

Evaluation of anchovy fishery by-catch meal as a partial fishmeal replacement in diets for juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂)

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Abstract. This study evaluated the effects of replacing 30% of fishmeal (FM) protein with fishery by-catch meal (FBCM) sourced from a local anchovy-processing facility in Sabah, Malaysia, in diets for juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂). The proximate composition, amino acid profile, fatty acid profile, and mineral composition of FBCM were compared with those of FM. Two isoproteic (50% crude protein) and isolipidic (12% crude lipid) diets were formulated, namely FM100, containing 100% of dietary protein from FM, and FMFBCM, in which 30% of FM protein was replaced with FBCM protein. Juvenile hybrid grouper (body weight: 16.50±0.05 g) were fed the experimental diets for eight weeks. FBCM contained significantly higher ($p < 0.05$) ash content and significantly lower ($p < 0.05$) crude protein and crude lipid contents than FM. However, the amino acid and fatty acid profiles did not differ significantly between FM and FBCM, whereas FBCM contained significantly higher levels of macrominerals than FM. Fish fed the FMFBCM diet showed comparable growth performance, feed utilization, survival, body condition indices, and whole-body proximate composition to those fed the FM100 diet. Under the conditions of this eight-week feeding trial, replacement of 30% of FM protein with FBCM protein was tolerated by juvenile hybrid grouper without significant differences in the measured growth, feed utilization, survival, body condition, and whole-body proximate composition endpoints. Further studies evaluating higher replacement levels, longer feeding periods, and additional physiological and health-related indicators are warranted.
Keywords: alternative protein, fishery by-products, fishmeal replacement, growth performance, marine carnivorous fish.

Introduction. Fisheries have long played a vital role in supporting the global food supply and ensuring food security. Nevertheless, fishery by-catch, defined as non-target species or individuals captured during fishing operations, has become a major concern for both the fishing industry and the marine environment. These unwanted catches are often disposed of as waste through landfilling, ocean dumping, or incineration, resulting in a significant loss of resource value and adverse impacts on marine ecosystems (Haque et al., 2026). According to records from the Food and Agriculture Organization (FAO), the annual volume of discards from global marine capture fisheries has been estimated at 9.1 million tonnes, representing approximately 10.8% of the average annual catch between 2010 and 2014 (Pérez Roda et al., 2019). In Sabah, Malaysia, by-catch has long been a persistent issue in the local anchovy fishing industry, with a substantial proportion being

discarded as waste through incineration or sea dumping (Liew, personal communication with the local fishermen). Although by-catch cannot be entirely avoided, its effective utilization as a resource could represent a practical and worthwhile solution.

Aquaculture has expanded rapidly in recent decades as a strategy to meet the growing demand for aquatic products while alleviating fishing pressure on wild stocks. Global aquaculture production reached 130.9 million tonnes in 2022, valued at USD 313 billion, accounting for 59% of total fisheries and aquaculture production (FAO, 2024). Notably, more than 70% of global aquaculture production depends on the supply of externally provided feed inputs (Tacon & Metian, 2015). In aquaculture, feed costs represent the largest operational expenditure, accounting for approximately 75-90% of total production costs (Hidir et al., 2026). This high cost is largely attributed to the reliance on expensive protein-rich ingredients, particularly fishmeal (FM), which remains the primary protein source in aquafeeds due to its excellent nutritional profile, high palatability, and superior digestibility (Hussain et al., 2024). However, the continued reliance on FM raises concerns regarding sustainability and resource availability, highlighting the urgent need for alternative protein sources.

Among cultured marine fish species, groupers (genus *Epinephelus*) are considered highly valuable because of their desirable flesh quality and strong market demand. In 2006, successful hybridization between tiger grouper (*Epinephelus fuscoguttatus*) and giant grouper (*E. lanceolatus*) was achieved at Universiti Malaysia Sabah, producing a hybrid (hereafter referred to as TGGG) characterized by rapid growth, improved survival, and the absence of deformities compared with its parental species (Ch'ng & Senoo, 2008). Since then, TGGG has become an important species in grouper aquaculture and is widely consumed in seafood markets (Shapawi et al., 2019). As a carnivorous species, TGGG requires relatively high-protein diets, for which FM has traditionally been an important protein ingredient (Shapawi et al., 2019). The reliance on FM can contribute to increased feed costs and concerns regarding the sustainability of feed resources. Consequently, identifying suitable alternative protein ingredients that can partially replace FM while maintaining adequate nutrient supply and fish performance is of particular interest for grouper aquaculture.

