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# Protein, energy, and amino acids digestibility of Spirulina (*Arthrospira platensis*) fed to snubnose pompano *Trachinotus blochii* and sobaity seabream *Sparidentex hasta*

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Spirulina possesses multifunctional dietary properties, making it a promising ingredient in aquaculture feed formulations as a substitute for conventional feed components. In this study, we determined the apparent digestibility coefficients (ADCs) of Spirulina to understand the nutrient availability in feeds. The ADCs for crude protein, energy, and amino acids in Spirulina were evluated for snubnose pompano *Trachinotus blochii* (706.2 ± 121.2 g) and sobaity seabream *Sparidentex hasta* (200 ± 50.2 g). Both fish species were fed either a basal diet (D1), which serves as a reference diet with 55.8% crude protein and 9.8% crude lipid content, or a test diet (D2), containing dried Spirulina powder as the test ingredient, with 56.9% crude protein and 8.8% crude lipid. The feeding period lasted 15 days before collecting fecal samples. The Spirulina was found to be palatable for both snubnose pompano and sobaity seabream, and average feed intake showed no significant differences between the basal and test diets in either species. Diet apparent digestibility coefficients (DADC) for protein among the test diets fed to snubnose pompano and sobaity seabream were 83.9 and 83.2%, respectively. Ingredient apparent digestibility coefficient (IADC) for protein for the Spirulina ingredients was significantly higher in sobaity seabream (81.6%) than snubnose pompano (74.7%). The amino acid digestibility values were significantly higher in sobaity seabream, which reflects the higher protein ingredient digestibility for the sobaity seabream. Conversely, the amino acid digestibility values were lower in the snubnose pompano, corresponding to a lower protein digestibility of the ingredients used in the diet. The findings obtained from the present study provide valuable guidance for considering Spirulina as a dietary feed ingredient for specific fish species. Additionally, the findings highlight clear species-specific differences in Spirulina digestibility, revealing that sobaity seabream has better protein and amino acid digestibility than snubnose pompano.

KEYWORDS

***Trachinotus blochii*, *Sparidentex hasta*, ingredient apparent digestibility coefficient, diet apparent digestibility coefficient, palatability, Spirulina, microalgae**

# 1 Introduction

Spirulina, a planktonic filamentous blue-green microalgae, has recently gained increasing attention as a promising ingredient in fish feeds due to its high protein value, low level of anti-nutritional compounds, and potential health benefits (Belay et al., 1996; Tibbetts et al., 2023). With a widening gap between the supply and demand of high-quality nutritional ingredients for aquafeed production, the aquaculture industry is continuously exploring sustainable and cost-effective substitutes for conventional ingredients (AlMulhim et al., 2023). The multifunctional dietary properties of Spirulina make it a potential ingredient in aquaculture feed formulations, functioning as a substitute for conventional feed ingredients (Mamun et al., 2023). The bioactive compounds present in Spirulina can enhance the immune response of fish, decrease mortality, and help with disease resistance (Ravi et al., 2010; Rana et al., 2024). Hence, including Spirulina in fish feed can potentially lead to improved fish health, reduced costs associated with disease treatment, and a lower risk of developing antibiotic resistance (Mamun et al., 2023; Rahman et al., 2023).

Numerous studies have been conducted with a wide range of finfish species to explore the potential of replacing fishmeal with Spirulina as a dietary ingredient (Jiang et al., 2022; Rosenau et al., 2021; Rosenau et al., 2022). However, there has been relatively little assessment of marine finfish species in determining the apparent nutrient digestibility of Spirulina as an ingredient (Jiang et al., 2022; Barroso et al., 2021; Riche et al., 2017; Burr et al., 2011). Apparent digestibility trials provide valuable insights into using Spirulina as a feed ingredient for specific fish species. The digestibility of feed ingredients has a direct influence on the bioaccessibility and uptake of vital nutrients such as proteins, carbohydrates, fats, vitamins, and minerals (Annamalai et al., 2021; Lall and Kaushik, 2021). Therefore, incorporating highly digestible ingredients into fish feed offers significant potential for promoting fish growth rates, improving feed efficiency, and further enhancing environmental sustainability within the aquaculture industry (Estévez et al., 2022; Glencross et al., 2007).

