*Borneo Journal of Resource Science and Technology (2026), 16(1): 1-9*
*DOI: https:doi.org/10.33736/bjrst.10397.2026*

# Evaluating Soy Protein Concentrate as A Sustainable Alternative to Fish Meal in Hybrid Grouper (*Epinephelus fuscoguttatus* ♀ X *E. lanceolatus* ♂) Diets with Phytase Inclusion

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Received: 30 July 2025 Accepted: 13 April 2026 Published: 30 June 2026

## ABSTRACT

Hybrid grouper (*Epinephelus fuscoguttatus* ♀ x *E. lanceolatus* ♂) is a prominent aquaculture species in Southeast Asia. To reduce reliance on fish meal (FM), soy protein concentrate (SPC) is being considered as a plant-based alternative. This study evaluates the effects of partial replacement with 30% SPC, with or without phytase, on growth, feed utilisation, nutrient digestibility, and intestinal morphology in juvenile hybrid grouper. Four diets were tested: a control with only FM, and three others where 30% of the FM was replaced by SPC, each with 0, 1000, or 2000 FTU/kg of phytase. After 12-week feeding experiment, there were no significant differences in growth or feed utilization between fish on the control and SPC-based diets. Nutrient digestibility and intestinal morphology were also similar, regardless of phytase. These results show that SPC can partially replace FM in hybrid grouper diets without harming growth or gut health. Adding phytase at the tested levels did not provide beneficial effects, thus supplementation of the enzyme in SPC-based diets is not necessary under this dietary formulation and culture condition.

Keywords: growth, hybrid grouper, nutrient digestibility, phytase, soy protein concentrate



## INTRODUCTION

Hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂) has become an important aquaculture species in Southeast Asia due to its rapid growth, strong environmental tolerance and high market value. However, the continued reliance on fish meal (FM) as the primary protein source in carnivorous fish diets has raised concerns related to sustainability, cost and resource availability. As a result, plant-based protein ingredients have been increasingly explored as alternatives to FM in aquafeeds.

Soy protein concentrate (SPC) is widely regarded as a promising plant protein source because of its relatively high protein content, improved digestibility and reduced levels of anti-nutritional factors compared to conventional soybean meal. Despite these advantages, SPC still contains phytate, an anti-nutritional compound that can bind phosphorus and other nutrients, thereby limiting their bioavailability in fish (Makkar and Becker, 2009; Kumar *et al*., 2012). Poor utilization of phytate-bound phosphorus may not only reduce growth efficiency but also increase phosphorus discharge into the culture environment, contributing to eutrophication (Costenaro-Ferreira and Della Flora, 2017; Flores-Moreno *et al*., 2024).

Phytase supplementation has been widely applied in aquafeeds to hydrolyse phytic acid and enhance nutrient availability from plant-based ingredients. Previous studies have demonstrated that dietary phytase can improve feed utilization, nutrient digestibility and growth performance in several marine fish species fed soybean-based diets (Biswas *et al*., 2007; Shapawi *et al*., 2013; Hussain *et al*., 2017). For example, inclusion of phytase at 2000 FTU/kg improved feed utilization in tiger grouper fed diets containing 30% defatted soybean meal (Shapawi *et al*., 2013), while Rachmansyah *et al*. (2005) reported enhanced utilization of soybean-

based diets in humpback grouper with phytase inclusion at 750 FTU/kg. Improvements in nutrient digestibility and growth have also been observed in black sea bream and European seabass fed plant-based diets supplemented with phytase (Kalhoro *et al*., 2018; Flores-Moreno *et al*., 2024).

In hybrid grouper, however, the response to plant protein inclusion appears to be species-specific. Previous studies have shown that replacing more than 30% of FM protein with SPC negatively affected growth performance, feed utilization and nutrient digestibility (Mohd Faudzi *et al*., 2017). Similar inclusion thresholds have been reported for defatted soybean meal in both tiger grouper and hybrid grouper (Shapawi *et al*., 2013; Mohd Faudzi *et al*., 2022). Although beneficial effects of phytase supplementation have been reported in several carnivorous fish species, the optimal level and effectiveness of phytase in SPC-based diets for hybrid grouper remain unclear. Therefore, the present study aimed to evaluate the effects of dietary phytase supplementation at different inclusion levels in SPC-based diets on growth, survival, feed utilization, nutrient digestibility and intestinal condition of juvenile hybrid grouper.

