

Evaluation of nutritional value and quality of aquafeed ingredients for Malabar snapper (*Lutjanus malabaricus*)

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ABSTRACT

Fish meal is considered the benchmark for aquafeed ingredients, but its finite supply and rising demand necessitate exploring alternative protein sources. This study evaluated the digestible nutrient value and cost-effectiveness of ten protein ingredients for Malabar snapper (*Lutjanus malabaricus*). Test diets were formulated by replacing 30 % reference diet with test ingredients: wheat gluten (WG), soybean meal (SBM), soy protein concentrate (SPC), corn gluten meal (CGM), poultry feather meal (PFM), squid liver meal (SLM), a single cell protein (SCP), two black soldier fly larvae meals (BSF1 and BSF2), and fish meal (FM) individually. Fish were fed to apparent satiation over 56 days at 29.56 °C. FM supported the highest growth and feed intake among the tested ingredients. Although the digestible essential amino acid profile of SCP resembled FM more closely than the other raw materials tested, it had the second lowest growth rate, similar to PFM's ($P > 0.05$). SLM, SPC, CGM, SCP and PFM exhibited significantly lower daily feed intake (g day^{-1}) than FM before Day 7. After Day 7, feeding responses of SCP and PFM were negative. Variations in growth performance and initial feeding responses of the tested ingredients could be explained by their positive association with digestible histidine, lysine, and methionine levels. These findings indicated potential palatability issues or nutrient deficits that limited the use of these raw materials at 30 % dietary inclusion level. Growth performance and initial feeding responses of fish are typically evaluated based on the measured outcomes of the whole diet rather than the test ingredient. Specific ingredient productivity values and quality indexes were calculated to evaluate ingredient qualities independently from the reference diet matrix. These indexes enabled the development of a cost-effective index (CEI) to evaluate ingredient qualities coherently for cost-benefit analysis. Among the ingredients investigated, SBM, CGM, and FM were identified as having the greatest cost-benefit potential. The nutritional values of alternative proteins and the CEI established in this study provide insights into ingredient qualities, highlighting the importance of considering the effect of ingredient qualities on cost, feeding response and growth performance when formulating species-specific diets for Malabar snapper.

1. Introduction

Fish meal (FM) is considered the ideal aquafeed ingredient due to its high digestible protein content, exceptional palatability, and well-balanced essential amino acid profile that supports optimal fish growth (Hardy and Tacon, 2002; Shepherd and Jackson, 2013). While fed aquaculture has grown steadily year-on-year, global fish meal availability has stagnated since the 1990s (Tacon and Metian, 2008; Naylor et al., 2009; FAO, 2024). Therefore, FM makes up a progressively diminished proportion of total aquafeed ingredients, necessitating the development of cost-effective alternative protein sources to meet the

digestible nutrient demand required for sustainable fish growth.

Complete or partial substitutions of FM in aquafeeds with alternatives have faced varying challenges, often requiring compromise in either feed cost, nutritive value, palatability, or fish growth performance (Hua et al., 2019; Aragão et al., 2022; Macusi et al., 2023; Hussain et al., 2024; Serra et al., 2024). For example, plant-based proteins are commonly used to replace fish meal in formulated aquafeeds due to their low price and high protein content (Gatlin III et al., 2007). However, the presence of anti-nutritional factors, non-starch polysaccharides and deficiencies in essential amino acids (i.e., lysine and methionine) necessitate prudent management of their maximum inclusion level and

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strategic use of diverse raw materials to balance and mitigate the negative impacts of feed intake, digestibility and fish growth (Gatlin III et al., 2007; Hardy, 2010; Glencross et al., 2020). Moreover, the agricultural industry itself has constraints on expanding production without demanding additional land, freshwater and phosphorous resources (Malcorps et al., 2019), advocating the need to explore other alternatives such as insects, uncommon animal by-products (i.e., poultry feather meal and squid liver meal), and single-cell proteins (SCP). Although these emerging alternatives show promise as substitutes for FM, their supply and production require additional time and scale before being commercially viable (Hua et al., 2019).

The limited availability of FM and the heterogeneous nutritional qualities of alternative ingredients have driven raw material research for many commercially important species, such as rainbow trout (*Oncorhynchus mykiss*) (Lee et al., 2020; Tefal et al., 2024), Atlantic salmon (*Salmon salar*) (Belghit et al., 2019; Egerton et al., 2020), and Asian seabass (*Lates calcarifer*) (Le Boucher et al., 2024; Santillan et al., 2024). In snappers (*Lutjanus* sp.), dietary evaluations have focused on tuna by-products, pet-grade poultry by-products, canola meal, corn gluten meal (CGM) and soybean meal (SBM) in spotted rose snapper (*L. guttatus*) (Silva-Carrillo et al., 2012; Hernández et al., 2014a; Hernández et al., 2014b; Hernández et al., 2020; Hernández et al., 2021). Similarly, the potential of animal by-products and defatted SBM to replace FM has been studied in mangrove red snapper (*L. argentimaculatus*) (Catacutan and Pagador, 2004; Jamil et al., 2007).

However, research on the nutritive value and dietary effects of aquafeed ingredients in Malabar snapper (*Lutjanus malabaricus*) remains limited. Malabar snapper is a commercially valuable food fish commonly distributed throughout the Indo-Pacific, including Singapore (Blaber et al., 2005; Shen et al., 2021; Takahashi et al., 2023). It is a primarily piscivorous species with a diet that includes crustaceans, cephalopods and other invertebrates (Kailola et al., 1993). Although the production of Malabar snapper has gained popularity, species-specific feeds remain unavailable. Its production primarily relied on generic marine feeds or feeds designed for other species, which are neither cost-effective nor optimised for growth in Malabar snapper. Commercial feeds often list FM and SBM as the primary protein sources. However, the dietary effects of FM, SBM and other potential alternatives are unknown in Malabar snapper, and thorough quality characterisation of these aquafeed ingredients is needed to determine their suitability for aquafeed development based on their nutritional value and cost (Glencross et al., 2007).

Evaluating ingredient quality is challenging as multiple must be considered, with cost and nutrient bioavailability being the most critical (Glencross, 2020). The direct approach is to compare the ingredient cost of digestible protein by estimating the ingredient's protein apparent digestibility coefficient (ADC_i) using the diet replacement method (Bureau et al., 1999). However, digestibility studies typically do not measure the ingredient effects on production parameters such as growth performance and feed utilisation (Hernández et al., 2015; Ortiz-Chura et al., 2018; Rahman et al., 2023). These parameters are often evaluated in a dose-response study using practical diets which are balanced nutritionally and energetically and contain varying levels of test ingredients (Campos et al., 2017; Caimi et al., 2021; Karapanagiotidis et al., 2023). However, a few challenges arise with dose-response studies for ingredient quality evaluation. First, the observed effects reflect the impact of the entire diet rather than the specific test ingredient. Secondly, balancing the nutrition and energy content of the experimental diets limits the ability to isolate and quantify the apparent effects of the test ingredient, as other ingredients are proportionally adjusted. This complicates the interpretation of observable outcomes, such as growth performance, as they can be equivalently explained by the proportional levels of other ingredients. Moreover, the absence of a unified index for comparing the distinct qualities of ingredients coherently makes comparing different ingredients onerous. Therefore, to formulate sustainable and cost-effective diets for Malabar snapper, this study aims to

evaluate the quality of ten ingredients, including four plant meals, two animal by-products, two black soldier fly (*Hermetia illucens*) larvae meals (BSF), a SCP and a high-quality FM using the diet replacement method. Firstly, the ingredient nutrient ADC_i is evaluated to determine the cost of digestible protein. Next, the ingredient's effect on growth performance and feed utilisation will be assessed independently of the reference matrix by calculating the ingredient productivity value. Finally, a simple cost efficiency index (CEI) will be developed and proposed to compare the cost-benefit of different ingredients.

2. Material and methods

2.1. Experimental diets

A practical reference diet (REF) was formulated to resemble commercial generic marine fish feeds commonly used for Malabar snapper (Table 1). To evaluate the partial apparent digestibility values of ten test ingredients (FM, wheat gluten (WG), defatted soybean meal (SBM), soy protein concentrate (SPC), corn gluten meal (CGM), defatted black soldier fly larvae meal (BSF1 and BSF2), poultry feather meal (PFM), squid liver meal (SLM), and single-cell protein (SCP) derived from microbial protein utilising methane), ten test diets were designed to contain 30 % test ingredients and 70 % reference diet mash. However,

Table 1
Ingredient composition of reference mash (g kg⁻¹, as fed-basis).

Ingredients (g kg ⁻¹)	REF Mash
Fish meal ^a	350
Wheat gluten ^b	130
Soybean meal ^c	180
Soybean concentrate ^d	50
Corn Gluten Meal ^e	60
Wheat Flour ^f	10
Corn Starch ^g	115
Fish Oil ^h	75
Vitamin mix ⁱ	7.5
Mineral mix ^j	7.5
MDCP	9.0
Betaine HCL ^k	2.0
Choline chloride ^l	1.0
Vitamin C phosphate(35 %) ^k	1.0
Mold inhibitor ^l	0.8
Antioxidant ^m	0.2
Yttrium Oxide ⁿ	1.0
Total	1000

[†] Provide per kg: Vitamin A, 2000 MIU; Vitamin D3, 400 MIU; Vitamin E, 20 g; Vitamin B2, 5 g; Vitamin K3, 2 g; Nicotinic Acid, 15 g; Calcium Pantothenate, 10 g; Folic acid, 0.5 g; Vitamin B1, 2 g; Vitamin B6, 2 g; Vitamin B12, 10 mg.

[‡] Provide per kg: Iron, 100 g; Copper 10 g; Manganese, 70 g; Zinc, 80 g; Cobalt, 300 mg; Iodine, 1000 mg.

^a FF Classic, FF SKAGEN A/S, Denmark.

^b Vital wheat gluten, Malindra group, USA.

^c Hipro soybean meal, Argentina.

^d X-soy 200, CJ selecta, Brazil.

^e Cargill, USA.

^f Little shephard brand, Prima Ltd., Singapore.

^g Daesang corporation, Korea.

^h Anchovy oil, Indonesia.

ⁱ Vistabet, AB Vista, United Kingdom.

^j Choline Chloride, 60 % corn cob, Shandong Jujia Biotech Co., Ltd., China.

^k L-ascorbate-2-polyphosphate, Rovimix Stay-C® 35, DSM, Netherlands.

