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Effects of organic acids dietary supplementation in soybean meal-based diets on growth performance and nutrient utilization in hybrid grouper (*Epinephelus fuscoguttatus* x *E. lanceolatus*) juveniles

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ABSTRACT

A 10-week feeding experiment was carried out to study the effects of supplementing citric and butyric acids in soybean meal (SBM) based diets for hybrid grouper (*Epinephelus fuscoguttatus* x *E. lanceolatus*) juveniles. Seven experimental diets were formulated to replace fishmeal (FM) with SBM at the levels of 30 % (SBM30) and 40 % (SBM40) as a base to substitute FM, SBM30 with the supplementation of 3 % citric acid and 1 % butyric acid (SBM30 +CA+BA), SBM40 with the supplementation of 3 % citric acid (SBM40 +CA), SBM40 with the supplementation of 1 % butyric acid (SBM40 +BA), SBM40 with the supplementation of 3 % citric acid and 1 % butyric acid (SBM40 +CA+BA) and with FM diet being the control. Triplicate groups of 16 fish (11.50 ± 0.6 g) in 21 tanks were fed respective experimental diets. Results showed that the highest weight gain, specific growth rate and protein efficiency ratio was recorded in fish fed SBM30 +CA+BA but it was not significantly different from fish fed control FM diet ($p > 0.05$) while fish fed SBM40 showed the lowest performance ($p > 0.05$). However, comparing the SBM40 treatments, fish fed SBM40 +CA showed the highest ($p > 0.05$). No significant differences were observed among the treatments for hepatosomatic index. Meanwhile, for the viscera-somatic index, fish fed SBM40 +BA was significantly lowest while FM, SBM30 and SBM30 +CA+BA showed no significant differences. Between the SBM40 treatments, SBM40 +CA showed the highest but no significant differences with SBM40 and SBM40 +CA+BA. Fish fed SBM40 +CA+BA also showed increased crude protein for whole body composition and apparent digestibility coefficient of protein. Based on the results, 30 % replacement of SBM with the combination of citric and butyric acid showed better growth while for 40 % replacement of SBM, supplementation of citric acid proved to give better results compared to butyric acid and without the supplementation of organic acid.

1. Introduction

High-protein aquafeed for carnivorous fish is still highly dependent on fishmeal (Hua et al., 2019; Davies et al., 2021). Fishmeal (FM) is derived from wild-captured foraging fish, and this is an unsustainable source. Global demand for fish products is expected to increase significantly over the next 35 years due to population growth (Gasco et al., 2018). A population of 9.7 billion is predicted by the year 2050 hence the demand for food supply is estimated to increase by 25 – 70 % (Hua et al., 2019). The production of FM from wild-captured forage fish has shown a decreasing trend over the last two decades however it is still an important feed component for many carnivorous fish (Turchini et al., 2019). Approximately 4.5 million tons of FM is produced annually with 69 % of it used in aquafeeds (Auchterlonie, 2018) and the total production of aquafeeds is expected to increase by 75 % from 49.7 million tons in 2015–87.1 million tons in 2025 (Tacon and Metian, 2015).

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Therefore, alternatives such as plant-based protein sources were explored. Among all that were studied, soybean meal (SBM) had a higher potential to be used as a FM replacement (Ye et al., 2019). SBM has a more balanced amino acid profile, available abundantly, has lower cost and a higher crude protein content of about 43 – 48 % compared to other plant-based protein sources (Yanti et al., 2019). However, the disadvantage of replacing SBM is that it contains antinutrients and has a low methionine content, which has been reported in previous studies and caused negative effects on fish in general (Gaylord et al., 2007; Lim et al., 2011). Due to this, it imposes the limitation to be used in diet formulations for carnivorous fish, especially at high levels (Ye et al., 2019). Various types of feed additives with SBM replacement are being studied where natural additives such as organic acids are favoured (Dada, 2012). Organic acids are weak acids with a carboxylic acid group (R-COOH), intermediates in the breakdown of carbohydrates, amino acids and lipid are used for their nutritional value and antibacterial properties in animal feed (Melaku et al., 2021). Organic acids have been supplemented into animal diets since the ban on the use of antibiotics and it is selected because of their ability to maintain gut barrier cellular integrity, modulate intestinal microbiota and improve digestion and nutrient absorption rate which contributes to improved growth (Melaku et al., 2021). Organic acids such as citric and butyric acids are functional feed additives that have been studied extensively on livestock (Hassaan et al., 2017; Soltan et al., 2017).