## MATERIALS AND METHODS

### Diets Formulation

A total of four diets containing 50% protein and 12% lipid were formulated (Mohd Faudzi *et al*., 2017; Yong *et al*., 2019). Meanwhile, the control diet consisted solely of FM (protein: 74.8%, lipid: 6.5%) as the protein source. In other treatment diets, 30% of SPC (protein: 60.4%, lipid: 0.1%) was used to replace FM partially. Phytase (Natuphos® 10000G, BASF) was included at 0 ($SPC_0$), 1000 ($SPC_{1000}$), and 2000 ($SPC_{2000}$) FTU/kg in the SPC-based diets, respectively (Mohd Faudzi *et al*., 2022). Approximately 0.5% of chromium oxide was added to the diets as an inert marker for digestibility analysis. The ingredients (Table 1) were thoroughly mixed, pelleted, oven-dried at 40 ˚C, and stored at 4 ˚C until the experiment commenced.

### Experimental Procedures

Feeding experiment was carried out at the fish hatchery of Universiti Malaysia Sabah. Each 100 L conical fiberglass tank was stocked with 20 fish (initial body weight: 5.8 ± 0.1 g). The tanks were operated under a continuous flow-through water system, and each dietary treatment was tested in 3 replicates. The fish were fed to apparent satiation at 0830 and 1430 hours. The feed intake was carefully recorded, and any uneaten feed was collected to ensure the actual feed intake was accurately calculated for the whole 12-week duration. The water quality was maintained at 27.6 – 30.3 ˚C (temperature), 4.5 – 5.9 mg/L (dissolved oxygen), 6.2 – 7.1 (pH) and 28 – 31 ppt (salinity).

Fish were measured biweekly to assess body weight gain [Eq. (1)], specific growth rate (SGR) [Eq. (2)] and survival [Eq. (3)]. Additionally, the feed conversion ratio (FCR) [Eq. (4)], protein efficiency ratio (PER) [Eq. (5)] and net protein utilization [Eq. (6)] were also determined.

$$\text{Weight gain (\%)} = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}} \times 100 \quad \text{Eq. (1)}$$

$$\text{Specific growth rate (SGR) (\% day}^{-1}\text{)} = \frac{\text{Ln Final body weight (g)} - \text{Ln Initial body weight (g)}}{\text{Time (days)}} \times 100 \quad \text{Eq. (2)}$$

$$\text{Survival (\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100 \quad \text{Eq. (3)}$$

$$\text{Feed intake (g)} = \text{Total feed intake for 12 weeks}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Wet weight gain (g)}} \quad \text{Eq. (4)}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Wet weight gain (g)}}{\text{Total protein intake (g)}} \quad \text{Eq. (5)}$$

$$\text{Net protein utilization (NPU)} = \frac{\text{Final fish body protein} - \text{Initial fish body protein}}{\text{Total protein intake}} \times 100 \quad \text{Eq. (6)}$$

During feeding trial, faecal samples were taken 3 hours after feeding (Mohd Faudzi *et al*., 2022). The collected faeces were cleaned and kept at -80 ˚C for biochemical analysis. At the end of trial, the entire bodies of fish ($n$ = 6) were sampled for biochemical analysis. Additionally, sample of intestine from fish ($n$ = 3) in respective treatment group were taken for histological examination.

**Biochemical Analysis**

**Amino Acids Composition**

The amino acids analysis was done for ingredients and diets. The samples were hydrolyzed using hydrochloric acid, 6N (temperature: 110 ˚C) for 24 hours (Ovissipour *et al*., 2010). High-performance liquid chromatography (Shimadzu Corporation, Japan) was used to analyze the samples. Shimadzu Class-VPTM software (Version 6.1) was then used to integrate, identify, and quantify the peaks by comparing them to the butyric acid and amino acid standards (Sigma, USA).