^l Funginat, Norel animal nutrition, Singapore.

^m Butylated hydroxytoluene (BHT), 2 %; Butylated hydroxyanisole (BHA), 0.5 %; Ethoxyquin, 3.2 %; Haltox, Zargo, Singapore.

ⁿ Yttrium(III) oxide, REacton, 99.9 %, CAS: 1314-36-9, Thermo Fisher Scientific, Singapore.

due to a calculation error during diet preparation, the inclusion level for FM, WG, SBM, SPC, CGM, SCP, BSF1 and BSF2 was slightly lower at 28.39%. PFM and SLM ingredients were successfully incorporated at the intended 30% inclusion level. The macronutrient composition, energy content, and amino acid profile of the test diets and ingredients were analysed to determine the apparent digestibility coefficient (ADC) of both diets and ingredients (Tables 2 and 3). All diets were produced at the pilot feed mill at the Marine Aquaculture Centre (MAC), Singapore Food Agency, Singapore. Ingredients were milled and sieved below 500 µm. Dry powdered ingredients were mixed homogeneously for 30 min using a 100 L horizontal paddle mixer (Kong Shiang Engineering Pte Ltd., Singapore) before extrusion. The experimental diets were extruded using a co-rotational twin-screw extruder (Evolum 25, Clextal) using a 7 mm diet insert with the highest temperature setting of 120 °C at the end of the barrel. Post-extrudate pellets were dried below 10% moisture content using a fluidised dryer at 70 °C for at least 40 min. The dried pellets were de-dusted by sieving before fish oil was vacuum coated at 200 mbar of absolute pressure. The experimental diets were cool at ambient temperature before storage at 4 °C.

2.2. Fish handling and feeding

The experiment was conducted at MAC for 56 days from October to December 2022. Juvenile Malabar snappers with an average body weight (ABW) of 14 g were procured from a local hatchery (Prime Aquaculture Pte Ltd., Singapore). The fish were quarantined for two weeks and grown out to experimental size for 70 days using two 20 m³ flow-through tanks at MAC. Upon reaching the experimental size, fish were transported to MAC's recirculating aquaculture system (RAS). The

experimental RAS consists of 40 replicated tanks of 1 m³. Each tank has a swirl separator for faecal collection, LED lighting and a water measuring probe for real-time monitoring of dissolved oxygen (DO), salinity and temperature. Fish were acclimatised to the system for a week and fed to apparent satiation using a commercial diet (Uni-president M504, Vietnam) before the start of the trial. At the start of the experiment (Day 0), fish were graded manually, and a total of 1320 fish with a mean body weight of 133.8 ± 1.5 g were randomly distributed to one of the 33 experimental tanks. The REF and 10 test diets were tested in triplicate tanks ($n = 3$), and the treatment replicates were randomised using the sample function in R using Dplyr (R Core Team, 2022). Each tank was hand-fed to apparent satiation twice daily at 9:30 and 14:30. Each feeding session was conducted and completed within an hour. Apparent satiation was determined when fish began to lose interest in feeding, and any uneaten pellets observed at the bottom of the tank were removed. The total feed intake was calculated by deducting any uneaten feed from the total feed offered. The tank water exchange rate was maintained at 150% per hour, and a 12-h light and dark period was maintained throughout the trial. Average water temperature was 29.56 ± 0.28 °C. The daily average DO level ranged from 5.02 to 7.85 mg/L. Water salinity ranged from 29 to 32 ppm. Ozone level in the system was maintained below 340 mV, and pH was maintained between 7.4 and 8.0 by supplementing sodium bicarbonate. Ammonia, nitrite, and nitrate concentrations in the system were tested daily using API® saltwater master test kit, and the measured levels throughout the experiment were below 0.5 mg/L, 0.5 mg/L, 160 mg/L and, respectively.

Table 2

Nutrients, energy content and amino acid profile of reference and 10 test diets fed to Malabar snapper (*L. malabaricus*).

	Diets										
	REF	FM	WG	SBM	SPC	CGM	SCP	BSF1	BSF2	PFM	SLM
Nutrient composition (% DM)											
Dry matter (% as fed)	93.7	92.5	90.0	92.3	93.9	92.7	93.0	94.3	95.1	92.3	93.8
CP	54.5	60.9	63.6	52.9	57.4	58.9	60.0	55.4	56.8	66.7	54.7
CF	14.5	13.5	11.8	12.1	11.0	11.7	13.0	14.1	12.9	11.9	14.9
Fibre	1.8	2.1	1.0	2.5	1.1	1.1	1.2	5.4	5.0	1.2	7.2
Carb [†]	22.0	14.9	18.0	26.8	23.3	22.4	18.6	20.9	21.2	13.7	20.3
Ash	8.9	10.6	6.6	8.2	8.3	7.0	8.4	9.6	9.1	7.7	10.1
GE [‡] (kJ.g ⁻¹)	22.4	22.3	22.8	21.9	21.9	22.4	22.5	22.2	22.2	22.8	22.3
Phosphorus (g kg ⁻¹)	13	16.1	9.8	11.3	11.5	11.1	15.3	13.4	12.5	10.1	11.4
Digestible protein and fat (% DM)											
DP	50.2	57.2	60.4	49.0	53.5	54.6	55.3	49.7	51.9	55.6	46.3
DF	13.5	12.7	11.1	11.5	10.3	10.6	12.1	13.2	12.1	10.4	13.8
Essential and conditional amino acids (g kg⁻¹; DM)											
Arginine	31.1	36.0	31.0	33.7	40.8	29.6	36.1	30.1	33.4	41.3	22.7
Cysteine	6.6	6.9	10.8	6.7	8.4	8.2	6.9	5.8	5.8	13.0	5.6
Histidine	13.4	15.2	14.2	13.8	16.1	13.7	14.0	12.9	15.5	11.4	9.6
Isoleucine	24.4	28.2	27.7	24.9	29.6	25.9	27.5	22.8	25.2	31.5	19.1
Leucine	45.8	50.7	51.0	44.8	53.2	66.9	49.4	40.2	45.1	55.2	34.4
Lysine	24.1	35.8	21.4	26.0	28.2	20.5	34.0	27.2	26.9	20.7	13.5
Methionine	13.4	16.7	14.0	12.2	14.0	14.5	15.4	11.0	13.4	11.9	10.2
Phenylalanine	26.4	28.5	32.8	26.9	32.3	32.5	28.6	23.7	27.0	32.5	19.9
Threonine	21.0	25.0	21.5	21.3	25.5	22.4	24.5	19.8	22.3	27.6	16.0
Tyrosine	20.6	22.9	24.1	21.0	24.7	25.9	22.8	23.8	28.2	24.2	15.6
Valine	27.1	31.6	29.8	27.0	31.8	28.5	34.1	29.5	32.4	39.3	21.1
Non-essential amino acids (g kg⁻¹; DM)											
Alanine	29.7	36.2	27.5	28.2	32.5	39.7	36.9	30.2	32.9	34.5	22.8
Aspartic Acid	46.6	55.4	40.3	51.1	60.8	46.7	51.5	45.3	51.9	51.2	35.1
Glutamic Acid	112.1	111.3	174.4	109.4	126.8	125.2	103.3	91.4	100.2	108.1	79.8
Glycine	28.9	36.0	23.4	26.5	29.2	27.1	30.1	25.3	30.3	42.0	13.6
Proline	35.6	34.4	60.2	35.6	39.4	44.4	34.3	32.3	34.5	53.9	27.1
Serine	24.7	26.9	28.4	25.7	30.5	28.7	24.7	22.3	24.9	46.9	17.5

DM: dry matter; CP: crude protein; CF: crude fat; Carb: carbohydrate; GE: gross energy; DP: digestible protein; DF: digestible fat; REF: reference; FM: fish meal; WG: wheat gluten; SBM: soybean meal; SPC: soy protein concentrate; CGM: corn gluten meal; BSF1 and BSF2: black soldier fly larvae meal 1 and 2; PFM: poultry feather meal, SLM: squid liver meal.

[†] Calculated as: 100% - (CP% + CF% + Ash% + Moisture%).

[‡] Calculated from mean GE values of protein, fat and carbohydrate (23.6, 39.5 and 17.2 kJ.g⁻¹, respectively).

Table 3
Nutrients, energy content and amino acid profile of 10 test ingredients.

	Test Ingredients									
	FM	WG	SBM	SPC	CGM	SCP	BSF1	BSF2	PFM	SLM
Proximate nutrient composition (% DM)										
DM (% as is)	90.3	92.7	91.3	91.7	91.2	93.6	98.2	93.1	95.4	95.4
CP	77.6	86.3	49.9	65.6	68.6	73.5	56.3	60.0	93.1	55.5
CF	11.9	5.7	3.9	2.5	6.0	9.4	13.4	9.5	6.2	16.7
Fibre	1.9	0.3	2.9	6.0	1.3	0.3	14.0	11.4	1.9	13.9
Carb [†]	0.0	7.2	39.0	23.8	20.8	8.6	18.8	20.8	0.0	16.6
Ash	16.6	0.8	7.1	8.1	4.6	8.5	11.4	9.7	2.9	11.2
GE [‡] (kJ g ⁻¹)	23.0	23.9	20.1	20.6	22.1	22.5	21.8	21.5	24.4	22.5
Phosphorus (g kg ⁻¹)	24.2	1.8	7.1	8.2	5.9	20.0	14.4	10.6	3.9	7.0
Digestible protein and fat (% DM)										
DP	68.0	79.5	43.2	57.5	58.6	63.3	46.7	50.2	63.5	36.0
DF	10.5	5.5	4.2	2.6	4.3	8.1	12.6	8.6	3.4	14.7
Essential and conditional amino acids (g kg⁻¹; DM)										
Arginine	48.5	28.5	38.0	50.8	25.8	45.2	33.7	37.3	66.9	8.4
Cysteine	6.9	16.2	6.8	8.4	12.0	4.6	2.5	4.5	25.1	1.8
Histidine	19.6	17.4	13.3	17.9	15.1	14.7	16.3	21.0	7.0	3.8
Isoleucine	36.1	31.8	24.5	33.0	29.8	31.5	23.4	26.8	49.1	9.3
Leucine	61.2	57.5	39.6	53.5	116.3	52.5	36.3	41.1	79.7	14.8
Lysine	60.9	11.1	27.0	37.6	11.4	38.6	26.4	35.7	16.7	5.6
Methionine	25.1	13.6	8.1	10.2	18.1	17.5	10.1	12.5	7.9	4.7
Phenylalanine	32.7	42.8	26.3	36.3	45.5	29.9	22.8	27.3	48.1	8.9
Threonine	34.7	21.0	20.5	27.7	25.0	30.7	21.0	23.9	42.6	7.9
Tyrosine	27.7	31.2	20.0	26.3	38.9	26.2	36.5	44.7	33.0	6.9
Valine	42.4	32.6	25.1	32.9	32.0	47.2	34.2	37.8	71.4	10.0
Non-essential amino acids (g kg⁻¹; DM)										
Alanine	50.9	21.4	22.9	31.0	64.1	51.0	38.4	38.9	47.2	12.7
Aspartic Acid	76.1	27.1	59.4	81.9	45.8	61.2	51.8	63.0	65.3	18.5
Glutamic Acid	106.4	308.5	91.1	123.2	152.5	72.6	65.2	64.3	103.9	30.0
Glycine	49.5	28.0	21.9	30.1	21.6	36.3	31.9	31.9	79.8	13.5
Proline	31.9	105.5	25.8	34.9	64.8	25.9	29.8	30.6	99.2	10.4
Serine	33.2	37.6	25.3	34.9	36.3	23.4	21.2	23.4	98.1	8.0
Total amino acids (% CP)										
Total amino acids	95.9	96.4	99.3	102.2	110.1	82.9	89.1	94.1	101.1	31.6

