of the challenges in maintaining a species in a production system is establishing an effective diet that promotes optimal zootechnical development in a shorter time frame, leading to the production of high-quality fish in large quantities and in a sustainable manner (Lisboa et al., 2020). As a result, there is a growing interest in non-traditional ingredients that can be included in feed formulations to support animal growth and reduce manufacturing costs. Various additives have been researched for their potential incorporation into aquaculture feeds, including those of plant origin such as extracts (e.g., garlic extract) (Mattos et al., 2021) or animal origin (e.g., propolis extract) (Mattos et al., 2017), as well as seaweed flours.

One of the seaweed-based ingredients with high nutritional value is the flour of *Schizochytrium* sp., a member of the Thraustochytriaceae family (Luying et al., 2008). *Schizochytrium* sp. is a spherical unicellular organism found worldwide in marine estuarine habitats (Haman 2012) and can contain 25–45% fatty acids, with triacylglycerols making up to 70% of its dry weight (Ashford et al., 2000).

Due to its high fatty acid content and rapid growth rate, *Schizochytrium* sp. is considered a potential alternative to fish oils in marine fish feed production (Li et al., 2009). Algae are a natural food source for many fish species, providing essential nutrients like proteins, fatty acids, minerals, and vitamins, making them a promising nutrient source for fish feed at various life stages (Mingyang & Qiang, 2023).

The fish production chain has experienced significant growth in recent decades, leading to a higher demand for feed for cultivated organisms, with fish meals being a major component. However, current production levels are insufficient to meet the demand for animal feed. To enhance economic efficiency and promote sustainable aquaculture, researchers are exploring alternative feeds that can partially replace fish meal and oils.

This study aimed to assess the effects of incorporating *Schizochytrium* sp. seaweed flour into the diet of juvenile dusky groupers and its influence on the zootechnical performance of the species reared in a water recirculation system.

Materials and Methods

The experiment took place at the Laboratory of Nutrition and Aquatic Organism Propagation (Lanpoa) at the Federal Institute of Espírito Santo, IFES/Campus Piúma. A total of 120 juvenile dusky groupers with an average initial weight of 20.77 ± 4.32 g were used in the experiment. These fish were obtained from a commercial marine fish fingerling laboratory. They were then transported to Lanpoa in a Transfish box (model Trevisan, 400 L) with 400 liters of water at a salinity of 35 and a temperature of 24.5 °C. Upon arrival at Lanpoa, the fish were acclimated to the laboratory conditions and housed in a 5,000 L tank. They were fed a control diet twice a day until the start of the experiment. No mortality was observed during the study period.

Experimental diet

As there is limited information available on the nutritional needs of the species, the experimental diets were formulated based on guidelines for marine fish. The feeds were produced using the industrial extrusion method. The experiment consisted of three treatments with four replications each: treatment 1, control diet (C) without seaweed meal; treatment 2, diet (D2) with 2% seaweed meal enrichment; and treatment 3, diet (D4) with 4% seaweed meal enrichment. The ingredients and guaranteed levels can be found in Table 1.

Bromatological analyses

The following values were determined: total carbohydrates, crude protein, ethereal extract by acid hydrolysis, and total fatty acid constituents including docosahexaenoic acid (DHA), arachidonic acid (ARA), and eicosapentaenoic acid (EPA). The analyses were conducted at the Animal Science Laboratory of Universidade Estadual do Norte Fluminense Darcy Ribeiro. The results of the bromatological analyses are presented in Table 1.

Table 1. Experimental diets with their respective levels of inclusion in relation to ingredients and bromatological analysis of the feed.

Ingredients (g/100g)	Control	D2	D4
Broken rice	20	20	20
Viscera meal	20	20	20
Soy protein concentrate	16.94	16.44	15.94
Ground corn	15.5	15	14.5
Meat meal	10	10	10
Isolated soy protein	6.1	6.1	6.1
Fish flour	5	5	5
Marine fish oil	4.46	3.46	2.46
Vitamin premix	1	1	1
Mineral premix	1	1	1
Seaweed meal	0	2	4
Total	100	100	100
Bromatological analysis (g/100g)			
Total carbohydrates	26.14	29.85	25.19
Crude protein	42.25	40.72	44.00
Ethereal extract	12.24	12.22	13.25
Docosahexaenoic acid (DHA)	0.31	0.62	1.05
Arachidonic acid (ARA)	0.11	0.11	0.11
Eicosapentaenoic acid (EPA)	0.08	0.07	0.06

Experimental design

The experiment followed a Completely Randomized Design (CRD) with 12 experimental units. The replicates were randomly assigned through a prior draw. Each experimental unit consisted of a 1,000-liter circular polyethylene tank housing 10 fish, totaling 120 fish for the entire experiment, which lasted 55 days.