In this context, fishery by-catch represents a potentially valuable but underutilized protein resource for aquaculture feed production. However, the nutritional suitability of by-catch-derived meal may be influenced by its heterogeneous species composition and the inclusion of non-target materials, which can result in variability in its biochemical composition. In particular, a relatively high ash content may reduce the concentration of protein and energy-yielding nutrients in the meal and may therefore limit its capacity to replace FM on a protein basis (Asimi et al., 2023). Despite this potential, limited information is available on the nutritional characteristics of heterogeneous fishery by-catch meal (FBCM) derived from anchovy-processing operations and its suitability for partial FM protein replacement in juvenile hybrid grouper diets. Thus, it remains unclear whether such a variable by-catch-derived ingredient can provide sufficient nutrients to support growth and nutrient utilization when incorporated into the diet.

Therefore, the present study had two objectives. First, the proximate composition, amino acid profile, fatty acid profile, and mineral composition of anchovy FBCM were characterized and compared with those of FM to identify potential nutritional similarities and limitations. Second, an eight-week feeding trial was conducted to evaluate the effects of replacing 30% of FM protein with FBCM protein on the growth performance, feed utilization, survival, body condition indices, and whole-body proximate composition of juvenile hybrid grouper. The 30% replacement level was selected as a moderate partial replacement level to assess the suitability of FBCM as a dietary protein ingredient while limiting the potential nutritional effects associated with its heterogeneous composition and relatively high mineral fraction. It was hypothesized that FBCM would differ from FM in proximate and mineral composition but would provide broadly comparable amino acid and fatty acid profiles, while partial replacement of 30% of FM protein with FBCM protein would not adversely affect growth performance, feed utilization, survival, body condition, or whole-body proximate composition under the experimental conditions tested.

Material and Method

Preparation of fishery by-catch meal. Sun-dried fishery by-catch (FBC) was obtained from a local anchovy-processing facility in Kimanis, Sabah, in June 2024. The anchovy and associated by-catch were harvested using 'bagang' in the coastal waters of Kimanis and subsequently sun-dried at the processing facility. The 'bagang' is a lift-net fishing method operated at night from bamboo or wooden platforms constructed in nearshore waters, where artificial lights are used to attract fish before they are captured using lift nets. The collected FBC samples were transported to the laboratory at the Borneo Marine Institute, Universiti Malaysia Sabah, for processing. Upon arrival, the FBC was first screened to remove foreign materials, including coral fragments, rocks, seashells, and other debris. Visual inspection for species identification indicated that the FBC comprised a mixture of more than 23 by-catch species, including mackerels, breams, scads, sardines, and other fish species, together with small amounts of squid, shrimp, and crab (detailed species composition reported in Liew et al., in press). Following screening, the fish specimens were collectively oven-dried at 45°C for 16 h to remove excess moisture. The dried materials were then ground into a fine powder, sieved, and homogenized to produce FBCM, which was stored at −20°C until further use.

Preparation of experimental diets. Two isoproteic (crude protein: 50%) and isolipidic (crude lipid: 12%) pelleted diets were formulated using FM and FBCM. The control diet was designated as FM100 (FM served as the sole protein source). The treatment diet was designated as FMFBCM (30% of the FM protein was replaced by FBCM). The formulations of the experimental diets are presented in Table 1.

Table 1

Formulation and proximate composition of the experimental diets

Ingredient (g 100 g^{-1})	*FM100*	*FMFBCM*
Fishmeal[a]	72.01	50.41
Fishery by-catch meal[b]	0.00	23.31
Dietary lipid[c]	8.86	9.31
Alpha cellulose[d]	0.40	0.40
Tapioca starch[e]	10.73	8.57
CMC[f]	1.50	1.50
Vitamin premix[g]	3.00	3.00
Mineral premix[h]	2.00	2.00
DCP[i]	1.00	1.00
Chromic oxide[j]	0.50	0.50
Total	100.00	100.00
Proximate analysis		
Crude protein (% dry matter)	50.97±0.38	49.64±0.04
Crude lipid (% dry matter)	12.25±0.06	12.37±0.04
Moisture (%)	8.40±0.05	8.86±0.04
Ash (% dry matter)	17.30±0.06	19.10±0.10