Snubnose pompano (*Trachinotus blochii*) and sobaity seabream (*Sparidentex hasta*) are commercially important fish species known for their fast growth rates and high market demand (Mapunda et al., 2021; Zehra et al., 2024). The majority of carnivorous fish tend to digest the nutrients and energy in feed ingredients derived from animal origin better than from plant origin (Sullivan and Reigh, 1995; Lee, 2002). The culturing of carnivorous fish species such as snubnose pompano and sobaity seabream using feed incorporating plant-based ingredients remains challenging due to their high carbohydrate content and palatability issues (AlMulhim et al., 2023; Ragaza et al., 2020; Vélez-Calabria et al., 2021). The incorporation of amino acid digestibility coefficients into fish feed formulations has emerged as a significant advancement in formulation strategy (Sørensen et al., 2002). Therefore, assessing the digestibility of nutrients and amino acids in ingredients holds paramount importance in the formulation of feeds (Glencross et al., 2007). Having a better understanding of the nutrient and amino acid digestibility coefficients of Spirulina in these highly valued species is essential for optimizing feed formulations, as it directly contributes to improving their growth performance and overall health (Sørensen et al., 2016). In this study, we aimed to evaluate the apparent digestibility coefficients for protein, energy, amino acids, and the palatability of Spirulina fed to snubnose pompano and sobaity seabream as dietary feed ingredients. The findings will provide valuable insights into the nutrient utilization and efficacy of Spirulina as a feed ingredient for these important fish species, contributing to the development of sustainable and nutritionally balanced aquafeeds.

Abbreviations: ADC, apparent digestibility coefficients; ANOVA, analysis of variance; DADC, diet apparent digestibility coefficients; IADC, ingredient apparent digestibility coefficients.

# 2 Materials and methods

## 2.1 Diet preparation

Whole dried Spirulina powder was purchased from Hunan Zhengdi Biological Resources Development Co., Ltd., China, for use in the experimental diet. All other ingredients were sourced from the Arabian Agricultural Service Company (ARASCO), Saudi Arabia. A basal mash was formulated with a nutrient content of 55.8% crude protein, 9% crude lipid, and 0.1% yttrium oxide as an inert marker. The basal mash was prepared and thoroughly mixed to serve as the basis for all the experimental diets. The test ingredient was added at a 30% inclusion level with a corresponding sub-sample of the basal mash (70%) to make up 100% of the diet mix, respectively, as outlined in Table 1. Each of the diets was processed by cooking expansion extrusion using a Coperion twin screw extruder machine ZSK 27MvPLUS (CoperionGmbh, Stuttgart, Germany) at King Abdullah University of Science and Technology fish feed mill, Saudi Arabia. The experimental diets were processed through a 6-mm diameter die. The proximate compositions and amino acid content of test diets and Spirulina ingredients are presented in Table 2.

## 2.2 Fish handling and experimental condition

The experimental protocol and methodology of fish handling in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of the King Abdullah University of Science and Technology (KAUST) with IACUC no.: 17IACUC17. The digestibility experiment was undertaken at the Centre for Marine Oceanographic

TABLE 1 Formulation of reference diet (D1) and test diet (D2).

| Ingredients (g/kg) | Reference diet (D1) | Spirulina diet (D2) |
|---|---|---|
| Fishmeal | 200 | 140 |
| Fish oil | 75 | 53 |
| Wheat meal | 164 | 115 |
| Wheat gluten | 300 | 210 |
| Soybean meal | 249 | 174 |
| Spirulina | 0 | 300 |
| Vit and Min premix (INVIVO 1%) | 10 | 7 |
| Choline | 1 | 0.7 |
| Yttrium oxide | 1 | 0.7 |

TABLE 2 Proximate composition of diets and Spirulina (% dry matter).

| Proximate composition | Reference diet (D1) | Spirulina diet (D2) | Spirulina ingredient |
|---|---|---|---|
| Dry matter | 94.0 ± 0.0 | 97.2 ± 0.0 | 94.1 ± 0.1 |
| Crude protein | 55.8 ± 2.1 | 56.9 ± 2.5 | 65.7 ± 0.9 |
| Crude lipid | 9.8 ± 0.7 | 8.8 ± 0.3 | 0.77 ± 0.1 |
| Carbohydrate | 28.6 ± 2.1 | 31.5 ± 2.3 | 22.1 ± 0.9 |
| Energy | 21.9 ± 0.12 | 22.3 ± 0.1 | 19.6 ± 0.02 |
| Amino acids (g/100 g) | | | |
| Alanine | 2.07 | 3.27 | 4.35 |
| Arginine | 2.30 | 3.35 | 3.74 |
| Aspargine | 3.01 | 4.45 | 5.25 |
| Cysteine | 0.30 | 0.50 | 0.31 |
| Glutamine | 11.53 | 13.11 | 7.35 |
| Glycine | 2.05 | 2.86 | 2.71 |
| Histidine | 1.07 | 1.37 | 1.02 |
| Isoleucine | 1.80 | 2.36 | 3.01 |
| Leucine | 3.62 | 4.95 | 5.24 |
| Lysine | 1.94 | 3.16 | 2.7 |
| Methionine | 0.46 | 1.24 | 1.56 |
| Phenyalanine | 3.91 | 3.73 | 3.71 |
| Proline | 3.90 | 4.34 | 2.21 |
| Serine | 2.23 | 2.75 | 2.89 |
| Threonine | 1.55 | 2.28 | 3.00 |
| Tyrosine | 1.48 | 1.95 | 2.42 |
| Valine | 2.03 | 3.09 | 3.45 |