**Proximate Composition**

The Association of Official Analytical Chemists (AOAC) (1999) approach was used to analyze the proximate composition of fish and diets. The moisture content was measured after 6 hours of oven drying at 105 ˚C. The dried samples were burned for 6 hours at 550 ˚C in the furnace to determine the ash content. A Kjeldahl system (Kjeltec ™ 2300, Foss, Germany) and Soxhlet extraction (Soxtec ™ 2043, Foss, Germany) were used to analyse the protein and lipid, respectively.

**Apparent Digestibility Coefficients (ADC) of Nutrients**

Amidol method (Egsgaard, 1948) was used to assess the phosphorus in diets and faeces. The samples were measured by spectrophotometer set to 750 nm of wavelength. The chromic oxide was quantified by the acid digestion method following Furukawa and Tsukahara (1966). The following formula [Eq. (7)] was used to determine the nutrient's apparent digestibility coefficients:

$$\text{ADCs of nutrient} = 100 \times \left[1 - \left(\frac{\%\ \text{faeces nutrient}}{\%\ \text{dietary nutrient}}\right) \times \left(\frac{\%\ \text{dietary chromic oxide}}{\text{faeces chromic oxide}}\right)\right] \quad \text{Eq. (7)}$$

**Histological Examination**

The intestinal samples ($n$ = 6) were taken from the middle part after stomach and preserved in Bouin's solution for 24 hours. The samples were then dehydrated, sectioned and stained with hematoxylin and eosin (Mohd Faudzi *et al*., 2022). Images were captured using a digital microscope and measurements of intestinal diameter (Id) and villus height (Vh) were taken. The Id:Vh ratio was then calculated.

**Statistical Analysis**

One-way ANOVA was used to examine data on survival, growth, feed utilization, nutrient digestibility and intestinal morphology. Tukey's multiple range tests were applied for post-hoc comparison of treatment means. IBM SPSS Statistics version 21.0 was used for all statistical analysis with the level of significance set at $P<0.05$.

**RESULTS**

The proximate composition showed that the nutrition composition of diets met the recommended requirement of the hybrid grouper (Table 1). Meanwhile, Table 2 showed the amino acids composition of FM, SPC and diets. The amount of aspartic acid, glutamic acid, leucine and tyrosine increased when FM was replaced with SPC at 30% level in SPC-based diets. On the other hand, the amount of threonine, glycine, alanine, cystine, methionine and lysine decreased when 30% of SPC was used to replace FM. Other amino acids were almost similar among the diets.

**Table 1.** The ingredients (g $kg^{-1}$ dry weight basis) and proximate composition (%) of diets fed to hybrid grouper

| Ingredients | Diets | | | |
|---|---|---|---|---|
| | Control | $SPC_0$ | $SPC_{1000}$ | $SPC_{2000}$ |
| Fish meal | 668.0 | 396.0 | 396.0 | 396.0 |
| Soy protein concentrate | - | 248.0 | 248.0 | 248.0 |
| Corn gluten meal | - | 80.0 | 80.0 | 80.0 |
| Fish oil | 62.0 | 70.0 | 70.0 | 70.0 |
| Lecithin | - | 20.0 | 20.0 | 20.0 |
| Taurine | - | 12.0 | 12.0 | 12.0 |
| Mineral[a] | 15.0 | 20.0 | 20.0 | 20.0 |
| Vitamin[b] | 30.0 | 30.0 | 30.0 | 30.0 |
| Dicalcium phosphate | 10.0 | 10.0 | 10.0 | 10.0 |
| Carboxyl methyl cellulose (CMC) | 20.0 | 20.0 | 20.0 | 20.0 |
| Alpha starch | 196.0 | 94.0 | 94.0 | 94.0 |
| Phytase (Natuphos® 10000G, BASF) | 0.0 | 0.0 | 0.1 | 0.2 |
| Chromic oxide | 5.0 | 5.0 | 5.0 | 5.0 |
| *Proximate Composition* | | | | |
| Protein (%) | 50.1 | 50.2 | 50.2 | 50.1 |
| Lipid (%) | 12.6 | 12.0 | 12.3 | 12.3 |
| Moisture (%) | 3.5 | 3.4 | 3.6 | 3.8 |
| Ash (%) | 12.8 | 11.8 | 11.9 | 11.7 |
| Fiber (%) | 0.7 | 0.8 | 0.8 | 0.8 |
| Nitrogen-free extract (NFE) | 20.3 | 21.8 | 21.2 | 21.3 |
| Energy (MJ $kg^{-1}$) | 19.3 | 19.7 | 19.7 | 19.6 |
| Protein:Energy (g $MJ^{-1}$) | 25.9 | 25.5 | 25.5 | 25.6 |