DM: Dry matter; CP: Crude protein; CF: Crude fat; Carb: Carbohydrate; GE: gross energy; FM: fish meal; WG: wheat gluten; SBM: soybean meal; SPC: soy protein concentrate; CGM: corn gluten meal; BSF1 and BSF2: black soldier fly larvae meal 1 and 2; PFM: poultry feather meal, SLM: squid liver meal.

[†] Calculated as: 100 % - (CP% + CF% + Ash% + Moisture%).

[‡] Calculated from mean GE values of protein, fat and carbohydrate (23.6, 39.5 and 17.2 kJ g⁻¹, respectively).

2.3. Faecal sample collection and processing for digestibility

Fish were acclimatised to the experimental diets and feeding regimes for a week prior to daily faecal sample collection, according to Cho et al. (1982), with some modifications. An hour after the last feeding, all tanks and swirl separators were cleaned daily to minimise and prevent any contamination from uneaten feeds in the faecal samples. Overnight faeces samples settled in the swirl separator were collected the following morning using a modified 300 mL centrifuge bottle prior to the first feeding. The collected faecal matter was pelleted using a refrigerated centrifuge (Centrifuge 5920 R, Eppendorf) at 3000 RCF for 15 min at 4 °C. Daily wet faecal pellets were pooled by tank and stored at -20 °C before freeze-drying. The freeze-dried samples were sieved through a 0.3 mm mesh to remove contaminating fish scales and stored at -20 °C before chemical analysis.

2.4. Ingredient cost

The costs of ingredients were based on Incoterms Free-On-Board (FOB) prices per MT or Ex-works (EXW) prices per MT in USD (Supplementary Table 1). Information on the prices of ingredients was obtained in two ways. FOB prices of ingredients traded as commodities, such as FM, SBM, SPC, WG, and CGM, were provided by 4SEA Consulting Ltd. by sourcing from www.intratec.co.th and www.herbavita.eu. The information was accessed and obtained in November 2023. EXW prices and information for other ingredients, such as SCP, BSF1, BSF2, PFM, and SLM, were provided directly by suppliers or distributors and have been deidentified for commercial reasons.

2.5. Chemical analyses

The crude protein (CP), crude fat (CF), amino acid composition and phosphorus of test ingredients, diets and freeze-dried faecal samples were determined by Eurofins, an accredited analytical service laboratory (Eurofins Food Testing Singapore Pte Ltd., Singapore). CP (Dumas; %N x 6.25) was analysed following AOAC 968.06 and 992.15, and CF (acid hydrolysis) was analysed following AOAC 922.06 and 954.02. Amino acid testing was analysed using HPLC according to Barkholt and Jensen (1989), Schuster (1988), Henderson et al. (2000) and Henderson and Brooks (2010). Phosphorus contents were determined using inductively coupled plasma with an optical emission spectroscopy detector (ICP-OES) following AOAC 984.27, 985.01, and 2011.14. Moisture content and ash of test ingredients, diets, and freeze-dried faecal samples were determined in triplicate per sample following AOAC 930.15 and 942.05, respectively. Dietary fibre contents were determined by Pacific Lab Services following AOAC 978.10. Yttrium contents in diets and faecal samples were determined by Pacific Lab Services using Pacific Lab Method 4.3, ICP-OES. Carbohydrate was determined by nutrient difference: Carbohydrate = 100 % - (% CP + % CF + % ash + % moisture) (Rahman et al., 2016). Gross energy (GE) was calculated using the mean energetic values of protein, fat and carbohydrate of 23.6, 39.5 and 17.2 kJ g⁻¹, respectively, according to Blaxter (1989).

2.6. Data calculations

The growth rate was determined by thermal growth coefficient (TGC) and specific growth rate (SGR). TGC was calculated following the

equation by Jobling (2003):

$$TGC = \left[\left(FBW^{\left(\frac{1}{3}\right)} - IBW^{\left(\frac{1}{3}\right)} \right) \div (T \times d) \right] \times 1000$$

SGR is calculated following the equation by Hopkins (1992):

$$SGR = (\ln FBW - \ln IBW) \div d \times 100$$

where FBW and IBW are the final and initial body weights (g), T is the mean temperature of the trial ($^{\circ}\text{C}$), and d is the number of days.

The total feed intake (TFI, g), feed efficiency (FE, %) and initial feeding response of the first 7 days (IFR, %BW day $^{-1}$) were calculated by the following equations:

$$TFI = \text{total feed offered} - \text{uneaten feed}$$

$$FE = (FBW - IBW) \div TFI \times 100$$

$$IFR = (\text{TFI of first 7 days}) \div IBW \times 100 \div d$$

where FBW and IBW are the final and initial body weights (g), and d is the number of days.

The apparent digestibility coefficients (ADC) for proximate composition, amino acids and energy in diets were calculated according to the equation following Cho et al. (1982):

$$ADC = [1 - (N_F/N_D \times Y_D/Y_F)] \times 100$$

where N_D and N_F represent either % nutrient, % amino acids or kJ g $^{-1}$ GE of the diet and faecal matter, respectively. Y_D and Y_F represent the % Yttrium incorporated as a digestion-inert marker of the diet and faecal matter.

The partial apparent digestibility coefficients of test ingredients (ADC $_i$) for proximate composition, amino acids and energy were calculated according to Bureau et al. (1999):

$$ADC_i = ADC_D + (ADC_D - ADC_R) \times [((1 - s) \times N_R) \div (s \times N_i)]$$

where ADC $_D$ is the apparent digestibility coefficient of the test diet, ADC $_R$ is the average value of the triplicated apparent digestibility coefficient of the reference diet, N_R is the nutrient content in the reference diet, N_i is the nutrient content in the test ingredient, and s is the proportion of test ingredient added to the test diet. In this study, s for FM, WG, SBM, SPC, CGM, BSF1, BSF2 and SPC was 0.2839, whereas s for PFM and SLM was 0.3.

To assess the % change of a parameter in the test diet (Para $_D$) relative to the parameter of reference diet (Para $_{Ref}$), the initial feeding response productive value (IFRPV) and TGC productive value (TGCPV) for test diet (D) and reference diet (Ref) was calculated with the parameter productive value (ParaPV) formula:

$$ParaPV_D = [Para_D - \text{meanPara}_{Ref}] \div \text{meanPara}_{Ref} \times 100$$

$$ParaPV_{Ref} = [Para_{Ref} - \text{meanPara}_{Ref}] \div \text{meanPara}_{Ref} \times 100$$

Ingredient productivity values of parameters (Para) such as initial feeding response (IFRPV $_i$) and TGC (TGCPV $_i$) relative to the proportion (s) in reference diet are calculated analogously to ADC $_i$.

$$ParaPV_i = ParaPV_D + (ParaPV_D - \text{meanParaPV}_{Ref}) \times [((1 - s) \times \text{meanPara}_{Ref}) \div (s \times Para_D)]$$

where Para is the parameter of a target such as feed intake or TGC. Para $_D$ is the parameter's value of the test diet. Para $_R$ is the parameter's value of the reference diet. ParaPV $_D$ is the delta of the Para $_D$ relative to the

meanPara $_{Ref}$. ParaPV $_{Ref}$ is the proportion of the Para $_{Ref}$ to the mean-Para $_{Ref}$. ParaPV $_i$ is the target ingredient parameter productivity value relative to the reference diet. In this study, s for FM, WG, SBM, SPC, CGM, BSF1, BSF2 and SPC was 0.2839, whereas s for PFM and SLM was 0.3.

The digestible nutrient content of the diet or ingredient is calculated by multiplying the nutrient content of the diet or ingredient with the corresponding nutrient ADC of the diet or ingredient. The cost of ingredient digestible nutrients (\$ kg $^{-1}$) is calculated by dividing the price of ingredients (FOB or EXW; \$ kg $^{-1}$, USD) with ingredient digestible nutrients (g kg $^{-1}$) \times 1000. Price differences between FOB and EXW were assumed to be negligible (FOB - EXW = \$0). The relative DP cost index (DPCI $_i$), the relative initial feeding response index (IFRI $_i$) and the relative growth index (GI $_i$) are calculated by normalising the reciprocal cost of DP (1/DPC $_i$), normalising AFPV $_i$ and normalising TGCPV $_i$, respectively. Normalisation is calculated by the following equation: normalised value = (value-min)/(max-min).

Cost-benefit of ingredients is quantified by using the cost-efficiency index (CEI $_i$) with the following equation:

$$CEI_i = \frac{DPCI_i \times C_{DPCI_i} + IFRI_i \times C_{IFRI_i} + GI_i \times C_{GI_i}}{(C_{DPCI_i} + C_{IFRI_i} + C_{GI_i})}$$

which C_{DPCI_i} , C_{IFRI_i} and C_{GI_i} is the contribution of their respective indexes; i is the test ingredient. The contribution weightage is determined by the coefficient of determination (R^2) between IFRI $_i$ with DPCI $_i$ and GI $_i$ with DPCI $_i$, which C_{IFRI_i} and C_{GI_i} were calculated by $1 - R^2$. In this study, C_{DPCI_i} is 1, C_{IFRI_i} is 0.8056 and C_{GI_i} is 0.80417.