Research (CMOR) Laboratory (King Abdullah University of Science and Technology, Thuwal, KSA). Snubnose pompano and sobaity seabream were obtained from the Jeddah Fisheries Research Centre (JFRC; Jeddah, Kingdom of Saudi Arabia) and held in holding tanks (2000 L), and were fed with a commercial diet (MarineFish; ARASCO, Al Kharj, Kingdom of Saudi Arabia) for 4 weeks before being acclimated to smaller circular, fiberglass experimental tanks (1,100 L). The flow-through aquaria system was supplied with filtered seawater at a salinity of approximately 42 ppt and a dissolved oxygen level of approximately 5.62 ± 0.23 mg/L (mean ± S.D.), delivered at a flow rate of approximately 5 L/min. Two separate experimental trials were conducted to assess apparent digestibility, one focusing on the snubnose pompano species and the other specifically on the sobaity seabream. Ten snubnose pompano and thirty-five sobaity seabream with an initial average body weight of 706.2 ± 121.2 g and 200 ± 50.2 g, respectively, were stocked for the respective trials. Following acclimation, the fish were allocated their dietary treatments, each with three replicates. In total, each experimental trial spanned a duration of 4 weeks to ensure an adequate quantity of fecal matter was collected for chemical analysis.

## 2.3 Fish feeding and fecal collection

In each experimental trial, fish were hand-fed to satiation once daily over a 2-h period (0900–1,100). After feeding, uneaten feed was collected by sieving the outflow water from the tank standpipe. The fish were allowed to acclimatize to their allocated diet for 15 days before fecal collection, and feces were collected using the stripping technique previously reported by Glencross (2011) and Blyth et al. (2015). Fish were sedated using AQUI-S (Aquatic Anaesthetics) (20 ppm), and the feces were removed from the distal intestine by applying gentle abdominal pressure. The hands of the person stripping the fish were rinsed with water between each fish to ensure that the feces were not contaminated by urine or mucous. The fecal sample was collected in a small labeled plastic vial and stored in a freezer at −80°C. Stripped feces were collected from 1,400 to 1800 h over a single day, with each fish only being stripped once. Feed intake was recorded daily, and the uneaten pellets remaining in the tanks were removed and air-dried, and the pellets were weighed to calculate daily fish feed intake. The effects on the palatability of diets were determined by the method described by Glencross et al. (2007) through feed intake data of fish fed with new diets for the first 10 days.

## 2.4 Chemical analysis

The experimental diets and fecal matter were analyzed in the Analytical Chemistry Core Laboratory at King Abdullah University of Science and Technology. Proximate compositions of the diets, ingredients, and fecal matter were analyzed based on AOAC (Association of Official Agricultural Chemists) methods (AOAC, 2005). Dry matter was calculated by gravimetric analysis following oven drying at 105°C for 24 h (AOAC, 2005; Method 930.15). Protein levels were calculated from the determination of total nitrogen using the combustion Dumas method in an elemental analyzer (CHNS/O – Flash 2000, FlashSmart™ Elemental Analyser, ThermoFisher Scientific) (AOAC, 2005; Method 990.03). Total lipids were determined gravimetrically following extraction of the lipids using the chloroform:methanol method (AOAC, 2005; Method 983.23). Gross ash content was determined gravimetrically following the loss of mass after the combustion of a sample in a muffle furnace at 550°C for 12 h (AOAC, 2005; Method 942.05). Gross energy was determined by adiabatic bomb calorimetry. Amino acids for test ingredients and diets were determined using an amino acid analyzer with an ion exchange column (Hitachi, Amino acid analyzer, Japan) (AOAC, 2005; Method 982.30). Total yttrium concentrations were determined after mixed acid digestion using inductively coupled plasma optical emission spectrophotometry (Agilent 5,110 ICP-OES) (AOAC, 2005; Method 985.01).