[a]Mineral premix, g $kg^{-1}$ dry weight (Dexchem Industries Sdn. Bhd., Malaysia): $CaH_4O_8P_2$ 270.98 g; $C_6H_{10}CaO_6$ 327 g; $FeSO_4$ 25 g; $MgSO_4$ 132 g; KCl 50 g; KI 0.15 g; $CuSO_4$ 0.785 g; $MnO_2$ 0.8 g; $CoCO_3$ 1 g; ZnO 3 g; $Na_2SeO_3$ 0.011 g; $CaCO_3$ 129.27 g

[b]Vitamin premix, g $kg^{-1}$ dry weight (Dexchem Industries Sdn. Bhd., Malaysia): $C_6H_8O_6$ 45 g; $C_6H_{12}O_6$ 5 g; $C_5H_{14}ClNO$ 75 g; $C_6H_5NO_2$ 4.5 g; $C_{17}H_{20}N_4O_6$ 1 g; $C_8H_{11}NO_3$ HCl 1 g; $C_{12}H_{17}N_4OS$ HCl 0.92 g; $C_{18}H_{32}CaN_2O_{10}$ 3 g; $C_{22}H_{32}O_2$ 0.6 g; vitamin D3 0.08 g; $C_{11}H_8O_2$ 1.67 g; $C_{31}H_{52}O_3$ 8 g; $C_{10}H_{16}N_2O_3S$ 0.02 g; $C_{19}H_{19}N_7O_6$ 0.09 g; $C_{63}H_{88}CoN_{14}O_{14}P$ 0.001 g; $C_6H_{10}O_5$

Growth, survival and feed utilization of juvenile hybrid grouper fed with the diets for 12 weeks are shown in Table 3. Growth of the fish was not significantly affected ($p>0.05$) by SPC-based diets. No significant changes ($p>0.05$) were observed on total feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU) when the fish fed with SPC and control diets. All treatments resulted in a 100.0% fish survival.

The ADC of nutrients in the present study are shown in Table 4. No significant effect ($p>0.05$) was observed on ADC of protein, lipid, and phosphorus when the fish fed with SPC and control diets. The results on the intestinal condition are shown in Figure 1 and Table 5. Feeding the fish with SPC-based diets did not alter the intestinal condition. The intestine diameter (Id), villus height (Vh) and Id/Vh ratio did not significantly improve ($p>0.05$) when phytase was included in SPC-based diets.

**Table 2.** The composition (g $kg^{-1}$ dry weight basis) of amino acids in fish meal (FM), soy protein concentrate (SPC) and diets

| Amino Acids | FM | SPC | Diets | | | |
|---|---|---|---|---|---|---|
| | | | Control | $SPC_0$ | $SPC_{1000}$ | $SPC_{2000}$ |
| Aspartic acid | 78.84 | 102.69 | 49.41 | 52.52 | 51.62 | 51.80 |
| Threonine | 34.11 | 24.29 | 22.01 | 18.82 | 19.65 | 19.84 |
| Serine | 37.20 | 30.48 | 23.68 | 24.54 | 24.39 | 24.42 |
| Glutamic acid | 114.21 | 140.38 | 73.08 | 93.78 | 94.32 | 93.65 |
| Glycine | 54.94 | 21.49 | 38.23 | 30.82 | 31.13 | 31.74 |
| Alanine | 51.80 | 29.82 | 35.22 | 30.88 | 31.64 | 31.25 |
| Cystine | 83.50 | 36.20 | 53.16 | 48.65 | 47.06 | 47.23 |
| Methionine | 21.46 | 5.98 | 13.08 | 9.26 | 8.65 | 9.36 |
| Isoleucine | 36.03 | 27.23 | 23.37 | 24.32 | 25.64 | 25.29 |
| Leucine | 62.62 | 39.17 | 41.28 | 45.16 | 44.25 | 43.43 |
| Tyrosine | 25.90 | 13.40 | 13.91 | 16.50 | 15.53 | 16.67 |
| Phenylalanine | 33.00 | 24.78 | 21.19 | 23.39 | 22.75 | 23.76 |
| Lysine | 72.11 | 29.56 | 48.41 | 40.12 | 38.28 | 39.90 |
| Arginine | 54.88 | 7.79 | 35.82 | 34.50 | 35.75 | 35.10 |