At the start of the experiment, all fish were anesthetized with eugenol (75 mg/L) following the method outlined by Vidal et al. (2008). They were then individually weighed on an analytical balance (± 0.001 g) and measured using a caliper (± 0.01 mm) to determine total length (TL) and quantify feed intake (FI). Fish in each treatment group were weighed and measured at 20, 42, and 55 days to track their intermediate and final development.

The experiment took place in a controlled laboratory environment, shielded from external factors like sunlight and wind. Artificial lighting was used to maintain a 12-hour light and 12-hour dark cycle throughout the study. The animals were fed three times a day (at 9:00 a.m., 1:00 p.m., and 5:00 p.m.) until they were full, with diets C, D2, and D4.

The experiment was carried out in a water recirculation system with tanks designed for filtering water using physical and biological methods. The water flow was consistent to ensure high oxygen levels and stable water quality. Aeration in the tanks was provided by an industrial blower connected to air pipes that distributed air to the experimental units.

The system was supplied with water collected by pumping directly from the sea and stored in a 15,000-L tank. Before being pumped into the experimental units, the water underwent disinfection (chlorine at 10 ppm) and sediment decantation.

Water quality parameters, including temperature ($^{\circ}\text{C}$), salinity, ammonia (mg/L), nitrite (mg/L), and pH, were monitored daily, and the tanks were cleaned daily. The experimental units were siphoned by gravity using a hose, and the walls were cleaned to prevent the accumulation of organic matter and bacterial biofilms. After siphoning and cleaning, any water lost during tank cleaning and evaporation was replaced.

The physicochemical parameters of the water obtained throughout the experiment remained within the ideal values for the species and did not influence the results of the treatments. The average values obtained during the experimental period are presented: Temperature ($^{\circ}\text{C}$) 26.5 ± 0.11 , Salinity (ppm) 34 ± 0.08 , Ammonia (mg/L) 0.25 ± 0.04 , Nitrite (mg/L) 0.25 ± 0.03 .

Analysis of variables

To evaluate the zootechnical indices, the calculated parameters were: weight gain (WG), specific growth rate (SGR), apparent feed conversion (AFC), and survival (S). These parameters were calculated using the following formulas:

$$\text{WG (g)} = \text{final weight (g)} - \text{initial weight (g)}$$

$$\text{SGR (\% day}^{-1}\text{)} = [(\text{Ln mean final weight} - \text{Ln mean initial weight}) / \text{days}] \times 100$$

$$\text{AFC} = \text{FI} / \text{WG}$$

$$\text{S (\%)} = (\text{final amount of fish} / \text{initial amount of fish}) \times 100.$$

Statistical analysis

Parameters between treatments were compared using analysis of variance (Anova), followed by Tukey's test ($p < 0.05$). Data were tested for normality (Shapiro-Wilk) and homoscedasticity (Bartlett). Statistical analysis was performed using the Statistica 7 software.

Results

After analyzing the data, it was confirmed that the control treatment had the highest average values for zootechnical performance variables, followed by treatments with 2% and 4% seaweed flour inclusion. The average values of zootechnical performance variables can be found in Table 2.

After 20 days of the experiment, it was observed that the 4% microalgae flour treatment resulted in a decrease in fish weight gain. Although there was a slight weight recovery at 40 days followed by a minor decline between 40 and 55 days, the fish did not reach their initial average weight. Even in the other treatments, the growth of the animals, although positive, was not significant. Statistical analysis revealed a significant difference in the specific growth rate (SGR) between treatments D4 and C, influenced by the initial weight loss. No statistical variances were found between D4 and D2, or between D2 and C. The Apparent Feed Conversion (AFA) showed a significant difference between treatments D2 and D4, but not between C and D2 or C and D4.

Table 2. Mean values for zootechnical performance variables and *Epinephelus marginatus* fed diets with seaweed meal *Schizochytrium* sp.

Initial Biometrics								
Treatment	Biomass (g)	Sd	FI (g)	Sd	WG (g)	SGR (%)	AFC	S (%)
C	794,09	±3,90	0	0	0	0	0	100
R2	815,41	±3,67	0	0	0	0	0	100
R4	882,59	±5,07	0	0	0	0	0	100
2nd Biometrics (20 days)								
Treatment	Biomass (g)	Sd	FI (g)	Sd	WG (g)	SGR (%)	AFC	S (%)
C1	845,64	±4,29	50,51	±1,00	51,55	0,31	0,98	100
R2	847,67	±4,98	46,39	±0,91	32,26	0,19	1,44	100
R4	756,64	±3,93	36,39	±0,74	-125,95	-0,77	-0,29	100
3rd Biometrics (40 days)								
Treatment	Biomass (g)	Sd	FI (g)	Sd	WG (g)	SGR (%)	AFC	S (%)
C1	924,56	±6,52	166,50	±1,10	130,47	0,38	1,28	100
R2	882,23	±6,12	143,57	±1,00	66,82	0,20	2,15	100
R4	793,91	±5,33	149,32	±0,92	-88,68	-0,26	-1,68	100
Final biometrics (55 days)								
Treatment	Biomass (g)	Sd	FI (g)	Sd	WG (g)	SGR (%)	AFC	S (%)
C1	892,11	±6,67 a	225,74	±1,01 a	98,02 a	0,21 a	2,30ab	97,5a
R2	855,70	±6,27 a	201,51	±0,90 a	40,29 a	0,09 ab	5,00 a	95 a
R4	757,05	±5,54 a	205,21	±0,86 a	-125,54a	-0,28 bc	-1,63b	95 a