## 2.5 Calculations

Feed intake per tank per day (FI, g/tank/day) was calculated using the formula (Equation 1):

$$FI\left(g/tank/day\right) = Total\ feed\ fed\ per\ tank\ per\ day - Uneaten\ feed\ per\ tank\ per\ day \tag{1}$$

Diet apparent digestibility coefficients (DADCs) and ingredient apparent digestibility coefficients (IADCs) were measured and calculated, respectively, according to the following formula (Equation 2) (Hardy and Kaushik, 2021):

$$DADC_{nutrient} = 1 - \left( \frac{Concentration\ of\ marker_{diet} * Nutrient_{faeces}}{Concentration\ of\ marker_{faeces} * Nutrient_{diet}} \right) \quad (2)$$

where the concentration of marker $_{diet}$ and concentration of marker$_{feces}$ represent the yttrium marker concentration in the test diet and feces, respectively, and Nutrient$_{diet}$ and Nutrient$_{feces}$ represent the nutritional content (dry matter, protein, lipid, or energy) of the test diet and feces, respectively.

The digestibility values for each of the test ingredients in the test diets examined in this study were calculated according to the following formula (Equation 3) (Hardy and Kaushik, 2021):

$$IADC_{ingredient} = \frac{\left( AD_{test} * Nutrient_{test} - \left( AD_{reference} * Nutrient_{reference} * 0.7 \right) \right)}{0.3 * Nutrient_{ingredient}} \quad (3)$$

where IADC$_{ingredient}$ is the digestibility of a given nutrient from the test ingredient included in the test diet at 30%. AD$_{test}$ is the apparent digestibility of the test diet. AD$_{reference}$ is the apparent digestibility of the reference diet, which makes up 70% of the test diet. Nutrient$_{ingredient}$, Nutrient$_{test}$, and Nutrient$_{reference}$ are the levels of the nutrient of interest in the ingredient, test diet, and reference diet, respectively (Sugiura et al., 1998). All raw material inclusion levels were corrected for dry matter contribution and the effects that this may have had on the actual ratio of reference diet to test ingredient.

## 2.6 Statistical analysis

All statistical analyses were performed using the software OriginPro, Version 2022b (OriginLab Corporation, Northampton, MA, USA). One-way analysis of variance (ANOVA) was used to compare the apparent digestibility coefficients of nutrients and energy for diet or ingredients among the two fish species. All data were tested for homogeneity of variances using Levene's test (Squared Deviations). Multiple comparison between groups was performed at the significance level of 0.05 using Tukey's test. All the data were expressed as mean ± SE (standard error).

# 3 Results

## 3.1 Palatability

Feed intake in the first 10 days by snubnose pompano and sobaity seabream was used as a proxy for measuring the palatability. Daily feed intake of snubnose pompano and sobaity seabream were presented in Figures 1a, 2a. The average feed intake of snubnose pompano fed with the test diet (13.2 ± 1.2 g/fish/day) is slightly higher than the basal diet (10.5 ± 1.1 g/fish/day). The average feed intake of sobaity seabream fed with a test diet (3.7 ± 0.2 g/fish/day) is similar to the basal diet (3.6 ± 0.3 g/fish/day). However, the average daily feed intake data of snubnose pompano and sobaity seabream showed no significant differences ($p > 0.05$) between the basal diet and test diet (Figures 1b, 2b).

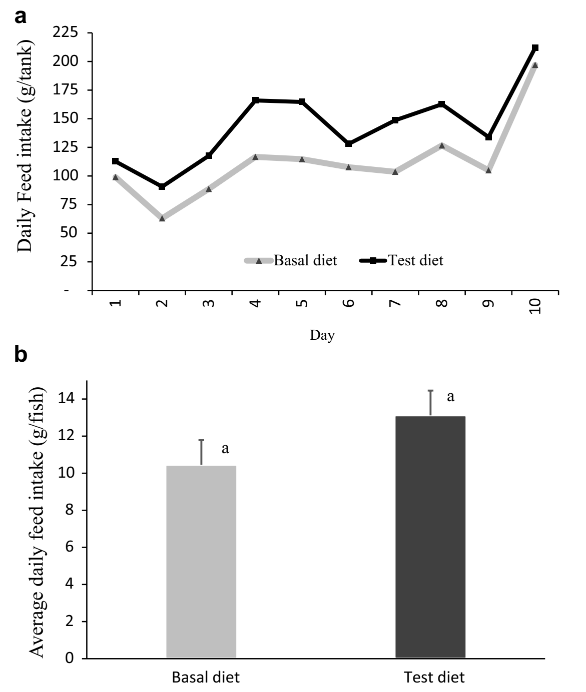


FIGURE 1
**(a)** Variations in daily feed intake of snubnose pompano fed basal diet and test diet. **(b)** Average daily feed intake of snubnose pompano.