**Table 3**. The performances of hybrid grouper after 12 weeks feeding on SPC diets with different phytase inclusion

| Parameter | Diets | | | |
|---|---|---|---|---|
| | Control | $SPC_0$ | $SPC_{1000}$ | $SPC_{2000}$ |
| Final BW (g) | 84.0 ± 11.1 | 108.7 ± 13.7 | 106.3 ± 4.6 | 94.7 ± 16.7 |
| Weight Gain (%) | 1312.1 ± 180.5 | 1768.9 ± 241.4 | 1711.8 ± 78.6 | 1521.2 ± 299.3 |
| SGR (%/day) | 3.2 ± 0.2 | 3.5 ± 0.2 | 3.5 ± 0.1 | 3.3 ± 0.2 |
| Survival (%) | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 |
| Total FI (g $fish^{-1}$) | 80.2 ± 5.2 | 102.2 ± 12.6 | 104.7 ± 6.5 | 96.5 ± 10.1 |
| FCR | 1.0 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.0 | 1.1 ± 0.1 |
| PER | 1.9 ± 0.2 | 2.0 ± 0.1 | 1.9 ± 0.1 | 1.8 ± 0.2 |
| NPU | 34.2 ± 2.2 | 34.6 ± 4.0 | 33.1 ± 2.1 | 32.2 ± 3.2 |

P<0.05 indicates a significant difference between the mean (±SE) values with different superscripts within the row

**Table 4**. Apparent digestibility coefficients (%) of hybrid grouper after 12 weeks feeding on SPC diets with different phytase inclusion

| Parameter | Diets | | | |
|---|---|---|---|---|
| | Control | $SPC_0$ | $SPC_{1000}$ | $SPC_{2000}$ |
| Protein | 97.6 ± 0.2 | 98.2 ± 0.5 | 98.5 ± 0.1 | 98.1 ± 0.7 |
| Lipid | 97.3 ± 0.4 | 97.7 ± 0.9 | 98.2 ± 0.4 | 97.4 ± 1.2 |
| Phosphorus | 68.5 ± 0.9 | 68.0 ± 2.9 | 69.8 ± 1.2 | 69.5 ± 2.9 |

p<0.05 indicates a significant difference between the mean (±SE) values with different superscripts within the row

**Table 5.** Measurement of hybrid grouper's intestinal morphometric after 12 weeks of feeding on SPC diets with different phytase inclusion

| Parameter | Diets | | | |
|---|---|---|---|---|
| | Control | $SPC_0$ | $SPC_{1000}$ | $SPC_{2000}$ |
| Intestine diameter (Id) (mm) | 1.9 ± 0.2 | 1.9 ± 0.1 | 1.9 ± 0.1 | 1.9 ± 0.1 |
| Villus height (Vh) (mm) | 0.7 ± 0.1 | 0.7 ± 0.1 | 0.7 ± 0.1 | 0.7 ± 0.0 |
| Id/Vh | 2.8 ± 0.1 | 2.8 ± 0.1 | 2.9 ± 0.1 | 2.8 ± 0.1 |

p<0.05 indicates a significant difference between the mean (±SE) values with different superscripts within the row