2.7. Statistical analysis

Data were analysed using IBM SPSS Statistics (Version 27). Residuals were assessed for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. Parameters that conformed to the assumptions of normality and homogeneity of variance were tested with one-way ANOVA. Post-hoc multiple comparisons between treatment groups, assuming homoscedasticity, were conducted using Tukey's HSD test to identify differences in mean values. Parameters that do not conform to normality assumptions were subjected to Kruskal-Wallis one-way ANOVA. Post-hoc pair-wise comparisons between treatment groups and FM were conducted using Dunn's test to identify differences in mean rank scores. The linear regression slopes of feeding response were compared using Student's t -test (two tailed). Statistical significance for all tests were set at $P < 0.05$.

3. Results

3.1. Growth performance and feed utilisation of Malabar snapper

After the 56-day trial, Malabar snapper remained healthy, with an average survival rate of 99.3 % (\pm SD 1.44 %) across all treatment groups (Table 4). Fish fed the REF and FM diets had the highest growth performance, while those fed the PFM and SCP diets grew the slowest and had only 34 % and 41 % of the weight gain of those fed the FM diet, respectively (Table 4). Between the two spectrum ends, no growth differences were observed between WG, SBM, SPC, BSF1 and BSF2 diets, and slightly lower growth rates were observed for CGM and SLM diets. A

high correlation between TGC and TFI was observed, $R^2 = 0.9218$. Fish-fed FM and REF diets had the highest TFI and FE, while the PFM diet had the lowest TFI of 112.3 g and FE of 56.5 %. Daily feed intakes (g fish $^{-1}$)

Table 4Growth and feed utilisation of red snapper (*L. malabaricus*)[†] after feeding REF and 10 test diets for 56 days trial period (n = 3).

Parameters	Diets											Pooled SEM	P-value
	REF	FM	WG	SBM	SPC	CGM	SCP	BSF1	BSF2	PFM	SLM		
IBW (g)	132.6	135.8	133.8	133.8	134.2	134.1	133.1	132.5	134.3	133.5	134.5	2.76	0.312
FBW (g)	309.7 ^a	324.5 ^a	267.9 ^{bc}	268.1 ^{bc}	255.7 ^{bcd}	247.2 ^{cd}	209.9 ^e	268.1 ^{bc}	271.0 ^b	197.1 ^e	234.3 ^{cd}	14.56	<0.001
BWG (g)	177.1 ^a	188.8 ^a	134.1 ^{bc}	134.4 ^{bc}	121.4 ^{bcd}	113.1 ^{cd}	76.8 ^e	135.7 ^b	136.7 ^b	63.6 ^e	99.9 ^d	14.15	<0.001
TGC	1.0 ^a	1.0 ^a	0.8 ^{bc}	0.8 ^b	0.7 ^{bc}	0.7 ^{cd}	0.5 ^e	0.8 ^b	0.8 ^b	0.4 ^e	0.6 ^d	0.07	<0.001
SGR (% day ⁻¹)	1.5 ^a	1.6 ^a	1.2 ^{bc}	1.2 ^{bc}	1.2 ^{bc}	1.1 ^{cd}	0.8 ^e	1.3 ^b	1.3 ^b	0.7 ^e	1.0 ^d	0.10	<0.001
TFI (g)	192.5 ^{ab}	195.1 ^a	165.6 ^{cd}	177.6 ^{bc}	162.6 ^{cde}	149.5 ^e	129.5 ^f	164.3 ^{cde}	166.6 ^{cd}	112.3 ^g	159.9 ^{de}	10.00	<0.001
FE (%)	92.0 ^a	96.7 ^a	81 ^b	75.6 ^b	74.7 ^b	75.6 ^b	59.2 ^c	82.5 ^b	82.1 ^b	56.5 ^c	62.4 ^c	5.49	<0.001
IFR (%BW day ⁻¹)	2.17 ^a	2.08 ^{ab}	2.03 ^{ab}	1.96 ^{bc}	1.82 ^{cd}	1.84 ^{cd}	1.79 ^{cd}	1.93 ^{bc}	1.93 ^{bc}	1.72 ^c	1.82 ^{cd}	0.11	<0.001
SR (%)	100.0	100.0	100.0	100.0	100.0	98.3	99.2	98.3	100.0	98.3	98.3	2.76	0.462

Values within parameters are mean, and values lacking common superscripted letters differ significantly (Turkey HSD, $P < 0.05$).

IBW: Initial body weight; FBW: final body weight; BWG: body weight gain; TGC: thermal growth coefficient; SGR: specific growth rate; TFI: total feed intake; FE: feed conversion efficiency; IFR: initial feeding response of first seven days; SR: survival rate; REF: Reference; FM: fish meal; WG: wheat gluten; SBM: soybean meal; SPC: soy protein concentrate; CGM: corn gluten meal; BSF1 and BSF2: black soldier fly larvae meal 1 and 2; PFM: poultry feather meal, SLM: squid liver meal.

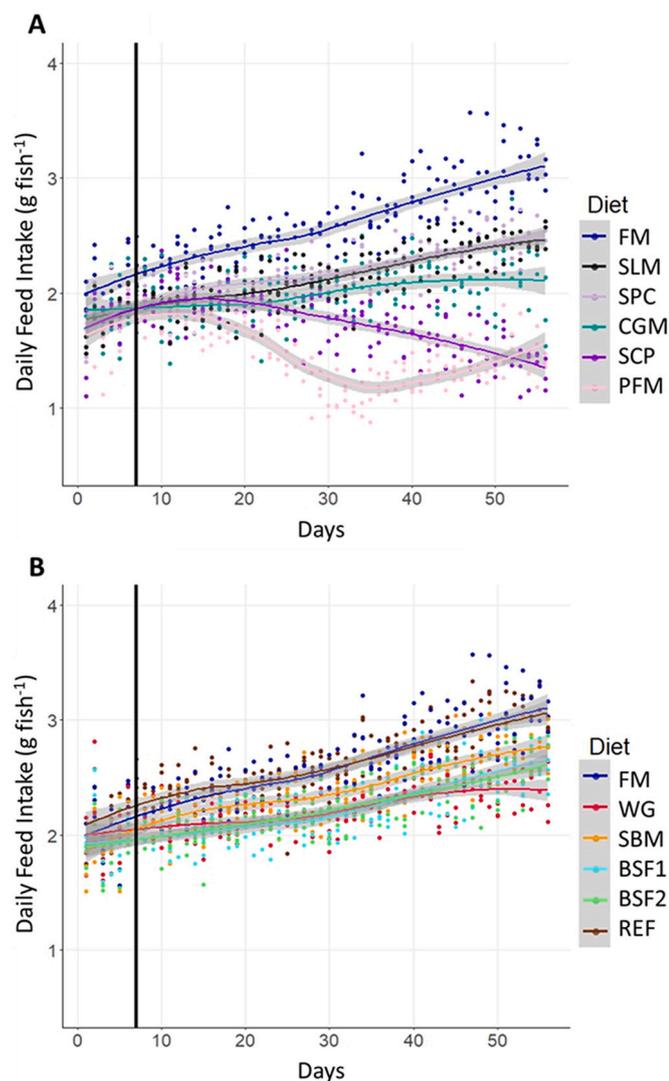


Fig. 1. Daily feed intake (g fish⁻¹) of the reference diet and 10 test diets over 56 days (n = 3). (A) Diets with daily feed intake that differed from diet FM on Day 7. (B) Diets with daily feed intake similar to diet FM on Day 7. Locally estimated scatterplot smoothing, Loess are plotted using formula $y \sim x$ and confidence interval level of 0.95. The black vertical line represents day 7 of the trial. FM: fish meal; WG: wheat gluten; SBM: soybean meal; SPC: soy protein concentrate; CGM: corn gluten meal; BSF1 and BSF2: black soldier fly larvae meal 1 and 2; PFM: poultry feather meal, SLM: squid liver meal, REF: reference.

of the trials' diets were plotted, and diets SPC, CGM, SCP, PFM, and SLM had significantly lower daily feed intake than diet FM at Day 7, based on 95 % confidence interval level (Fig. 1). These observations were further confirmed when the initial feeding response for the first seven days (IFR; %BW day⁻¹) was calculated, and significant differences were also observed in IFR between those diets and the FM diet (Table 4).

After Day 7, the feeding responses of the diets were evaluated by comparing the linear regression slopes from Day 8 to Day 56 (Table 5). This allowed for an independent comparison of the feeding rate (regression slope) of the diets, which, to some extent, excluded the effect of fish size. The feeding response after Day 7 of FM was significantly higher than all the diets, including the REF diet. Compared to the REF diet, the feeding responses of SBM, BSF1, BSF2 and SLM diets were similar ($P > 0.05$). On the contrary, the feeding responses of WG, SPC, CGM, SCP and PFM were significantly lower than REF diet ($P < 0.05$, Table 5). Notably, the feeding responses of SCP and PFM were negative compared to the rest of the diets.

3.2. Nutrient composition and bioavailability of test ingredients

The nutrition compositions varied between test ingredients (Table 3), which influenced the nutrient profile of the test diets when included at either 28.4 % or 30 % (Table 2). Crude protein (CP) levels in the test ingredients ranged from 49.9 % in SBM to 93.1 % in PFM, while crude fat (CF) content ranged from 2.5 % in SPC to 16.7 % in SLM (Table 3). These variations in nutrient composition resulted in CP levels in the test diets ranging from 52.9 % (SBM) to 66.7 % (PFM) and CF levels ranging from 11.0 % (SPC) to 14.9 % (SLM) (Table 2).

The apparent digestibility coefficient (ADC) of proximate composition and amino acid for the test ingredients were compared against FM (Table 6). Ingredient protein and energy ADC values of BSF1, BSF2, PFM and SLM were significantly lower than FM ($P < 0.05$). Separately, PFM fat ADC value was lower than FM ($P < 0.05$, Table 6). The amino acid ADC values are also heterogeneous between the test ingredients. All the ADC values of PFM and SLM amino acids were significantly lower compared to FM ($P < 0.05$). The extremely low levels of amino acid ADC of SLM (from 0 % to 20.3 %) were likely biased towards amino acids that were not measured (e.g., tryptophan) or contain other forms of nitrogen unbound to amino acids (i.e., histamine or ammonia) since only 31.6 % of the CP measured were amino acids (Table 3). The amino acids of plant-based ingredients (i.e., WG, SBM, SPC, and CGM) were highly digestible, with ADC values of over 90 %, similar to FM. The only exceptions were CGM's lysine and threonine ADC values, which were significantly lower ($P < 0.05$). Between the two BSF larvae meals, BSF1 had lower digestibility for all the amino acids except lysine, methionine and cysteine compared to FM ($P > 0.05$), whereas BSF2 had similar amino acid ADC values compared to FM for all amino acids except arginine, cysteine and alanine ($P < 0.05$). SCP amino acids were also

Table 5
Feeding response (%BW day⁻¹) from Day 8 to Day 56 after feeding REF and 10 test diets.