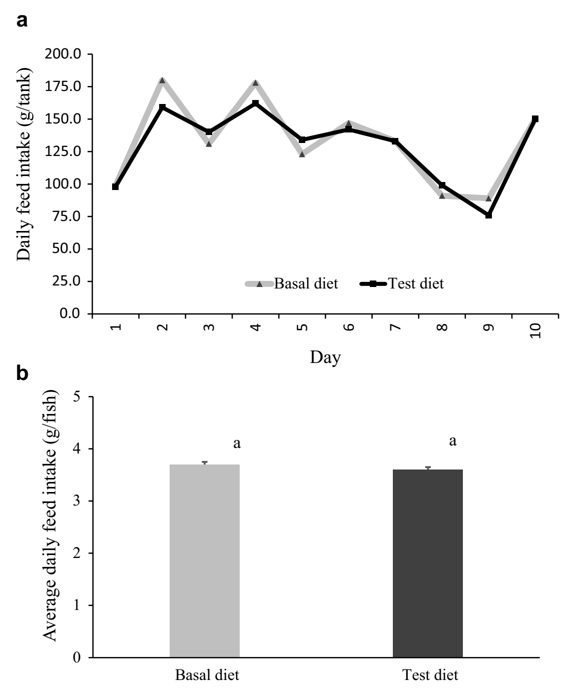


FIGURE 2
**(a)** Variations in daily feed intake of sobaity seabream fed basal diet and test diet. **(b)** Average daily feed intake of sobaity seabream.

## 3.2 Diet apparent digestibility coefficients (DADC)

Diet ADC values for protein, energy, and amino acids of the test diet (D2) consumed by snubnose pompano and mangrove red snapper are summarized in Table 3. The digestible protein value of the test diet (D2) was 83.9% for snubnose pompano, whereas the DADC value of 83.2% for sobaity seabream. The protein DADC values of the test diet measured showed no significant difference between the two species. No significant differences were observed in the energy digestibility values in the test diet consumed by snubnose pompano (76.4%) and sobaity seabream (76.6%). The DADC values for amino acid ranged between 80 and 100% for snubnose pompano, whereas in sobaity seabream, the DADC values ranged from 84 to 100%. Cysteine, histidine, methionine, proline, and tyrosine are 100% digestible in both species. The Spirulina diet produced no significant effects ($p > 0.05$) on a diet of apparent digestibility for amino acids in both tested species, snubnose pompano and sobaity seabream.

## 3.3 Ingredient apparent digestibility coefficients (IADCs)

Ingredient apparent digestibility coefficients for crude protein and energy in Spirulina as a test ingredient fed to snubnose pompano and sobaity seabream are shown in Table 4. The protein digestibility value for sobaity seabream at 81.6% was significantly higher than the IADC value measured in snubnose pompano (Table 4). The apparent energy digestibility of the Spirulina when fed to snubnose pompano and sobaity seabream was 96.0 and 64.3%, respectively. Snubose pompano had significantly ($p < 0.05$) higher energy ADC values for Spirulina than the measured apparent energy digestibility values in sobaity seabream. Spirulina ingredient had significant effects ($p > 0.05$) on ingredient apparent digestibility for all amino acids except arginine, isoleucine, and phenylalanine. The IADC values for amino acid ranged between −0.02 and 158% for snubnose pompano, whereas for sobaity seabream, amino acid digestibility values were between 82 and 147%. Substantial variations in ingredient amino acid digestibility between snubnose pompano and sobaity seabream were observed.

TABLE 3 Diet apparent digestibility coefficient (DADC) values for protein and energy in Spirulina for snubnose pompano and sobaity seabream.