[a] Local fish meal, Sandakan Fishmeal Sdn. Bhd., Sabah, Malaysia; Crude protein 69.43%; Crude lipid 7.14%; [b] Self-produced; Crude protein 64.35%; Crude lipid 4.66%; [c] Salmon fish oil, Hi-Chem Supplies Sdn. Bhd., Sabah, Malaysia; [d] Sigma-Aldrich® brand; [e] Cap Kapal ABC brand, Myxo Company Sdn. Bhd., Sabah, Malaysia; [f] Carboxymethyl cellulose, DChemie Malaysia Sdn. Bhd., Johor, Malaysia; [g] Vitamin premix: Vitamin A, 20 miu kg^{-1}; Vitamin D3, 4 miu kg^{-1}; Vitamin E, 52 g kg^{-1}; Vitamin K3, 4 g kg^{-1}; Vitamin B1, 4 g kg^{-1}; Vitamin B2, 10 g kg^{-1}; Vitamin B6, 6.4 g kg^{-1}; Vitamin B12, 40 mg kg^{-1}; Biotin, 200 mg kg^{-1}; Folic acid, 3.2 g kg^{-1}; Niacin, 80 g kg^{-1}; Pantothenic acid, 22.4 g kg^{-1}; Nutri Vet Livestock Sdn. Bhd., Negeri Sembilan, Malaysia; [h] Mineral premix (g kg^{-1} mixture): Zinc, 30; Manganese, 20; Ferrous, 80; Copper, 4; Iodine, 1.5; Selenium, 0.125; Cobalt, 0.15; Magnesium, 50; Nutri Vet Livestock Sdn. Bhd., Negeri Sembilan, Malaysia; [i] Dicalcium phosphate, R & M Chemicals brand; [j] HmbG Chemicals brand.

The diet formulation was adapted from previous studies with slight modifications (Shapawi et al., 2013, 2014). To prepare the diets, all ingredients were thoroughly mixed, and water was gradually added to form a homogeneous dough. The dough was

then extruded through a meat mincer fitted with a 3 mm die to produce continuous feed strands. The strands were collected and oven-dried at 45°C for 6 h. After cooling, the dried strands were manually broken into smaller pellets (approximately 5 mm in length), sealed in plastic bags, and stored at −20°C until further use.

Experimental fish and rearing conditions. Juvenile hybrid groupers were procured from a local fish farm and transferred to the Wet Laboratory of the Borneo Marine Institute, Universiti Malaysia Sabah. The fish were acclimatized for two weeks in a 1,000 L fiberglass tank. During the acclimation period, the fish were fed control diet (FM100) twice daily at 0900 and 1500 h. The tank bottom was cleaned daily by siphoning out feces, and approximately 50% of the water was exchanged to maintain the water quality.

After the acclimation period, 10 fish were randomly selected for the determination of initial whole-body proximate composition. The remaining fish were individually weighed, and a total of 108 fish (initial body weight: 16.50±0.05 g) were randomly distributed into six fiberglass tanks (60 cm length × 60 cm width × 40 cm height; approximately 150 L capacity) at a stocking density of 18 fish per tank. All tanks were supplied with continuous aeration and a flow-through seawater system. The feeding trial was conducted for eight weeks, during which each experimental diet was assigned to triplicate tanks.

The experimental procedures were adapted from Liew et al. (2026). To ensure optimal culture conditions, water quality parameters were monitored daily in each tank using a YSI Professional Plus multiparameter meter and maintained within the following ranges: temperature, 25.5-30.3°C; salinity, 29.77-33.74‰; dissolved oxygen, 5.06-5.83 mg L^{-1}; and pH, 7.35-7.75. Fish were hand-fed twice daily at 0800 and 1400 h to apparent satiation. Feed intake was estimated by collecting uneaten pellets after each feeding session (each feeding session lasts approximately 30 min), counting the number of remaining pellets, and multiplying the number by the average pellet weight (approximately 0.05 g). Feces and uneaten feed were siphoned out daily before feeding and after each feeding session. Mortalities were recorded and dead fish were removed promptly throughout the experiment. Fish were bulk-weighed every two weeks to monitor growth performance. At the end of the feeding trial, all fish were individually weighed. Subsequently, one fish was randomly sampled from each replicate tank with each dietary treatment (n = 3 per treatment) for determination of hepatosomatic index (HSI), viscerosomatic index (VSI), and whole-body proximate composition, with all samples analyzed individually. Based on the data collected, growth performance and feed utilization parameters, including weight gain (WG), specific growth rate (SGR), survival rate, feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), net protein utilization (NPU), condition factor (CF), HSI, and VSI, were calculated using the formulas as reported in Liew et al. (2026):