Calculation	REF	FM	WG	SBM	SPC	CGM	SCP	BSF1	BSF2	PFM	SLM
slope	0.016	0.019	0.009	0.014	0.011	0.006	-0.013	0.015	0.013	-0.010	0.013
n	147	147	147	147	147	147	147	147	147	147	147
SE(reg)	0.196	0.206	0.152	0.183	0.144	0.215	0.180	0.189	0.160	0.245	0.157
SE(slope)	0.0011	0.0012	0.0009	0.0011	0.0008	0.0013	0.0011	0.0011	0.0009	0.0014	0.0009
Against REF											
difference (slope)	-	-0.0035	0.0071	0.0018	0.0044	0.0097	0.0287	0.0005	0.0023	0.0261	0.0028
SE(difference)	-	0.0017	0.0014	0.0016	0.0014	0.0017	0.0016	0.0016	0.0015	0.0018	0.0015
t-stat	-	2.09 [†]	4.90	1.16	3.07	5.71	18.46	0.34	1.58	14.25	1.93
df	-	292	292	292	292	292	292	292	292	292	292
P-value	-	0.038	<0.001	0.246	0.002	<0.001	<0.001	0.732	0.115	<0.001	0.055
Against FM											
difference (slope)	0.0035	-	0.0106	0.0053	0.0078	0.0132	0.0321	0.0040	0.0058	0.0295	0.0063
SE(difference)	0.0017	-	0.0015	0.0016	0.0015	0.0017	0.0016	0.0016	0.0015	0.0019	0.0015
t-stat	2.09	-	7.07	3.29	5.34	7.57	20.13	2.46	3.81	15.83	4.16
df	292	-	292	292	292	292	292	292	292	292	292
P-value	0.038	-	<0.001	0.001	<0.001	<0.001	<0.001	0.014	<0.001	<0.001	<0.001

[†] Values were multiplied by -1.

Table 6
Macronutrient, energy and amino acid apparent digestibility coefficient of 10 test ingredients, including FM for Malabar snapper (*Lutjanus malabaricus*).

ADC [†] (%)	Test ingredients										Pooled SEM	P-value [‡]
	FM	WG	SBM	SPC	CGM	SCP	BSF1	BSF2	PFM	SLM		
Nutrient and energy												
DM	94.8	96.8	81.8	67.9*	84.9	100.0	79.6	83.7	64.3*	68.9*	1.31	0.009
CP	97.1	99.4	94.7	95.5	93.7	92.0	84.4**	89.8*	71.5**	68.1**	0.42	0.001
CF	98.6	100.0	100.0	100.0	77.6	92.3	95.5	97.2	57.2*	92.6	0.91	0.001
Carb	49.3	100.0	74	48.7	95.3	89.5	33.3	49.9	100.0*	23.7	5.11	0.013
GE (kJ.g ⁻¹)	98.9	100.0	89.6	86.3	91.9	91.7	79.7*	84.8*	69.3**	69.5**	0.60	0.002
Essential and conditional amino acids												
Arginine	98.4	99.7	97.6	98.6	94.2	93.3	74.8*	85.0*	74.3*	12.6**	49.6	0.001
Cysteine	97.2	100.0	95.7	98.1	94.2	94.6	88.1	78.2*	55.4*	12.4**	67.4	0.001
Histidine	99.3	99.8	97.1	98.5	94.7	92.7	88.6*	94.0	64.5**	0.0***	46.8	0.001
Isoleucine	98.8	100.0	94.5	96.5	93.2	94.3	89.0*	95.4	72.8**	3.9***	43.2	0.002
Leucine	98.7	100.0	93.8	96.0	96.2	92.2*	88.0*	95.1	70.7**	0.0***	44.9	0.001
Lysine	98.9	100.0	97.2	98.7	90.9*	99.0	96.6	95.9	55.5*	0.0**	73.6	0.005
Methionine	97.5	100.0	94.9	97.4	94.9	90.1	90.0	98.0	68.8*	0.0**	50.9	0.001
Phenylalanine	97.9	100.0	94.6	97.1	95.6	87.6*	89.3*	96.1	71.9**	5.3**	41.8	0.001
Threonine	98.9	98.7	93.1	96.1	92.2*	94.1	89.8**	95.2	67.3**	0.0***	45.5	0.002
Tyrosine	98.5	100.0	95.8	98.3	95.8	90.1*	90.2*	95.8	70.7**	3.4**	42.9	0.001
Valine	98.7	99.9	93.5	96.1	93.0	95.6	88.7*	94.5	73.2**	7.6***	42.7	0.002
Non-essential amino acids												
Alanine	98.3	98.6	92.6	95.3	95.5	93.4	81.4**	86.4*	69.7**	9.3***	43.1	0.001
Aspartic Acid	98.0	99.2	96.6	98.9	93.9	97.4	91.9*	97.3	69.5**	0.0**	45.3	0.005
Glutamic Acid	98.7	100.0	97.1	99.2	96.8	98.0	89.8*	95.7	70.8*	9.1**	41.4	0.001
Glycine	96.5	94.6	92.5	94.3	93.2	93.3	79.2*	90.9	76.0**	0.0**	62.0	0.008
Proline	97.0	100.0	96.2	97.7	96.6	95.5	87.9	93.9	73.9*	20.3**	41.7	0.002
Serine	97.7	100.0	95.5	97.7	95.4	92.1	89.4*	95.4	73.6**	0.0**	41.1	0.001

Dunn's Test, significant mean values ($n = 3$) different from FM are indicated by ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

DM: Dry matter; CP: Crude protein; CF: Crude fat; Carb: Carbohydrate; GE: gross energy.

[†] ADC values are mean. ADC values less than 0 % are rounded to 0 %, and values above 100 % are rounded to 100 % for practical reasons.

[‡] P-values are obtained from Kruskal-Wallis one-way ANOVA.

highly digestible, with ADC over 90 % for all amino acids, except phenylalanine, which had a slightly lower ADC of 87.6 % (Table 6).

The digestible amino acid values (% DP in DM) of the ten test ingredients were calculated, and the similarity of the test ingredients' amino acid profiles could be visualised in the principal component analysis (PCA) plot (Fig. 2 and Table 7). The top three components encompass 94.1 % of the total variance. From the PCA plot, SBM, SPC, BSF1, BSF2 and SCP clustered closer to FM, indicating their digestible amino acid profile resembled FM's. In contrast, WG, CGM and PFM clustered further away from FM, indicating the disparateness of their digestible amino acid profile compared to FM's. The similarities and dissimilarities of the test ingredients' digestible amino acid profiles to FM's are supported by the range of R² values between 0.20 and 0.90, in which SPC has the closest profile resembling FM (Table 7). Focusing on digestible essential amino acid (% DP, in DM), the proportion of each

test ingredient's value relative to FM was visualised using FM's value as a benchmark (Fig. 3). Digestible lysine and digestible methionine with cysteine (Met + Cys) of all the test ingredients were lower than FM except CGM, which had a higher content of Met + Cys and leucine. The digestible essential amino acids of SLM were mostly absent. Compared to FM, digestible histidine was limiting in PFM, whereas the proportion of all essential amino acids was lower in WG, except Phe + Tyr. Among the same ingredient types with different origins, slight differences were observed. BSF1 exhibited lower digestible threonine, whereas BSF2 had a higher tyrosine content when compared to FM ($P < 0.05$). Similarly, subtle differences between the digestible essential amino acids of SBM and SPC were observed. SPC had significantly higher digestible phenylalanine than FM, while SBM contained significantly lower digestible threonine than FM ($P < 0.05$, Table 7).

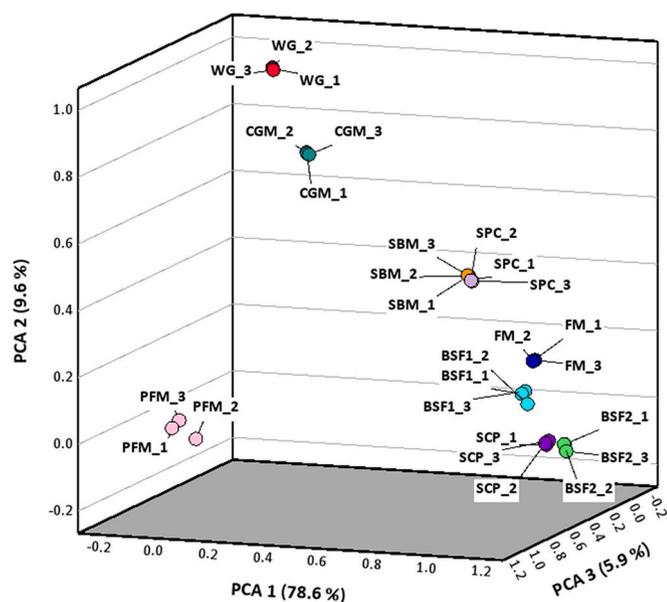


Fig. 2. Principal component analysis (PCA) of digestible amino acid (DAA) profile (% DP) of nine test ingredients, including FM ($n = 3$). From the PCA plot, 94.1 % of the total variance could be explained by the top three components. SLM was excluded from this PCA plot. FM: fish meal, WG: wheat gluten, SBM: soybean meal, SPC: soy protein concentrate, CGM: corn gluten meal, BSF1 and BSF2: black soldier fly larvae meal 1 and 2, PFM: poultry feather meal, SLM: squid liver meal.