Citric acid and its salt are among the most extensively studied organic acids in aquaculture, primarily due to their favourable flavour and high buffering capacity. These properties make citric acid particularly suitable for inclusion in aquafeeds, especially those with SBM as a FM replacement (Anthonius et al., 2018; Ng and Koh, 2018; Shah et al., 2015). During feeding, the hydrochloric acid concentration in the stomach decreases, increasing pH levels and potentially impairing pepsin activation and pancreatic enzyme secretion. This disruption negatively affects digestive capacity and fish growth. Citric acid, as an acidifier, helps maintain the acidic environment essential for pepsin activity and other enzyme functions critical for efficient protein hydrolysis (Fabay et al., 2022).

Much research has demonstrated the significant benefits of citric acid supplementation in various fish species. In red drum (Sciaenops ocellatus), a 1.5 % supplementation improved digestive enzyme activity (Castillo et al., 2014). In rainbow trout (Oncorhynchus mykiss), a 2 % citric acid inclusion in a 35 % SBM-based diet significantly enhanced growth performance, feed utilization, and protein digestibility. Similarly, in hybrid grouper (*E. fuscoguttatus* x *E. lanceolatus*), a 3 % citric acid inclusion in diets containing 5 % duckweed improved feed efficiency and maintained apparent digestibility coefficients comparable to control diets (Anthonius et al., 2018).

Studies on SBM as a replacement for FM in carnivorous fish have proven that the fish is intolerant to higher levels of SBM due to the antinutritional factors that cause enteritis. This enteritis then leads to poor fish growth and intestinal damage (Ke et al., 2022; Zhu et al., 2021). Butyric acid, another key organic acid, is a metabolite of the intestinal bacterium *Clostridium butyricum*. It serves as an energy source for intestinal epithelial cells, supporting cellular function and maintaining intestinal health (Ke et al., 2022). Unlike citric acid, studies on butyric acid in aquafeeds are relatively limited, but available research highlights its potential to mitigate SBM-induced enteritis, promote digestion, and maintain intestinal morphology (Liu et al., 2019; Lin and Cheng, 2017). Butyric acid supplementation also improved growth performance in yellow drum (*Nibea albiflora*), turbot (*Scophthalmus maximus*), and Pengze crucian carp (*Carassius auratus Pengze*) (Fang et al., 2021; Wu et al., 2020; Yu et al., 2021).

Furthermore, butyric acid reduces mucosal inflammation, oxidative stress, and enhances intestinal barrier function, contributing to overall health and performance. It also regulates the expression of intestinal inflammatory and growth-related genes, further reinforcing its importance in aquafeeds (Abdel-Tawwab et al., 2021; Wu et al., 2020). Together, citric acid and butyric acid show promising solutions for addressing SBM-associated challenges and advancing sustainable aquaculture.

Hybrid grouper is a marine carnivorous fish, and it is highly cultured particularly in Southeast Asia due to its fast growth and high market value (Shapawi et al., 2018). The grouper production in China has reported that it reached 192,045 tonnes in 2020 (China Fishery Statistics Yearbook, 2020). Therefore, the nutrition and feed for grouper have become important for grouper aquaculture and much progress has been seen regarding grouper nutrition over the past years (Zhao et al., 2021). Previous studies on SBM as FM replacement for hybrid grouper showed that higher replacement levels decreased the growth performance of the fish (Firdaus et al., 2016; Mohd Faudzi et al., 2017). In a study on the use of soy protein concentrate to replace FM, hybrid grouper recorded its best growth at, and it can tolerate up to 30 % replacement without affecting its growth (Mohd Faudzi et al., 2017). Inclusion of SBM at 40 % and above caused a significant decrease in the fish growth performance (Firdaus et al., 2016; Mohd Faudzi et al., 2017) hence 40 % of SBM is considered a high-level replacement for this species.

In the ongoing research on the utilization of SBM as FM replacement, there remains a gap in both the prevention of enteritis and improving the growth performance of the fish when fed higher levels of SBM. To the authors' knowledge, the supplementation effect of citric acid, butyric acid and the combination of both on hybrid grouper has not been reported. Hence, the present study aimed to investigate the supplementation of citric acid and butyric acid at (3 % and 1 % respectively) at optimal SBM replacement level (30 %) and a high-level replacement (40 %) on the growth performance, feed utilization, apparent digestibility coefficient, body condition and body composition of hybrid grouper juveniles. The present study was also conducted to help lessen the fishmeal burden in the industry, as well as promote growth of fish for a better grouper culture.