1. WG (%) = (Final – Initial fish weight) / Initial fish weight × 100
2. SGR (% d^{-1}) = [ln (Final fish weight) – ln (Initial fish weight)] / days × 100
3. Survival (%) = (Final number of fish / Initial number of fish) × 100
4. FI (g $fish^{-1}$) = (Total given feed – Total uneaten feed) / number of fish
5. FCR = Dry feed consumed / Wet weight gain
6. PER = Wet weight gain / Total protein intake
7. NPU = (Final – Initial fish body protein) / Total protein intake × 100
8. CF = (Final fish weight (g) / [Total length (cm)3] × 100
9. HSI = [Liver weight (g) / Fish weight (g)] × 100
10. VSI = [Viscera weight (g) / Fish weight (g)] × 100

All statistical analyses were performed using tank as the experimental unit, with three replicate tanks per dietary treatment (n = 3), and were based on tank-level replication rather than individual fish.

Analysis of proximate composition. The proximate composition of protein meals and experimental diets was determined using standard methods of the Association of Official Analytical Chemists (AOAC, 1990) with slight modifications based on the specifications and operating settings of the laboratory equipment. Moisture content was determined by drying samples to a constant weight at 105°C. Crude protein was analyzed using Kjeldahl method (Kjeltec 2300 system, FOSS, Denmark), while crude lipid was determined by ether extraction method using a Soxtec™ 2043 system (FOSS, Sweden). Ash content was measured by incineration in a muffle furnace at 550°C for 5 h.

Analysis of amino acid composition. High-performance liquid chromatography (HPLC) was used to determine the amino acid profile of the protein ingredients. Samples were hydrolyzed with 6 M HCl at 110°C for 24 h in sealed tubes. After hydrolysis, the samples were cooled, filtered through a 0.2 μm membrane filter, and diluted with distilled water to obtain solutions suitable for analysis. The amino acids were derivatized using o-phthaldialdehyde (OPA) prior to separation on an HPLC system equipped with an AdvanceBio AAA column (4.6 × 100 mm, 2.7 μm particle size; Agilent Technologies). Detection was performed using fluorescence at the appropriate excitation and emission wavelengths. Amino acid concentrations were quantified by comparing peak areas with those of known standards. All analyses were conducted in triplicate to ensure accuracy.

Analysis of fatty acid composition. Gas chromatography with flame ionization detection (GC-FID) was used to determine the fatty acid profile of the protein ingredients. Samples were first digested with hydrochloric acid (HCl), followed by lipid extraction using diethyl ether. The extracted lipids were then subjected to methylation to convert fatty acids into fatty acid methyl esters (FAMEs) before analyzed by GC-FID. All measurements were performed in triplicate to ensure accuracy.

Analysis of mineral composition. Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the mineral composition of the protein ingredients. Samples were digested using heavy metal-free nitric acid and heated for 30 min at a temperature not exceeding 120°C. The digested solution was allowed to cool and subsequently rinsed with two aliquots of 15 mL ultra-purified water. The solution was then filtered and diluted to volume in a 50 mL volumetric flask. Finally, the prepared samples were analyzed using ICP-MS to quantify mineral composition. All measurements were performed in triplicate to ensure accuracy.

Statistical analyses. All statistical analyses were performed using IBM SPSS Statistics version 26. Prior to analysis, data expressed as ratios and percentages were subjected to arcsine transformation. The normality of residuals and homogeneity of variances were verified using the Shapiro–Wilk and Levene's tests, respectively. When these assumptions were met, data were analyzed using independent t-test. Otherwise, the non-parametric Mann-Whitney U test was applied. Differences were considered statistically significant at $p < 0.05$.

Results

Proximate composition of fishmeal and fishery by-catch meal. The proximate composition of the two protein meals is presented in Table 2. FM exhibited a significantly higher ($p < 0.05$) crude protein content (69.43%) compared to FBCM (64.35%). Similarly, the crude lipid content of FM (7.14%) was significantly higher ($p < 0.05$) than that of FBCM (4.66%). No significant difference ($p > 0.05$) was observed in the moisture content between FM (6.90%) and FBCM (6.96%). In contrast, FBCM contained a significantly higher ($p < 0.05$) ash content (27.30%) than FM (19.60%).

Table 2

Proximate composition of fishmeal (FM) and fishery by-catch meal (FBCM)

Nutritional composition	*FM*	*FBCM*
Crude protein (% dry matter)	69.43±0.12[a]	64.35±0.44[b]
Crude lipid (% dry matter)	7.14±0.08[a]	4.66±0.13[b]
Moisture (%)	6.90±0.04	6.96±0.07
Ash (% dry matter)	19.60±0.10[a]	27.30±0.10[b]

*Values are presented as means±standard deviation; means in the same row with different superscripts show significantly different ($p < 0.05$) among each other.