3.3. Evaluation of test ingredients based on quality and cost-effectiveness

The quality of test ingredients was assessed using indexes developed for digestible protein cost, initial feeding response and growth (Fig. 4a, b, c). Across all ingredients, FM had the highest growth index, but its digestible protein cost index was significantly lower than those of SBM, SPC, CGM, and SCP. In contrast, its initial feeding response index was not significantly different from WG, SBM, BSF1 and BSF2. Focusing on the digestible protein cost index, CGM had the highest value, indicating the lowest digestible protein cost because the index is an inverted

normalised value of digestible protein cost. Although BSF2 was the most expensive, its digestible protein cost was similar to SLM and BSF1 and significantly different compared to other ingredients. The initial feeding response index revealed that PFM had the lowest value, though it was not significantly different from SPC, CGM, SCP, and SLM ($P > 0.05$). Growth index values were the lowest for PFM and SCP among all the ingredients. These wide variabilities of the ingredient indexes highlighted the complexity of evaluating ingredient quality comprehensively. Hence, the cost efficiency index (CEI) was developed to integrate these metrics into a single value. Based on CEI, SBM, FM, and CGM demonstrated the highest cost efficiency, while SLM, SCP, PFM, SPC, BSF1 and BSF2 had significantly lower CEI values (Fig. 4d).

Correlations between diet effects, ingredient productivity values, and their respective digestible nutrients were analysed (Fig. 5). Initial feeding responses for diet and ingredient were highly correlated with their TGC and TGC productive value, respectively. Interestingly, digestible protein and fat levels did not significantly influence early feed intake or growth. However, strong associations were observed between growth parameters and digestible levels of histidine, lysine and methionine ($P < 0.05$).

4. Discussion

4.1. Development of quality indexes for ingredient cost-benefit analysis

The value of an ingredient lies in the digestibility and bioavailability of its nutrients, making it essential to evaluate the nutritional value of an ingredient based on its digestible nutrient content (Glencross et al., 2007). The apparent digestibility coefficient of ingredients (ADCI) is a widely used metric, calculated as described by Bureau et al. (1999), and has been adapted to estimate ingredient nutrient productive value (Hardy and Brezas, 2021; Salehi et al., 2023). The ingenuity of the equation enabled the theoretical nutrient contributions of both the reference diet and the test ingredient to be calculated (Bureau and Hua, 2006). This allowed the representative test ingredient values to be comparable across studies and between ingredients under fixed assumptions and known caveats to interpret those values (Glencross et al., 2007; Glencross, 2009).

On the contrary, parameters resulting from feed utilisation, such as initial feeding response and TGC values, are measured outcomes from

Table 7
Digestible amino acid profile of 10 test ingredients (% DP⁻¹; DM).

Digestible amino acid (% DP ⁻¹ ; DM)	Test ingredients										Pooled SEM	P-value [†]
	FM	WG	SBM	SPC	CGM	SCP	BSF1	BSF2	PFM	SLM		
Essential and conditional amino acids												
Arginine	6.3	3.3	7.8	8.0	3.8	6.2	5.3	5.9	7.5	0.3*	0.20	<0.001
Cysteine	0.9	1.9	1.4	1.3	1.8	0.6	0.5	0.7	2.1	0.1	0.21	<0.001
Histidine	2.6	2.0	2.7	2.8	2.2	2.0	3.0	3.7	0.7	0.0*	0.06	<0.001
Isoleucine	4.7	3.7	4.9	5.1	4.3	4.4	4.4	4.8	5.4	0.1*	0.10	<0.001
Leucine	8.0	6.7	7.9	8.2	17.4	7.2	6.7	7.3	8.5	0.0*	0.14	<0.001
Lysine	8.0	1.3*	5.6	5.9	1.6*	5.6	5.4*	6.3	1.4*	0.0*	0.19	<0.001
Methionine	3.3	1.6*	1.6*	1.6*	2.7	2.3	1.9	2.3	0.8*	0.0*	0.04	<0.001
Phenylalanine	4.2	5.0	5.3	5.6*	6.8*	3.9	4.3	4.9	5.2	0.1	0.09	<0.001
Threonine	4.6	2.4*	4.0*	4.2	3.6*	4.3	4.0*	4.2	4.3	0.0*	0.09	0.001
Tyrosine	3.6	3.6	4.1	4.1	5.8	3.5	6.9	7.9*	3.5	0.1	0.12	<0.001
Valine	5.6	3.8	5.0	5.1	4.6	6.7	6.4	6.6	7.8	0.2*	0.13	<0.001
Non-essential amino acids												
Alanine	6.6	2.4*	4.5*	4.7	9.5	7.0	6.6	6.2	4.9	0.3*	0.12	<0.001
Aspartic Acid	9.9	3.1	12.1	12.9	6.7	8.8	10	11.4	6.8	0.1*	0.27	<0.001
Glutamic Acid	13.9	36.1	18.7	19.5	23	10.5	12.3	11.4	11.1	0.7*	0.40	<0.001
Glycine	6.3	3.1*	4.3*	4.5	3.1*	5.0	5.3	5.4	9.1	0.0*	0.39	<0.001
Proline	4.1	12.3*	5.3	5.4	9.7*	3.7	5.5	5.3	11.0*	0.6	0.19	<0.001
Serine	4.3	4.4	5.1	5.4	5.4	3.2	4.0	4.1	10.9*	0.0	0.14	<0.001
Correlation (R²)												
R ² (with FM)	1.00	0.44	0.85	0.85	0.50	0.90	0.81	0.76	0.24	0.20		

Dunn’s test, significant mean values ($n = 3$) different from FM are indicated by ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

[†] P-value are obtained from Kruskal-Wallis one-way ANOVA.

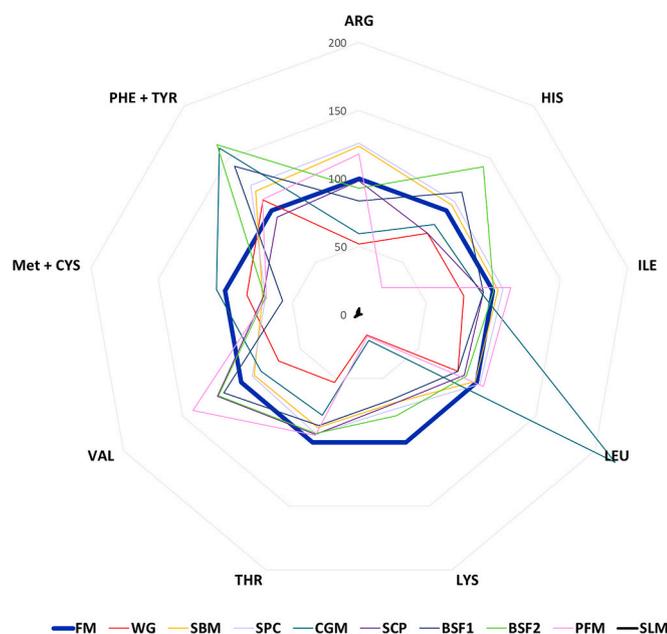


Fig. 3. Relative proportions of digestible essential amino acid profile (% DP) of nine test ingredients relative to FM's profile (% DP) ($n = 3$). The digestible essential amino acid values of each ingredient were calculated by dividing by the respective mean values of FM $\times 100$. The digestible essential amino acid values of FM are represented by the dark blue line at 100%. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

consuming diets and hence reflect the effects of the diets rather than the specific contributions of test ingredients. Consequently, those values are unsuitable for evaluating ingredient effects, especially when the test diets contain different proportions of test ingredients. To resolve this, ADC_i equation was modified to calculate the ingredient parameter's productive value. Calculating the productive values of the test diets and the reference diet is essential to determine the proportional difference between the test diets and reference diet, which are used to calculate ingredient productive values. The ingredient productive values calculated would be representative of the test ingredient effects, independent of the contribution of the reference diet. An ingredient quality could be evaluated not only by its cost and digestible nutrient content but also by its palatability and effects on fish after utilisation (Glencross et al., 2007). Palatability, which reflects the acceptability of taste and the likelihood of being consumed, is typically quantified by measuring dietary feed intake response using nutritionally balanced diets (Jobling et al., 1995). However, dietary palatability assessment or inference using isonitrogenous and isoenergetic diets with varying inclusion levels of a test ingredient or between different ingredients might be inconclusive since the inclusion level of other ingredients in the diet would be changed (e.g., lower FM content) to balance dietary nutrients and energy content, which can affect overall palatability and dietary feed intake (Arndt et al., 1999; Al-Souti et al., 2019; Karapanagiotidis et al., 2023; Samsing et al., 2024). In a gilthead seabream (*Sparus aurata*) study, a test diet containing 22.5% feather meal dietary level had higher palatability than a control diet without feather meal, but the higher palatability effect could equally be attributed to 13% SBM reduction in the test diet (Al-Souti et al., 2019). Furthermore, an ingredient's effect on palatability, such as defatted BSF, could be masked if the inclusion level of the test ingredient was too low. For example, in another gilthead seabream study, defatted BSF inclusion up to 10% had no significant impact on feed intake, but feed intake decreased when the diet contained 17.4% defatted BSF (Karapanagiotidis et al., 2023). The reduction in feed intake could also be explained by lower dietary inclusion levels of FM and wheat flour, highlighting the limitations of inferring

ingredient palatability based on a 'balanced nutrient' approach.

This study evaluated the test ingredient's effect on early feed response alongside an *in vivo* digestibility assay using the diet replacement method. This protocol was suggested by Barrows et al. (2017), who had adapted it from Glencross et al. (2007). The protocol was modified to assess feeding response after Day 7 rather than two weeks, strengthening the assumption that any observed differences in initial feeding response were driven by ingredient gustation rather than growth-related factors. The adjustment also aligned with the 7-day acclimation period allocated for fish to adjust to the experimental conditions before faecal collection. Significant changes in initial feeding response indexes were assumed to be proxies for changes in ingredient palatability or early onset of other factors affecting feed intake levels. Evaluating ingredient quality without balancing dietary nutrient content would also be advantageous in quantifying the ingredient quality in its 'as is' form. This was similarly applicable to the growth index, such that the observable effects would be caused solely by the test ingredient (i.e., nutrient and energy deficiency) without any influential effect contributed by other factors (i.e., nutrient supplementation). The strong correlation between TFI and TGC ($R^2 = 0.9218$, $P < 0.05$) highlights the interplay between feed intake and growth. However, this study did not intend to differentiate the effect of nutrient deficiency on these two parameters, which should be discussed together. Rather, a restricted pair-feeding design would be needed to segregate the effect of feed intake and growth, and further studies are warranted (Suresh et al., 2011; Vikeså et al., 2017).