| Proximate composition | Reference diet | Snubnose pompano | Sobaity seabream | *p*-value |
|---|---|---|---|---|
| DADC | | | | |
| Protein (%) | 89.6 ± 0.03 | 83.9 ± 0.03 | 83.2 ± 0.02 | 0.09 |
| Energy (%) | 70.5 ± 0.01 | 76.4 ± 0.04 | 76.6 ± 0.03 | 0.62 |
| Amino acids | | | | |
| Alanine | 0.92 ± 0.02 | 0.88 ± 0.01 | 0.91 ± 0.02 | 0.29 |
| Arginine | 1.00 ± 0.03 | 0.97 ± 0.03 | 0.95 ± 0.03 | 0.66 |
| Aspargine | 0.93 ± 0.03 | 0.89 ± 0.03 | 0.91 ± 0.02 | 0.52 |
| Cysteine | 0.87 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 | 0.99 |
| Glutamine | 0.97 ± 0.01 | 0.94 ± 0.01 | 0.96 ± 0.01 | 0.52 |
| Glycine | 0.91 ± 0.03 | 0.88 ± 0.01 | 0.90 ± 0.02 | 0.33 |
| Histidine | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 | 0.99 |
| Isoleucine | 0.96 ± 0.04 | 0.96 ± 0.04 | 0.92 ± 0.04 | 0.53 |
| Leucine | 0.96 ± 0.02 | 0.91 ± 0.01 | 0.93 ± 0.02 | 0.45 |
| Lysine | 0.91 ± 0.02 | 0.89 ± 0.01 | 0.92 ± 0.02 | 0.17 |
| Methionine | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 | 0.99 |
| Phenyalanine | 0.90 ± 0.03 | 0.80 ± 0.02 | 0.84 ± 0.04 | 0.36 |
| Proline | 0.97 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 | 0.99 |
| Serine | 0.92 ± 0.03 | 0.90 ± 0.02 | 0.92 ± 0.02 | 0.61 |
| Threonine | 0.90 ± 0.02 | 0.89 ± 0.02 | 0.90 ± 0.03 | 0.68 |
| Tyrosine | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 | 0.99 |
| Valine | 0.88 ± 0.03 | 0.88 ± 0.01 | 0.90 ± 0.02 | 0.51 |
| sAA | 0.95 ± 0.02 | 0.92 ± 0.01 | 0.93 ± 0.02 | 0.55 |

All values are Mean±SEM of three replicate analyses. Means in each column, sharing superscript letters, are not significant (one−way ANOVA; Tukey's HSD; $p < 0.05$). DADC; diet apparent digestibility coefficient.

## 4 Discussion

The quality of an ingredient has a crucial impact on feed palatability, and if it negatively impacts or reduces feed intake, it has limited potential as a feed ingredient (Glencross et al., 2007). The increasing interest in incorporating plant-based sources as proteinaceous ingredients necessitates an assessment of their impact on the palatability of fish diets (Vélez-Calabria et al., 2021). In this study, the feed intake data suggested that no significant differences were observed in snubnose pompano and sobaity seabream fed with basal and test diets, suggesting that Spirulina incorporation up to 30% did not impair palatability. These results align with findings Al-Souti et al. (2019), who observed improved palatability when algal meal was included at levels up to 35% in diets for gilthead seabream (*Sparus aurata*). Additionally, Ursu et al. (2014) demonstrated that algal ingredients, including Spirulina, provided favorable aroma and flavor properties, contributing to their acceptance in fish diets.

The results not only affirm Spirulina's acceptability as a palatable ingredient but also emphasize its potential as a highly digestible protein source for marine carnivorous fish species. Since palatability did not restrict feed intake in either species, assessing the nutrient digestibility and utilization efficiency becomes a key consideration when evaluating Spirulina's overall suitability as a feed ingredient. The apparent digestibility coefficients of Spirulina were determined based on the fecal collection using the stripping method (Blyth et al., 2015) instead of the settlement method. The most significant differences in apparent digestibility assessments between the stripping and settlement fecal collection (stripping and settlement) methods were observed in ingredients with higher carbohydrate levels (Glencross et al., 2005). Blyth et al. (2015) reported that the collection of feces by stripping produced more reliable and consistent ADC values than those obtained using the settlement technique for the high-carbohydrate content ingredients. Considering the prior works highlighting the advantages of the stripping method for fecal collection over the settlement technique for carbohydrate-rich ingredient, we opted the stripping method for Spirulina (with a carbohydrate content 22.1%) to ensure consistent ADC values and avoid discrepancies.

With marine finfish species, very few studies have been conducted, notably those with Arctic charr and Atlantic salmon (Burr et al., 2011), Florida pompano (Riche et al., 2017), and blunt snout bream (Jiang et al., 2022). In this study, the protein digestibility values for the

TABLE 4 Ingredient apparent digestibility coefficient values for protein and energy in Spirulina for snubnose pompano and sobaity seabream.