Amino acid composition of fishmeal and fishery by-catch meal. The amino acid composition of FM and FBCM is shown in Table 3. The amino acid profiles of FM and FBCM did not differ significantly from one another ($p > 0.05$). All amino acids detected in FM were also found in FBCM, although their concentrations varied between the two ingredients. FBCM exhibited higher levels of lysine (4.34%), methionine (2.01%), threonine (2.12%), tryptophan (1.49%), trans-4-Hydroxy-Proline (1.67%), alanine (1.64%), aspartic acid (4.76%), glutamic acid (7.20%), proline (6.04%), and serine (2.12%) compared to FM, which contained 3.59%, 0.75%, 1.44%, 1.19%, 1.62%, 1.63%, 4.27%, 7.07%, 5.06%, and 2.10%, respectively. However, all these differences were not statistically significant ($p > 0.05$).

Table 3

Amino acid composition of fishmeal and fishery by-catch meal (% dry matter)

Amino acids	*FM*	*FBCM*
Essential amino acid		
Valine	1.46±0.01	1.02±0.01
Arginine	9.49±0.01	7.04±0.01
Histidine	4.80±0.01	4.67±0.01
Isoleucine	1.07±0.01	0.74±0.01
Leucine	4.13±0.01	3.62±0.01
Lysine	3.59±0.01	4.34±0.01
Methionine	0.75±0.01	2.01±0.01
Phenylalanine	2.37±0.01	1.71±0.01
Threonine	1.44±0.01	2.12±0.01
Tryptophan	1.19±0.01	1.49±0.01
Non-essential amino acid		
trans-4-Hydroxy-Proline	1.62±0.01	1.67±0.01
Alanine	1.63±0.01	1.64±0.01
Aspartic acid	4.27±0.01	4.76±0.01
Cystine	2.82±0.01	1.23±0.01
Glutamic acid	7.07±0.01	7.20±0.01
Glutamine	0.61±0.01	0.47±0.01
Glycine	5.14±0.01	4.98±0.01
Proline	5.06±0.01	6.04±0.01
Serine	2.10±0.01	2.12±0.01
Tyrosine	3.45±0.01	2.76±0.01

*Values are presented as means±standard deviation.

Fatty acid composition of fishmeal and fishery by-catch meal. Table 4 presents the fatty acid composition of FM and FBCM. No significant differences were observed in the fatty acid profiles between FM and FBCM ($p > 0.05$). However, the fatty acid levels in FBCM were generally lower than those in FM. Furthermore, arachidic acid (0.1%) and behenic acid (0.1%) were detected in FM but were absent in FBCM.

Table 4

Fatty acid composition of fishmeal and fishery by-catch meal (% dry matter)

Fatty acids	*FM*	*FBCM*
Essential fatty acid		
Linoleic acid (C18:2n6c)	0.1±0.1	0.1±0.0
Arachidic acid (C20:0)	0.1±0.1	-
Eicosapentaenoic acid EPA (C20:5n3)	0.5±0.1	0.3±0.0
Docosahexaenoic acid DHA (C22:6n3)	0.9±0.1	0.7±0.1
Non-essential fatty acid		
Myristic acid (C14:0)	0.6±0.1	0.3±0.0
Palmitic acid (C16:0)	2.2±0.1	1.6±0.1
Palmitoleic acid (C16:1)	0.6±0.0	0.3±0.0
Stearic acid (C18:0)	0.9±0.0	0.8±0.0
Oleic acid (C18:1n9c)	0.8±0.1	0.5±0.1
Behenic acid (C22:0)	0.1±0.1	-
Tricosanoic acid (C23:0)	0.3±0.1	0.1±0.1

*Values are presented as means±standard deviation; (-) indicates not detected (less than 0.1 g/100 g).

Mineral composition of fishmeal and fishery by-catch meal. The mineral composition of the two protein meals is presented in Table 5. In general, FBCM contained significantly higher levels ($p < 0.05$) of macrominerals, including magnesium, calcium, sodium, potassium, phosphorus, and chloride, compared to FM. However, no significant difference ($p > 0.05$) was observed in sulphur content between FM (1.10%) and FBCM (0.90%). For microminerals, FBCM contained significantly higher levels ($p < 0.05$) of manganese (23.14 mg kg^{-1}) and zinc (62.72 mg kg^{-1}) than FM, which contained 20.46 mg kg^{-1} and 54.61 mg kg^{-1}, respectively. In contrast, FM exhibited significantly higher levels ($p < 0.05$) of iron (179.73 mg kg^{-1}) and copper (2.20 mg kg^{-1}) compared to FBCM, which contained 124.33 mg kg^{-1} iron and 1.73 mg kg^{-1} copper. Furthermore, chromium (0.78 mg kg^{-1}) was detected in FM but was not detected in FBCM.