The complexity of an ingredient quality could be visualised when the indexes of digestible protein cost, initial feeding response, and growth performance of the ingredients were compared (Fig. 4). Characterisation of ingredients based on their monetary value of digestible protein is useful for comparing ingredient cost and bioavailable nutrients at face value. However, it does not represent the 'true' ingredient quality. Based on the digestible protein cost in this study, SCP, PFM, and plant proteins (except WG) are more economical than FM, while BSF2 is the most expensive of the test ingredients evaluated. Given that SCP's digestible amino acid profile aligned closely with FM, selecting SCP as a viable alternative to replace FM would be intrinsic but misleading. This is because the initial feeding response and growth indexes place SCP among the lowest ranks and are not significantly different from PFM. The discrepancies in quality indexes highlighted the need for a holistic index to assess the cost-benefits of ingredients. Leveraging on the quality indexes, the 'cost-efficiency index' or CEI, was developed by assigning a contribution factor to each quality index. The assumptions and caveats of interpreting CEI will be discussed further in Section 4.4.

4.2. Interpretation of quality indexes and CEI for cost-benefit analysis

The CEI values were used to compare the cost-benefits of ten aqua-feed ingredients by integrating the quality indexes of digestible protein cost, initial feeding response, and growth, along with their respective contribution factors. Based on these assessments, SBM, CGM and WG were identified as suitable replacements for FM, while SPC and other ingredients (SCP, PFM, SLM, BSF1, and BSF2) exhibited significantly lower cost benefits than FM (Fig. 4). Among the soy-based ingredients, the SBM and SPC had nearly identical nutritional profiles but were limited by methionine deficiencies. SBM and SPC are known to contain antinutritional factors such as trypsin inhibitors, saponins and phytic acids (Kumar et al., 2020). Despite the nutritive limitations of soy-based products, their competitive pricing outweighs their potential adverse effects, making them potential alternatives to FM (Hardy, 2010; Kumar et al., 2020). However, SBM was more cost-effective than SPC, as SPC's 14.3% higher digestible protein content failed to justify its USD 0.5 kg^{-1} higher price, reducing its overall cost efficiency. Similarly, CGM is another excellent plant protein alternative as it contains few antinutritional factors but is known for being deficient in tryptophan compared to FM (Hernández et al., 2021; Islam et al., 2024). Although CGM's

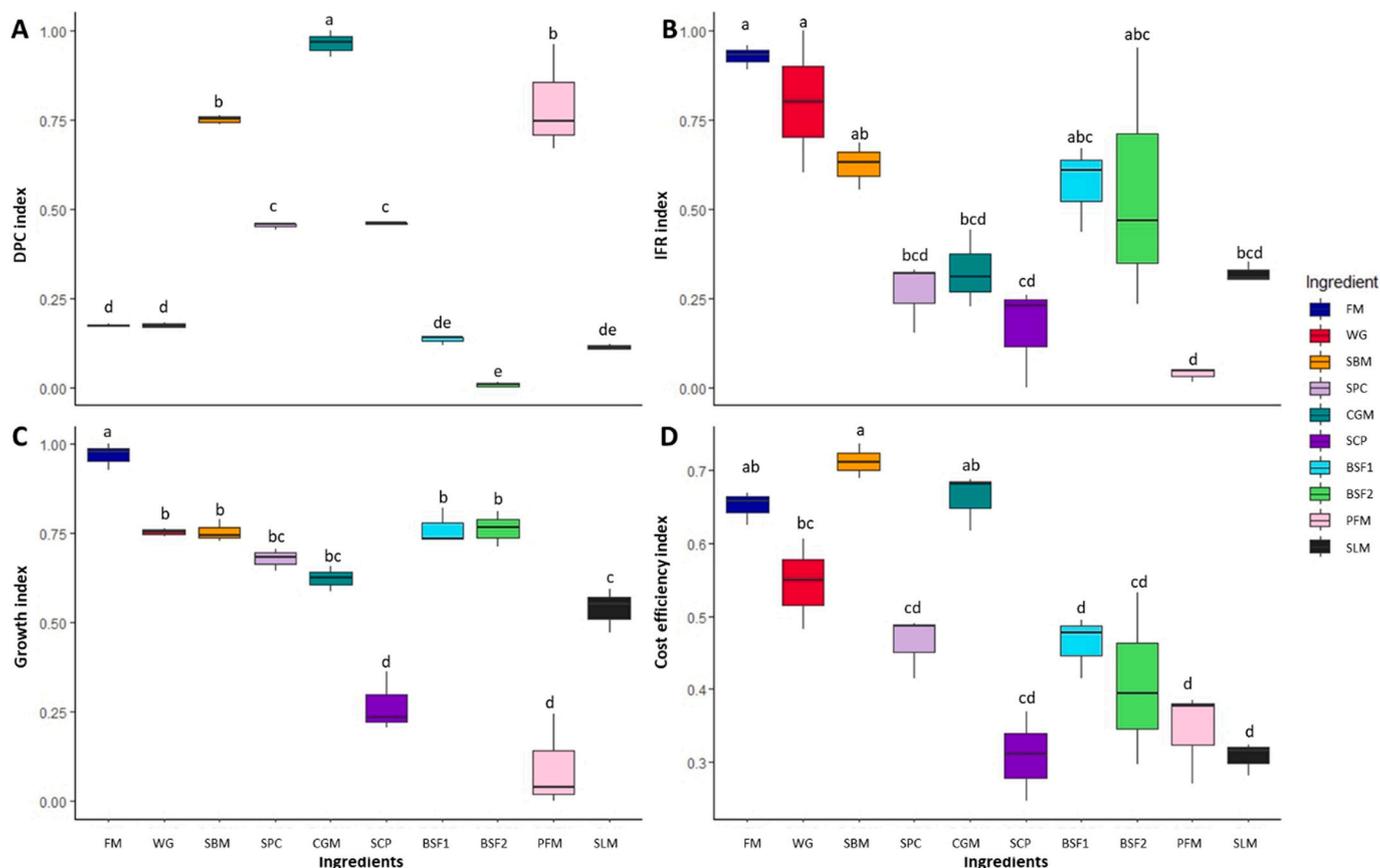


Fig. 4. Quality indexes of test ingredients (n = 3). (A) Digestible protein cost index (*DPCI*) of ingredients, (B) initial feeding response index (*IFRI*) of ingredients, (C) growth index (*GI*) of ingredients, and (D) cost efficiency index (*CEI*) of ingredients. *DPCI_i* is calculated by normalising $1/DPC_i$, *IFRI_i* is calculated by normalising $IFRPV_i$, *GI_i* is calculated by normalising $TGCPV_i$, *CEI* is calculated based on C_{DPCI_i} , C_{IFRI_i} and C_{GI_i} as 1, 0.8056 and 0.80417, respectively. The mean values lacking common superscripted letters differ significantly (Tukey HSD, $P < 0.05$). Error bars indicate a confidence level of 95 %. FM: fish meal; WG: wheat gluten; SBM: soybean meal; SPC: soy protein concentrate; CGM: corn gluten meal; BSF1 and BSF2: black soldier fly larvae meal 1 and 2; PFM: poultry feather meal, SLM: squid liver meal.

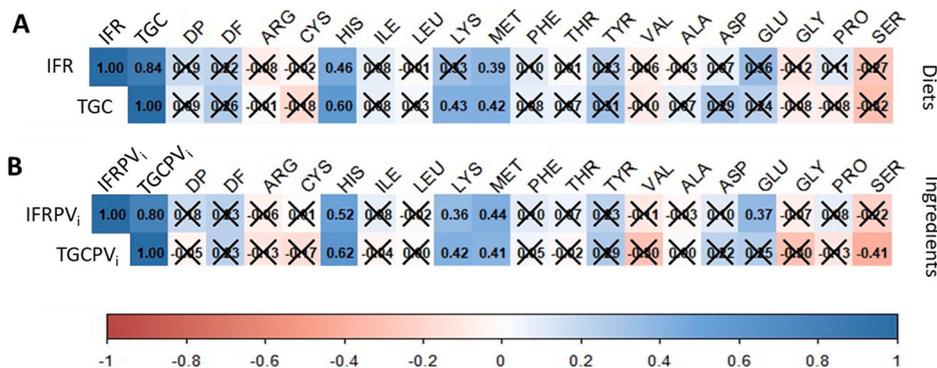


Fig. 5. Association of diets and ingredients with their respective digestible nutrients. (A) Correlation of dietary *IFR* at Day 7 (%BW day⁻¹) and *TGC* with dietary digestible nutrients and digestible amino acids. Correlation was performed with all test diets without REF. (B) Correlation of ingredient *IFRPV_i* and *TGCPV_i* with ingredient digestible nutrients and digestible amino acids content. Pearson correlation coefficient ‘R’ values were shown in the text within the coloured box. The correlation strength was illustrated by the colour tone in which the red represented negative correlations, and the blue represented positive correlations. Coefficient values that are not significant ($P > 0.05$) were marked with a ‘cross’. *IFR*: initial feeding response at Day 7; *IFRPV_i*: ingredient initial feeding response productive value; *TGC*: thermal growth coefficient; *TGCPV_i*: ingredient thermal growth coefficient productive value; *DP*: digestible protein; *DF*: digestible fat. REF: reference diet. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

digestible amino acid profile distinctly differed from FM’s, lacking digestible arginine, lysine, and methionine content (Figs. 2 and 3). Its low cost also made it a viable alternative ingredient, comparable to SBM in cost efficiency. However, unlike SBM, the feeding response after Day 7 of CGM was significantly lower than REF diet, highlighting the need

for the CGM’s inclusion level to be optimised. Separately, WG contained the highest digestible protein content of 85.7 % among the four plant proteins investigated in this study. WG is practically devoid of anti-nutritional factors and has been proposed as a suitable FM alternative by Apper et al. (2013). The imbalanced amino acid profile (EAA: NEAA =

3.5: 6.5) of WG, comprising mostly glutamic acid and lacking lysine, necessitates EAA supplementation when replacing FM with WG. In a rainbow trout study, WG could replace up to 50 % of FM's dietary protein in a diet that contained 64 % FM with proper supplementation of fish oil and essential amino acids (Storebakken et al., 2015). However, a reduced growth rate was also reported in Japanese seabass (*Lateolabrax japonicus*) after FM was replaced by WG when dietary FM content was below 20 % (Zhang et al., 2023). Although both WG and SBM required supplementation of EAA to replace FM, the digestible protein cost of WG was similar to FM, which made it a less cost-efficient aquafeed ingredient than SBM.