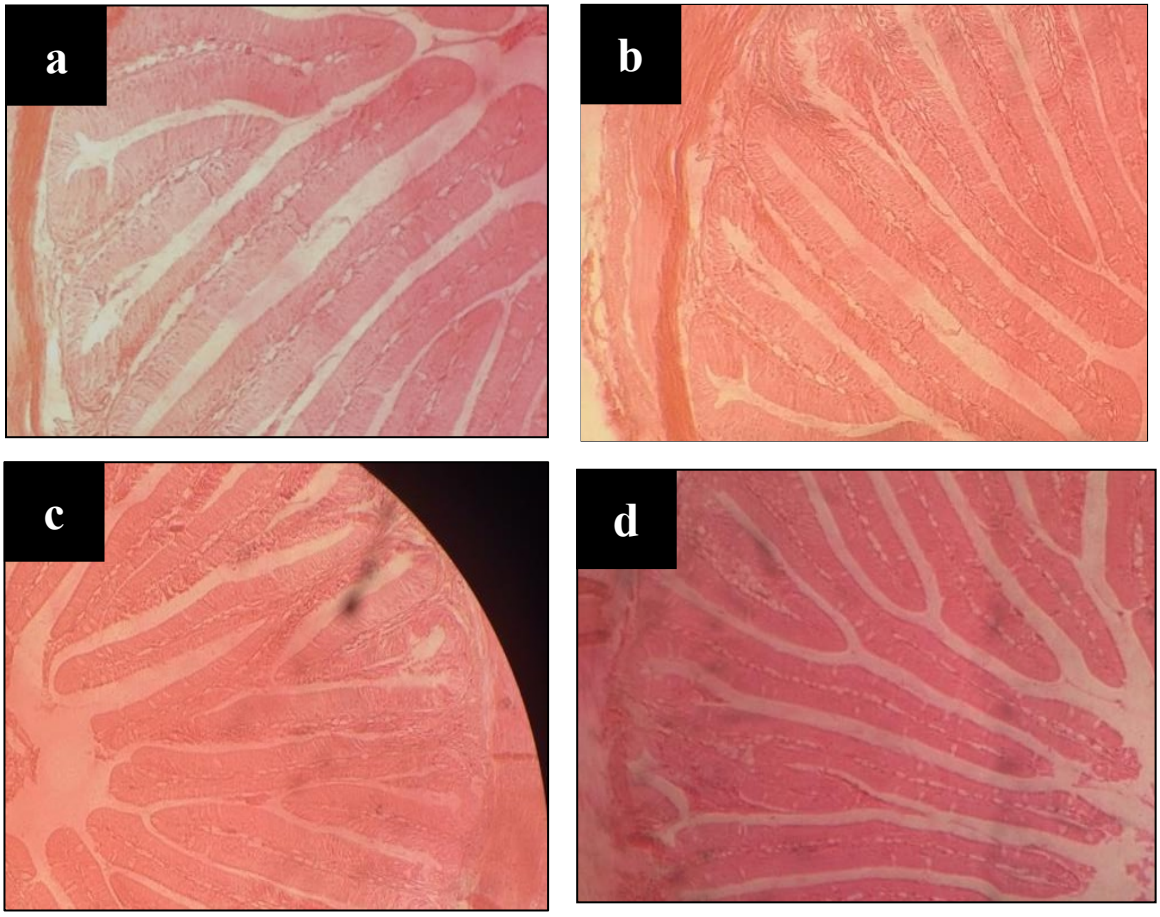


**Figure 1.** Tissue section of hybrid grouper stained with hematoxylin and eosin-stained. (a) Fish fed CON. (b) Fish fed SPC0. (c) Fish fed SPC1000. (d) Fish fed SPC2000

## DISCUSSION

The study revealed that the growth performance, survival or feed utilization of juvenile hybrid grouper were nor adversely affect by the partial replacement of fish meal with 30% soy protein concentrate (SPC). Although no statistically significant differences were detected among treatments, fish fed SPC-based diets exhibited numerically comparable or slightly higher growth relative to those fed the control diet. This suggests that hybrid grouper are able to efficiently utilize SPC when included at an appropriate level, without compromising growth potential.

The absence of growth depression in fish fed SPC-based diets may be partly attributed to the balanced nutritional formulation and the amino acid profile of the diets. Although replacement of fish meal with SPC altered the concentrations of certain amino acids, the overall dietary amino acid composition remained within acceptable ranges for hybrid grouper (Mohd Faudzi *et al*., 2017; Wu *et al*., 2017; Mohd Faudzi *et al*., 2022). Increases in aspartic acid, glutamic acid, leucine and tyrosine in SPC-based diets may have supported metabolic and protein synthesis processes, while reductions in some essential amino acids, such as methionine and lysine, did not appear to reach levels that limited growth. This indicates that the hybrid grouper possesses a degree of nutritional flexibility, allowing it to tolerate moderate changes in dietary amino acid composition without adverse effects on performance.