Table 5

Mineral composition of fishmeal and fishery by-catch meal

Minerals	*FM*	*FBCM*
Macrominerals (mg kg^{-1})		
Magnesium	2954.05±95.68^{a}	3314.67±43.19^{b}
Calcium	6583.85±0.44^{a}	10352.47±62.90^{b}
Sodium	13267.82±94.90^{a}	50587.83±196.09^{b}
Potassium	6546.44±6.38 ^{a}	14126.60±85.36^{b}
Phosphorus	27426.99±182.66^{a}	52290.13±127.21^{b}
Chloride	171.23±1.45^{a}	484.30±0.30^{b}
¤Sulphur (%)	1.10±0.10	0.90±0.00
Microminerals (mg kg^{-1})		
Manganese	20.46±0.17^{a}	23.14±0.07^{b}
Iron	179.73±0.87^{a}	124.33±0.34^{b}
Copper	2.20±0.03^{a}	1.73±0.05^{b}
Zinc	54.61±0.31^{a}	62.72±0.13^{b}
Chromium	0.78±0.01	-

*Values are presented as means±standard deviation; All minerals were presented in mg kg^{-1}, except for ¤Sulphur presented in %; means in the same row with different superscripts show significantly different ($p < 0.05$) among each other; (-) indicates not detected (less than 0.01 mg kg^{-1}).

Growth performance, feed utilization, body condition, and whole-body proximate composition of fish. The growth performance, feed utilization, body condition, and whole-body proximate composition of hybrid grouper juveniles fed different experimental diets are summarized in Table 6. Fish fed the FM100 diet exhibited slightly higher growth performance, in terms of final body weight (42.05 g $fish^{-1}$), WG (154.49%), and SGR (1.66% day^{-1}), compared with those fed the FMFBCM diet (40.88 g $fish^{-1}$, 147.15%, and 1.61% day^{-1}, respectively), although these differences were not

significant ($p > 0.05$). In contrast, survival rate was lower in fish fed the FM100 diet (57.41%) than in those fed the FMFBCM diet (72.22%), but this difference was also not significant ($p > 0.05$). For feed utilization, fish fed the FMFBCM diet showed higher FI (34.78 g fish^{-1}) and a higher feed conversion ratio (FCR; 1.43) compared with those fed the FM100 diet (32.65 g fish^{-1} and 1.29, respectively), although these differences were not significant ($p > 0.05$). In contrast, the PER was higher in fish fed the FM100 diet (1.52) than in those fed the FMFBCM diet (1.41), but again without significant difference ($p > 0.05$). Notably, NPU was significantly higher ($p < 0.05$) in fish fed FM100 (21.35) compared with those fed FMFBCM (17.41). CF, HSI, and VSI were not affected by dietary treatments ($p > 0.05$). Similarly, whole-body proximate composition analysis revealed no significant differences ($p > 0.05$) between treatments in crude protein (61.29% and 61.66%), crude lipid (14.90% and 14.23%), moisture (75.56% and 77.28%), and ash (15.13% and 16.95%) contents for fish fed the FM100 and FMFBCM diets, respectively.

Table 6

Growth performance, feed utilization, body condition and whole-body proximate composition of hybrid grouper juveniles fed FM100 and FMFBCM diets

Parameters	*Initial*	*FM100*	*FMFBCM*
	Growth performance		
IBW (g fish^{-1})	-	16.52±0.08	16.54±0.06
FBW (g fish^{-1})	-	42.05±4.83	40.88±2.03
WG (%)	-	154.49±28.36	147.15±11.90
SGR (% day^{-1})	-	1.66±0.21	1.61±0.09
Survival (%)	-	57.41±11.57	72.22±16.67
	Feed utilization		
FI (g fish^{-1})	-	32.65±3.65	34.78±0.61
FCR	-	1.29±0.11	1.43±0.09
PER	-	1.52±0.13	1.41±0.09
NPU	-	21.35±1.35^{a}	17.41±1.32^{b}
	Body condition		
CF	-	1.99±0.12	1.99±0.02
HSI	-	0.99±0.16	0.90±0.25
VSI	-	8.40±1.54	8.09±1.22
	Whole-body proximate composition		
Crude protein (% dry matter)	58.92±0.69	61.29±1.86	61.66±1.17
Crude lipid (% dry matter)	16.78±0.43	14.90±1.98	14.23±0.92
Moisture (%)	72.06±0.49	75.56±1.29	77.28±0.41
Ash (% dry matter)	18.01±0.98	15.13±1.41	16.95±1.88

*Values are presented as means±standard deviation; means in the same row with different superscripts show significantly different ($p < 0.05$) among each other; (-) indicates not applicable.