FI: Feed intake; Sd: Standard deviation; WG: Weight gain; SGR: Specific growth rate; AFC: Apparent feed conversion; S: Survival. Different letters indicate statistically significant differences between means.

Discussion

While no significant differences were observed in the zootechnical performance of dusky groupers across most variables, the findings of this study contrast with other literature that has utilized microalgae-based products. Although the data presented here do not show positive outcomes for the majority of the variables examined, previous research has highlighted the effectiveness of incorporating *Schizochytrium* sp. in fish feed. Studies have indicated that oil extracted from seaweeds can yield significant results when replacing marine fish oil in the diet of Atlantic salmon (*Salmo salar*) without compromising growth (Miller et al., 2007).

Additionally, dry algae *Schizochytrium* sp. has been identified as a valuable source of 22:6 n-3 for *Sparus aurata* (Ganuza et al., 2008). In a study on channel catfish (*Ictalurus punctatus*), diets containing 1.0% and 1.5% of dry algae showed superior results in terms of weight gain when compared to those containing 0% and 0.5% of dry algae; however, values of 2% inclusion did not differ from those of the other treatments (Li et al., 2009). Nile tilapia (*Oreochromis niloticus*), in a fish oil replacement test for *Schizochytrium* sp., resulted in improved weight gain and feed conversion rates (Sarker et al., 2016).

In most of the treatments evaluated during the experiment, there were no significant differences observed in the pattern of growth and weight gain over the experimental period. The treatments showed final values that were similar to the initial values, except for specific growth rate, which differed between the treatment with 4% microalgae flour and the control treatment, and for apparent feed conversion, which differed between the treatment with 2% inclusion and 4%, both influenced by the initial weight loss of the fish in the treatment with higher inclusion levels. Ramos et al. (2012) reported different results in their study on growth and weight gain, where they tested commercial feed for carnivorous fish and fresh food for grouper (*E. marginatus*) with an average size of 7cm and weight of 5.4g. Over a 60-day period, the groupers more than doubled their weight and increased in size by approximately 30%. In contrast, López & Castello-Orvay (2003) studied the effect of salinity and temperature on groupers with an average initial size of 40g and achieved a final weight of 450g after 15 months. These findings support the notion that species of the Serranidae family, such as the dusky grouper, exhibit slow growth rates. Bruslé (1985) estimated that dusky groupers can take around 3.3 years to reach a weight of 400g in the wild.

Negative weight gain values were observed in the 4% seaweed flour treatment after the twentieth day of the experiment. Aride et al. (2021) also reported similar negative values in a study on the effect of photoperiod on species performance, indicating that weight loss was not uncommon for the dusky grouper during the experimental period.

Throughout the experiment, active feeding behavior was observed in all treatments at various feeding times (8 am, 1 pm, and 5 pm). Juveniles were seen feeding on the surface, searching for floating feed pellets in the water. Despite this feeding activity, the efficiency of feed utilization, or feed conversion, was found to be high in all treatments. However, the diets used did not effectively promote growth in the control treatment or with different levels of seaweed meal inclusion. In fact, the inclusion of 4% algae flour may lead to weight loss in the species under evaluation.

The survival rates of the treatments in the experiment were high at the end of the experimental period, with the control treatment showing the highest average survival rate as there was no inclusion of seaweed flour. The other treatments also had a 95% survival rate. This performance is considered satisfactory, especially considering that groupers in the early stages of cultivation can exhibit cannibalistic behavior, which could potentially reduce final survival rates significantly (Cunha et al., 2009). In terms of feeding behavior, Gibran (2007) noted that groupers are carnivorous and exhibit benthic behavior in their natural environment, feeding primarily at dawn and dusk. However, in the experimental culture setting, the groupers were active throughout the day and fed on the surface, capturing floating feed, which is different from their natural feeding behavior.

Conclusion

The addition of seaweed meal in grouper feed has no significant positive impact on most performance variables and survival rates in the study. Specifically, including 2% seaweed meal did not show any difference compared to the control group with 0% seaweed meal. However, the inclusion of 4% seaweed meal did affect the initial weight loss of the fish. Future research should explore a wider range of seaweed meal inclusion levels in the feed, along with assessing digestibility and storage conditions, to gain a better understanding of its nutritional effects on the species.

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