Animal by-product meals, such as hydrolysed PFM and SLM are promising candidates since they contain more than 50 % protein (Table 2), and their digestible proteins are more economical than FM (Fig. 3). However, the reduced feed intake, negative feeding responses after Day 7 and slower growth might be explained by the lower digestible lysine, methionine and histidine content in PFM (Campos et al., 2017; Jasour et al., 2017). The recommended dietary inclusion level of PFM seldom exceeds 20 %, regardless of preprocessing methods by either fermentation by *Bacillus subtilis*, alkaline treatment, or hydrolysis (Hasan et al., 1997; Al-Souti et al., 2019; Adelina et al., 2020). Hence, the negative feeding response post Day 7 PFM was expected for PFM and the dietary inclusion level of PFM should be optimised for Malabar snapper. SLM has been used as a feed attractant but contains high levels of heavy metal cadmium, which can bioaccumulate in fish, impairing liver and kidney function (Jang et al., 2021). Interestingly, most of the crude protein (measured nitrogen) of the batch of SLM tested in this study were not amino acids since only 31.6 % (CP) of the total crude protein were amino acids (excluding tryptophan, which was not tested). The possibility of an analytical error could be dismissed since retests were performed, indicating that this batch of SLM contained other forms of nitrogen that were not bound to amino acids (i.e., ammonia). A study by Cho et al. (2005) evaluated squid liver meal™, which composed of 60 % SLM and 40 % SBM, and contained 50.8 % (CP) of EAA. Hence, there is a potential risk that the batch of SLM used in this was adulterated, and the results should be cautiously interpreted (Tangendjaja, 2022). Despite SLM's lack of digestible amino acids in this study, significantly higher growth and initial feeding response indexes than PFM and SCP were observed. Furthermore, the feeding response after Day 7 of SLM was not significantly different from REF diet. These supported that SLM at 30 % dietary inclusion had positive palatability effects, validating its function as a feed attractant (Xue and Cui, 2001; Nunes et al., 2006).

Emerging alternative proteins such as BSF and SCP from microbial protein utilising methane are touted as excellent feed sources since they contain high crude protein and have an excellent essential amino acid profile similar to FM (Barroso et al., 2014; Ritala et al., 2017; Hua et al., 2019; Jones et al., 2020). The prices of BSF1 and SCP are competitively lower than those of FM, which supports them as potential FM alternatives (Supplementary Table 1). However, the presence of chitin in BSF might explain the lower protein digestibility and reduced growth performance in this study since fish's ability to digest chitin remains ambiguous (Rust, 2002; Henry et al., 2015). Separately, high inclusion of SCP above 10 % in diet was known to cause palatability issues in some studies, which could explain SCP's lower feed intake and negative feeding response after Day 7 in this study (Jones et al., 2020; Samsing et al., 2024). The mechanism of SCP causing reduction in feeding response remained speculative. Some have suggested the presence of antinutritional factors in SCP, including high content of nucleic acids and the presence of cell wall or allergenic compound, might affect feed intake (Sharif et al., 2021; Pereira et al., 2022). While others have tested dietary additives such as fish hydrolysate and garlic extract to improve SCP's palatability with varying success, depending on fish species (Pilmer et al., 2022; Woolley et al., 2023). Significant differences in digestible amino acid profiles were observed between the two BSF meals in this study compared to FM, indicating higher nutritional value for

BSF2. However, since no significant growth and feed intake improvements of BSF2 over BSF1, further justifications are needed for the higher BSF2 price. The variance in nutritional quality in BSF larvae meals may be attributed to the types of feed substrates the insects were reared on or the processing methods used to separate insect oil from protein (Henry et al., 2015; Magalhães et al., 2017). Many of these emerging proteins are still optimising and upscaling their processes. Thus, it is important to consider that the ingredient quality only reflected the batch of meals used in this study and should not represent the quality of the whole industry.

4.3. Initial feeding response and growth are associated with histidine, lysine and methionine levels

Assessing the quality of multiple ingredients without balancing the dietary nutrients can provide valuable insights by simultaneously testing a wide range of digestible nutrient levels. The strong correlation between initial feeding response and growth indexes supports the phenomenon of early feed intake, which is potentially driven by palatability, contributing to fish growth (Morais, 2017). However, it remains unclear whether nutrient deficiencies in test ingredients reduced dietary feed intake and palatability, leading to poor growth, or whether poor growth caused reduced feed intake. A restricted pair-feeding strategy would be required to disentangle these effects, warranting further investigation. However, TGC showed a weaker correlation with initial feeding response than TFI, and therefore, the decision to evaluate ingredient cost-effectiveness using early feed intake of the first seven days was appropriate and a better fit in this context than TFI.

The initial feeding response and growth index differences were likely influenced by nutrient imbalance in the test ingredients and diets. Focusing on digestible essential amino acids of the ingredient, digestible histidine, lysine and methionine were identified to correlate significantly with the ingredient effects on initial feeding response and TGC (Fig. 5). Although correlation does not imply causation, lysine and methionine are widely recognised as the most limiting amino acids in FM alternatives, with deficiencies known to hinder growth performance and feed intake (Hardy, 2010; Hussain et al., 2024). The different ingredients investigated in this study resulted in a range of dietary histidine and digestible histidine from 0.96 to 1.61 %DM and 0.93 to 1.54 %DM, respectively. In other species, significant improvement in feed intake but not growth rate was observed in yellowtail (*Seriola quinqueradiata*) fingerlings when dietary histidine was increased to 1.9 %DM when supplemented with L-histidine HCl (Yamamoto et al., 2019), and the recommended digestible histidine was 1.6 to 2.0 %DM in yellowtail kingfish (*S. lalandi*) (Booth and Pirozzi, 2022). Given the relatively high dietary histidine requirement observed in yellowtail species, future studies should aim to determine the specific amino acid requirements for Malabar snapper, especially histidine, lysine, and methionine, and balance these dietary amino acids when FM alternatives are used in formulations. In addition to the amino acids investigated in this study, taurine is also increasingly recognised in marine diets to improve fish growth performance since plant-based ingredients generally lack taurine (Takagi et al., 2008; Takagi et al., 2011; Salze and Davis, 2015; Adeshina and Abdel-Tawwab, 2020; Kotzamanis et al., 2020). In other snappers, the absence of taurine supplementation in diets containing more than 30 % SBM had reduced weight gain and SGR, highlighting the importance of studying taurine requirement in Malabar snapper too (Hernandez et al., 2018; Walsh et al., 2021).

4.4. Assumptions used to interpret quality and cost-effective indexes

In this study, the CEI values were compared with the assumption that the differences in the FOB and EXW prices of the ingredients are negligible. This scenario is unlikely, and the prices of FOB are usually greater than EXW since suppliers offering FOB have to cover the costs of transportation, handling and customs clearance (Sources, 2024).

Furthermore, changes in feeding rates or differences in test ingredient inclusion levels have been assumed not to affect digestibility (Cho and Kaushik, 1990; Glencross, 2009). However, those assumptions might be misguided since ingredients with higher carbohydrate content have lower ADC_i when fed at higher feeding levels (Elesho et al., 2021). Lower protein digestibility has also been reported with increasing SBM dietary inclusion levels (Appleford and Anderson, 1997). Fish's ability to digest an ingredient depends on the amounts of digestive enzymes secreted from the stomach and pancreas, and its feeding habit (Bakke et al., 2010; Jiao et al., 2023). Changes in dietary protein levels or different sources of protein could have varying stimulatory effects on digestive enzyme activity (Santos et al., 2020; Murashita et al., 2022). Therefore, an ingredient with poor or no digestion-stimulating effects could still be digested in the presence of other ingredients (e.g., FM) within the diet. So, the digestibility of an ingredient could decrease at higher test ingredient inclusion levels since the FM levels in the diet were lowered correspondingly. In addition, the composition and the ratio of the reference diet and test ingredients in digestible trials also affect the ingredient digestibility coefficient.

The ADC_i had been investigated by including between 20 % to 50 % of test ingredients, with 30 % being the most used inclusion level (Glencross, 2009; Rahman et al., 2023; Barreto et al., 2024). The practical inclusion level of any ingredient is typically lower in commercial feed formulation to mitigate known risks associated with being heavily reliant on a single ingredient (Glencross, 2016). Some studies suggested that ingredient digestibility should be evaluated at commercial inclusion levels or at a range of inclusion levels to study the interactions between ingredient and reference formulation (Allan et al., 2000; Smith and Tabrett, 2004). Despite the various caveats of ADC_i assessment, the merits of assessing the effects of ingredient growth performance and feed utilisation concurrently with *in vivo* ingredient digestibility trials are conspicuous and should be conducted. Although the suggestions to investigate ingredients at commercial inclusion levels or different ranges were reasonable and warranted further studies, it is also important to consider that no significant difference in feed intake or growth when small amounts of an ingredient are added does not necessarily indicate that they are appropriate raw materials; rather, our ability to detect minute differences may be limited.

5. Conclusion

This study characterised the nutritional qualities of ten aquafeed ingredients by evaluating their digestible nutrients, growth performance and feed utilisation using the 70:30 diet replacement method. A cost-effective index was developed to comprehensively assess ingredient cost-benefit by taking into account ingredient digestible protein cost, initial feeding response and growth performance. Among the tested ingredients, FM was the only one that supported growth performance equivalent to the reference diet and exhibited the highest feeding response after Day 7. SBM, CGM, and FM were identified as the most cost-efficient ingredients. However, the low feeding response of CGM highlighted the need for its inclusion level to be optimised. The positive correlation between ingredients' initial feeding response and TGC productive values with digestible histidine, lysine and methionine highlights the critical role of these amino acids in improving feed intake and growth, and further studies are warranted to determine their requirement. The nutritional values and cost-efficiency index derived from this study provide valuable insights for formulating sustainable and cost-effective diets for Malabar snapper, contributing to a sustainable aquaculture industry for this fish species.

Ethical statements

All procedures related to the handling of fish were conducted in compliance with the guidelines using animals set by the National Advisory Committee on Laboratory Animal Research (NACLAR) for the

care and use of animals for scientific purposes in Singapore and were approved by James Cook University Singapore's Institutional Animal Care and Use Committee under ethics approval number: 2022-A017.

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CRedit authorship contribution statement

Si Yan Ngoh: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Xueyan Shen:** Writing – review & editing, Funding acquisition. **Leo Nankervis:** Writing – review & editing. **Isaiah Chia:** Writing – review & editing, Investigation. **Katheline Hua:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2025.742253>.

Data availability

Data will be made available from the corresponding author upon reasonable request.

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