Discussion. The nutritional characteristics of FBCM indicate both its potential and its principal limitation as a partial FM replacement ingredient. Although FBCM provided a substantial amount of crude protein (64.35%) and crude lipid (4.66%), both values were lower than those of FM (69.43% and 7.14%, respectively), while its ash content was markedly higher. The elevated ash content is consistent with the mineral-rich nature of FBCM and is likely related to its heterogeneous composition, which included different fish species and other by-catch materials. High ash levels can reduce the proportion of protein and energy-yielding nutrients in an ingredient and may also limit nutrient utilization when incorporated at higher dietary levels. Previous studies have similarly identified high ash content as a constraint on the use of animal-derived meals as FM substitutes (Asimi et al., 2023; Barreto et al., 2024; Saromines et al., 2025). Thus, the relatively high ash content of FBCM represents an important nutritional consideration when determining its inclusion level in aquafeeds.

Despite differences in proximate composition, the amino acid profiles of FBCM and FM were broadly comparable. The presence of the essential amino acids detected in FM in

FBCM suggests that the protein fraction of the heterogeneous by-catch retained a nutritional profile suitable for partial dietary replacement of FM. The comparable levels of lysine, methionine, threonine, and other essential amino acids are particularly relevant because these amino acids play important roles in tissue synthesis and growth and may become limiting when conventional protein ingredients are replaced (He et al., 2013; Aragão et al., 2025; Liaqat et al., 2025). However, amino acid concentration alone does not establish protein quality, as digestibility and amino acid availability were not determined in the present study. Therefore, the comparable amino acid profiles support the nutritional suitability of FBCM for the replacement level evaluated, but further digestibility studies are required to determine the extent to which its amino acids are available to the fish.

The fatty acid profiles of FBCM and FM were likewise broadly comparable, although the lower lipid content of FBCM resulted in numerically lower concentrations of several fatty acids. Importantly, FBCM retained long-chain polyunsaturated fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are nutritionally important for marine fish such as the groupers, as they possess limited ability to biosynthesize long-chain PUFAs from precursor fatty acids (Li et al., 2014; Xie et al., 2020). The presence of these fatty acids indicates that FBCM can contribute to the dietary supply of essential long-chain fatty acids when used as a partial FM replacement. Nevertheless, the present data do not establish whether FBCM can independently satisfy the quantitative fatty acid requirements of juvenile hybrid grouper, particularly at higher replacement levels. The comparable fish performance observed at the replacement level tested therefore suggests that the lower lipid and fatty acid concentrations of FBCM were not sufficiently restrictive under the present dietary formulation and experimental conditions.

The mineral profile provides further evidence that the heterogeneous composition and processing history of FBC influenced its nutritional characteristics. FBCM contained higher concentrations of several macrominerals than FM, including magnesium, calcium, sodium, potassium, phosphorus, and chloride. The elevated calcium and phosphorus concentrations may contribute to meeting the mineral requirements of marine organisms, particularly crustaceans such as crabs and shrimps, which require substantial amounts of minerals for exoskeleton formation and molting processes (He et al., 2025; Chen et al., 2026). In fish, *Epinephelus* groupers generally require approximately 6 g kg^{-1} of dietary calcium and phosphorus for optimal growth and skeletal development (Ye et al., 2006). Thus, the relatively high mineral content of FBCM may provide nutritional benefits; however, the contribution of individual minerals should be considered carefully because excessive levels of certain minerals may be undesirable in formulated diets.

In particular, the substantially higher sodium and chloride concentrations in FBCM are likely associated with the salted-water cooking step used during anchovy processing. This processing-related salt contribution is important because excessive dietary sodium and chloride may become a constraint when FBCM is included at higher levels, even though moderate dietary salt supplementation (approximately 1-4.5%) has been reported to benefit growth and feed utilization in some cultured fish, including salmonids and Asian seabass, *Lates calcarifer* (Harpaz et al., 2005; Salman, 2009; Tavares-Dias, 2021). The mineral profile of FBCM should be considered together with its processing history when assessing its suitability as an aquafeed ingredient. Processing strategies such as desalination may help reduce excessive salt concentrations, whereas methods such as de-boning or selective removal of high-ash materials could potentially reduce the overall mineral fraction (Mohd Nor et al., 2025).