| Proximate composition | Snubnose pompano | Sobaity seabream | *p*-value |
|---|---|---|---|
| IADC | | | |
| Protein (%) | 74.7 ± 0.02[b] | 81.6 ± 0.02[a] | 0.01 |
| Energy (%) | 96.0 ± 0.12[a] | 64.3 ± 0.11[b] | 0.01 |
| Amino acids | | | |
| Alanine | 0.58 ± 0.02[b] | 0.86 ± 0.05[a] | 0.01 |
| Arginine | 0.56 ± 0.08[a] | 0.85 ± 0.08[a] | 0.06 |
| Aspargine | 0.52 ± 0.07[b] | 0.85 ± 0.06[a] | 0.02 |
| Cysteine | 0.60 ± 0.00[b] | 1.30 ± 0.00[a] | 0.01 |
| Glutamine | −0.02 ± 0.07[b] | 0.85 ± 0.06[a] | 0.01 |
| Glycine | 0.49 ± 0.04[b] | 0.82 ± 0.07[a] | 0.02 |
| Histidine | 0.36 ± 0.00[b] | 1.00 ± 0.00[a] | 0.01 |
| Isoleucine | 0.69 ± 0.10[a] | 0.86 ± 0.10[a] | 0.30 |
| Leucine | 0.48 ± 0.03[b] | 0.83 ± 0.05[a] | 0.01 |
| Lysine | 0.45 ± 0.03[b] | 0.85 ± 0.07[a] | 0.01 |
| Methionine | 0.43 ± 0.00[b] | 1.00 ± 0.00[a] | 0.01 |
| Phenyalanine | 0.56 ± 0.06[a] | 0.72 ± 0.12[a] | 0.30 |
| Proline | 1.58 ± 0.00[b] | 1.14 ± 0.00[a] | 0.01 |
| Serine | 0.43 ± 0.05[b] | 0.88 ± 0.07[a] | 0.01 |
| Threonine | 0.55 ± 0.04[b] | 1.20 ± 0.06[a] | 0.01 |
| Tyrosine | −0.61 ± 0.00[b] | 1.26 ± 0.00[a] | 0.01 |
| Valine | 0.53 ± 0.03[b] | 1.47 ± 0.07[a] | 0.01 |
| sAA | 0.44 ± 0.03[b] | 0.87 ± 0.06[a] | 0.01 |

All values are Mean±SEM of three replicate analyses. Means in each column, sharing superscript letters, are not significant (one−way ANOVA; Tukey's HSD; $p < 0.05$). IADC; ingredient apparent digestibility coefficient.

test diet were similar for both species at 83.9% for snubnose pompano and 83.2% for sobaity seabream, suggesting that the protein in the test diet was digested by both species to a similar extent. The observations of the present study support the earlier research reported by Mohamed et al. (2024) that there are clear commonalities in the digestibility of different diets between certain carnivorous species. Zehra et al. (2024) reported that the protein digestibility coefficients for protein sources of plant origin when fed to sobaity seabream were approximately 85%. It is worth noting that diets including Spirulina had protein digestibility values of 89.8% for the early phase of Atlantic salmon (Tibbetts et al., 2023) and 93.4% for rainbow trout (Cerri et al., 2021). Similar to the diet protein digestibility, the energy DADC values were also comparable between the two species, with snubnose pompano at 76.4% and sobaity seabream at 76.6%. The lack of significant difference ($p > 0.05$) in energy digestibility suggests that both species are equally efficient in digesting the energy content of the test diet. The amino acids diet digestibilities were not significantly different among species and all exceeded 84%. Amino acid digestibility values of the test diet containing Spirulina for sobaity seabream were consistent with the digestibility results reported for juvenile blunt snout bream (Jiang et al., 2022) and early phase of Atlantic salmon (Tibbetts et al., 2023). The higher protein ADC values represent that snubnose pompano and sobaity seabream appear to digest and absorb protein and amino acids from algal-based diets (Burr et al., 2011).

The experiment results provide critical insight into the ingredient apparent digestibility coefficients for protein, energy, and amino acids in Spirulina as a test ingredient fed to snubnose pompano and sobaity seabream. The protein digestibility of Spirulina was significantly higher in sobaity seabream (81.6%) than in snubnose pompano (74.7%). The higher value in sobaity seabream, which is known for its efficient protein utilization, suggests that Spirulina is not only highly digestible but also that species-specific differences may play a role in protein utilization efficiency. The lower protein digestibility observed in snubnose pompano compared to sobaity seabream in the present study further supports the importance of evaluating ingredient digestibility on a species-specific basis when formulating aquafeeds. The ingredient protein digestibility value of Spirulina is similar to the value obtained for Florida pompano (73.2%) (Riche et al., 2017). The protein digestibility of Spirulina was reported to be 84.7% in Atlantic salmon and 82.2% in Arctic charr (Burr et al., 2011). The apparent energy digestibility (IADC) of Spirulina was significantly higher in snubnose pompano (96.0%) than sobaity seabream (64.3%). The energy digestibility values of Spirulina were reported to be 82.5% in Atlantic salmon and 82.7% in Arctic charr (Burr et al., 2011). The apparent digestibility coefficients (IADCs) of individual amino acids from Spirulina differed significantly between snubnose pompano (*Trachinotus blochii*) and sobaity seabream (*Sparidentex hasta*), indicating species-specific variations in amino acid utilization. In general, sobaity seabream exhibited higher essential amino acid digestibility values than snubnose pompano. This observation aligns with previous reports suggesting that the digestibility of amino acids can vary considerably between fish species due to differences in digestive physiology, enzyme activity, and nutrient absorption capabilities (García-Meilán et al., 2023; Natale et al., 2025).