Feed intake did not differ significantly among treatments, indicating that inclusion of SPC did not reduce diet palatability. This finding contrasts with reports in some carnivorous fish species where plant protein inclusion resulted in reduced feed intake and poorer growth performance (Shapawi *et al*., 2013; Zhang *et al*., 2023). The comparable feed intake observed in the present study suggests that hybrid grouper readily accepted SPC-based diets, which may be related to the reduced anti-nutritional factor

content of SPC compared to conventional soybean meal, as well as the hybrid vigor characteristics of this species. Consequently, similar feed conversion ratio, protein efficiency ratio and net protein utilization across treatments further support the suitability of SPC as a partial replacement for fish meal in hybrid grouper diets.

Nutrient digestibility remained high and was not significantly affected by SPC inclusion or phytase supplementation. The apparent digestibility coefficients of protein and lipid exceeded 97% across all treatments, indicating efficient nutrient utilization. Previous studies reported reduced digestibility when higher levels of SPC or defatted soybean meal were used (Mohd Faudzi *et al*., 2017; Chen *et al*., 2019), however, the present findings suggest that inclusion at 30% does not impair digestive efficiency. Variability in digestibility responses among studies may be influenced by differences in fish size, physiological condition, ingredient quality and experimental conditions.

Phosphorus digestibility was also unaffected by phytase supplementation in the present study. Although phytase has been shown to enhance phosphorus availability in various fish species, its effectiveness is known to depend on several factors, including dietary phytate content, enzyme activity, fish species and gut environment. The lack of response to phytase supplementation observed here suggests that the phytate level in the SPC-based diets may have been insufficient to limit phosphorus utilization, or that endogenous digestive processes in hybrid grouper were already adequate to release available phosphorus. As a result, additional phytase inclusion did not provide measurable benefits under the present dietary formulation.

The intestinal morphology of hybrid grouper was not altered by SPC inclusion or phytase supplementation. Measurements of intestinal diameter, villus height and their ratio remained consistent across all treatments, indicating that intestinal structure and absorptive capacity were maintained. Preservation of normal intestinal morphology suggests that SPC inclusion at 30% did not induce inflammatory responses or structural damage to the intestinal epithelium. This contrasts with reports in certain pure grouper species (tiger grouper and giant grouper) where higher inclusion levels of plant protein sources resulted in shortened villi and intestinal inflammation (Chor *et al*., 2015; Garcia-Ortega *et al*., 2016). The present findings further support the notion that hybrid grouper exhibit greater tolerance to plant-based ingredients, likely due to hybrid vigor and enhanced physiological adaptability.

Although anti-nutritional factors were not directly measured in the present study, the lack of adverse effects on growth, nutrient digestibility and intestinal morphology suggests that their levels in the SPC-based diets were below thresholds that negatively affect fish performance. Previous studies have shown that excessive phytate and other anti-nutritional compounds can impair nutrient utilization and growth; however, moderate inclusion levels, as applied in this study, appear to be well tolerated by hybrid grouper. The observed responses are consistent with earlier findings that hybrid grouper are less sensitive to plant protein inclusion compared to their parental species.

## CONCLUSION

The findings suggest that inclusion of phytase had no positive impact on the hybrid grouper's performances. However, SPC demonstrated promise as a partial replacement for FM, supporting comparable performance when included at 30% of the dietary protein. The study highlights the potential of SPC as a sustainable alternative protein source in hybrid grouper diets without the necessity for inclusion of phytase.

## ACKNOWLEDGEMENTS

The research was funded by the Higher Institution Centre of Excellence (HICoE) Research Grant Scheme of the Ministry of Higher Education, Malaysia (Approval Letter No. JPT (BKPI) 1000/016/018/35(2), Grant Code HIC2405). The authors acknowledged staff and research assistants of Fish Hatchery, Borneo Marine Research Institute, Universiti Malaysia Sabah, for the significant technical assistance throughout the research.

## ETHICAL STATEMENT

The fish were handled and cared in accordance with research purposes. Specific animal care protocols included the suitable stocking number, regular monitoring of water quality and careful

handling during feeding and sampling to reduce stress of fish.

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