The feeding trial provides evidence that the nutritional differences between FBCM and FM did not translate into significant differences in growth performance or the other measured biological endpoints at the replacement level evaluated. The comparable final body weight, WG, SGR, CF, HSI, VSI, survival, and whole-body proximate composition indicate that the FMFBCM diet was nutritionally adequate for juvenile hybrid grouper under the present experimental conditions. This response is consistent with the broadly comparable amino acid and fatty acid profiles of the two protein ingredients, suggesting that the nutrient composition of FBCM was sufficient to support normal growth when

incorporated at 30% FM protein replacement. The absence of a significant difference in survival further supports the lack of an apparent adverse effect of FBCM at the replacement level tested, although the slightly lower survival observed in the FM100 treatment warrants consideration of other factors that may have contributed to mortality. The slightly lower survival observed in the FM100 treatment was believed not considered to be diet-related, as mortality was accompanied by visible external injuries and aggressive interactions were frequently observed among size-variant fish. Such behaviour has been reported in grouper culture, particularly under conditions of size variation and social dominance (Lim et al., 2015).

The absence of significant differences should nevertheless be interpreted within the scope of the experimental design. Only one replacement level was evaluated, and the feeding period was limited to eight weeks. Consequently, the present results demonstrate tolerance to the tested level rather than establishing the maximum replacement level or the suitability of FBCM for broader FM replacement. The heterogeneous composition and relatively high ash and salt contents of FBCM may become increasingly important at higher inclusion levels. Evaluation of graded replacement levels, together with nutrient digestibility and physiological or health-related indicators, would therefore be necessary to determine the upper practical inclusion level of FBCM.

Although most feed utilization parameters did not differ significantly between treatments, the numerical differences observed among FCR, PER, and NPU may indicate some influence of the different ingredient composition on nutrient utilization. The higher ash content of the FMFBCM diet provides one possible explanation, as elevated dietary ash has been associated with reduced dry matter digestibility and nutrient utilization in fish (Salehi et al., 2023; Prakash et al., 2024). However, this interpretation should be treated cautiously because nutrient digestibility was not measured in the present study. The lack of significant differences in growth performance despite these numerical changes suggests that any effect of the higher ash content was insufficient to impair growth at the replacement level tested. Reducing the ash fraction of FBCM through appropriate pre-treatment may nevertheless improve its nutritional value and provide greater scope for its use at higher replacement levels.

Conclusions. Fishery by-catch meal (FBCM) can be considered as a partial fishmeal (FM) protein replacement ingredient in diets for juvenile hybrid grouper. Replacement of 30% of FM protein with FBCM did not result in statistically detectable differences in most measured growth performance, feed utilization, survival, body condition indices, or whole-body proximate composition parameters under the conditions of the present eight-week feeding trial. However, net protein utilization (NPU) was significantly lower in fish fed the FMFBCM diet, indicating that the replacement may have affected protein utilization despite the absence of statistically detectable differences in most other measured endpoints. The relatively high ash and mineral contents of FBCM should therefore be considered when evaluating its use as a dietary ingredient, particularly at higher replacement levels. The possible contribution of elevated dietary ash to the lower NPU remains a hypothesis, as nutrient digestibility and retention were not determined in the present study. Further studies involving graded FBCM replacement levels, longer feeding periods, and measurements of nutrient digestibility and retention are required to determine the nutritional limitations and practical upper inclusion level of FBCM. Nevertheless, the present findings indicate that 30% replacement of FM protein with FBCM did not produce statistically detectable impairment in most measured performance and body composition endpoints under the experimental conditions tested, supporting further investigation of FBCM as a locally derived ingredient for aquafeed production.

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Resources; HL: Writing - review & editing, Methodology; ASKY: Writing - review & editing, Methodology; RS: Writing - review & editing, Resources; LL: Writing - review & editing, Conceptualization, Validation, Supervision, Project administration, Funding acquisition.

Conflict of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Statement. All experimental procedures were conducted in accordance with the Code of Practice for the Care and Use of Animals for Scientific Purposes of Universiti Malaysia Sabah and were approved by the Universiti Malaysia Sabah Animal Ethics Committee (AEC0009/2024).

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