The ingredient amino acid digestibility values of snubnose pompano were notably lower when compared to Florida pompano (Riche et al., 2017). The digestibility values of amino acids of Spirulina fed to sobaity seabream were comparable to previously reported digestibility values for Spirulina fed to Florida pompano (Riche et al., 2017), Arctic charr (Burr et al., 2011), and Atlantic salmon (Tibbetts et al., 2023; Burr et al., 2011). The IADC values for essential amino acids such as arginine, leucine, lysine, methionine, threonine, and valine were significantly higher in sobaity seabream, often exceeding 100%, indicating near-complete absorption or potential overestimation due to endogenous amino acid contributions. The similar results of higher digestibility values (>100%) as observed in the present study have been reported for other species including Asian seabass (Ngo et al., 2015), Florida pompano (Riche et al., 2017), Arctic charr (Burr et al., 2011), and Atlantic salmon (Burr et al., 2011; Glencross et al., 2023). IADC values exceeding 100% sometimes occur because the calculation assumes additivity of the digestibility of all components in the diet, and there is no occurrence of interactions between the different ingredients. We, in fact, know that interactions occur, and these have been documented in various studies (Glencross et al., 2017; Irvin et al., 2016; Glencross et al., 2012). Despite the occurrence of these interactions, the determination of the IADC remains the best objective evaluation of the nutritional value of an ingredient, as it circumvents dietary formulation and intake effects that mask the value interpretation of ingredients when using a growth study approach. As demonstrated by Glencross (2020), the ingredient evaluation approach must account for IADC and palatability effects prior to assessing growth effects to avoid misinterpretation of the ingredients nutritional value.

In contrast, snubnose pompano exhibited notably lower digestibility coefficients for several amino acids, including leucine (0.48), lysine (0.45), methionine (0.43), and histidine (0.36), with the digestibility coefficient for tyrosine being negative (−0.61). Negative or unusually low amino acid digestibility values have been previously documented in studies where ingredient digestibility is assessed using indirect methods, particularly in marine fish species. They may reflect endogenous losses, amino acid imbalance, or incomplete digestion of certain amino acids (Glencross et al., 2023; Kaushik and Seiliez, 2010). This suggests that snubnose pompano may face challenges in fully utilizing certain amino acids from Spirulina, which may be because of the limitation in enzyme systems specific to this species. Considering the relatively lower digestibility coefficients observed for certain amino acids in snubnose pompano, it may be necessary to optimize diet formulations by supplementing limiting amino acids, such as methionine and lysine, to ensure balanced amino acid profiles and maximize growth performance.

## 5 Conclusion

In conclusion, the observations from the current study suggest that Spirulina is a promising feed ingredient for snubnose pompano and sobaity seabream. Spirulina as a test ingredient did not impact the palatability of the diet when fed to both fish species. The higher protein and amino acid ingredient digestibilities values suggested that Spirulina can be used as a potential ingredient for sobaity seabream. These results contribute to the understanding of how different fish species utilize novel feed ingredients and underscore the need for feed formulations based on digestible coefficients. However, future research should build upon these species-specific differences in Spirulina digestibility by investigating the physiological and digestive enzyme variations between snubnose pompano and sobaity seabream that may underlie these discrepancies. Detailed analysis of gut histology, intestinal enzyme activity (e.g., proteases, aminopeptidases), and microbiome composition could elucidate why sobaity seabream exhibits superior protein and amino acid digestibility. Furthermore, long-term growth trials should be conducted to evaluate the performance and nutrient utilization efficiency when Spirulina is incorporated at varying inclusion levels in practical diets for both species. These trials could assess optimal inclusion rates for maximizing growth, feed conversion efficiency, and overall health status. Additionally, given Spirulina's multifunctional properties, its potential immunomodulatory and antioxidant effects on both species should be examined under stress conditions such as crowding, temperature fluctuations, or disease challenges.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## Ethics statement

The animal study was approved by Institutional Animal Care and Use Committee. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

SA: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. SZ: Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. RS: Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. PM: Investigation, Methodology, Writing – original draft, Writing – review & editing. JL: Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. MA: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. AS: Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing. YH: Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing. BG: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. AM: Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

## Publisher's note



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