2. Materials and methods

The present study involving live animals was performed according to the Researcher's Guidelines on Code of Practice for The Care and Use of Animal for Scientific Purposes, Universiti Malaysia Sabah (UMS), Approval No. AEC007/2022, dated 24 May 2022.

2.1. Experimental diets

Experimental diets were formulated according to the requirements of hybrid grouper juveniles which consist of 50 % crude protein and 12 % crude lipid (Yong et al., 2019) (Table 1). This experiment consists of seven experimental diets as follows:

1. Full FM based diet as the control group.
2. Optimal level of SBM replacement at 30 % (SBM30) which serves as a base to substitute FM.
3. Higher level of SBM replacement at 40 % (SBM40) which also serves as a base to substitute FM in this experiment.
4. SBM30 diet supplemented with 3 % citric acid (CA) (Anthonius et al., 2018) and 1 % butyric acid (BA) (Lin and Cheng, 2017).
5. SBM40 diet supplemented with CA.
6. SBM40 diet supplemented with BA.
7. SBM40 diet supplemented with CA and BA.

As 30 % SBM is the optimal replacement for hybrid grouper (Yong et al., 2019), the inclusion of organic acid combination was decided instead. At 40 % SBM, it is considered a higher level of replacement, hence individual organic acid inclusions were tested to observe if there are differences between them. The seven experimental diets were denoted as FM, SBM30, SBM40, SBM30 +CA+BA, SBM40 +CA, SBM40 +BA and SBM40 +CA+BA from here onwards.

All dry ingredients of the experimental diets were mixed thoroughly

Table 1
Dry ingredients (g/100 g) and proximate composition (%) of experimental diets for hybrid grouper juveniles.

Ingredients (g)	Experimental diets						
	FM	**SBM30**	**SBM30 +CA+BA**	**SBM40**	**SBM40 +CA**	**SBM40 +BA**	**SBM40 +CA+BA**
Fishmeal[a]	66.94	46.86	46.86	40.17	40.17	40.17	40.17
SBM	0.00	29.04	29.04	38.72	38.72	38.72	38.72
Fish oil	5.81	7.43	7.43	7.97	7.97	7.97	7.97
Tapioca starch	19.75	9.16	5.16	5.64	2.64	4.64	1.64
Vitamin premix[b]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[c]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
CMC[d]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Citric acid	0.00	0.00	3.00	0.00	3.00	0.00	3.00
Butyric acid	0.00	0.00	1.00	0.00	0.00	1.00	1.00
Total (g)	100.0	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition (%)							
Dry matter	91.70	89.14	91.51	91.81	92.12	92.02	92.03
Crude protein	53.68	52.21	53.21	52.02	51.67	51.31	53.09
Crude lipid	13.00	12.62	12.21	11.96	12.26	12.11	12.56
Ash	13.95	12.50	13.41	13.24	13.22	13.85	12.84

[a] Danish fishmeal, TripleNine 999 Fish Protein, Denmark.
[b] Vitamin mixture (g/kg mixture): Inositol, 5.0, choline chloride, 75.0; niacin, 4.5; riboflavin, 1.0; pyridoxine HCl, 1.0 l; thiamine HCl, 0.92; D-calcium pantothenate, 3.0; retinyl acetate, 0.60; vitamin D3, 0.083; Menadione, 1.67; DL alpha tocopherol acetate, 8.0; D-biotin, 0.02; folic acid, 0.09; vitamin B12, 0.00135. All ingredients were diluted with alpha cellulose to 1 kg.
[c] Mineral mixture (g/kg mixture): Calcium phosphate monobasic, 270.98; Calcium lactate, 327.0; Ferrous sulphate, 25.0; Magnesium sulphate, 132.0; Potassium chloride, 50.0; Sodium chloride, 60.0; Potassium iodide, 0.15; Copper sulphate, 0.785; Manganese oxide, 0.8; Cobalt carbonate, 1.0; Zinc oxide, 3.0; Sodium selenite, 0.011; Calcium carbonate, 129.274.
[d] Carboxymethyl cellulose (CMC), Sigma.

before adding fish oil and water. Experimental diets were also added with 0.5 % chromic oxide as the inert marker for the estimation of apparent digestibility coefficients. The mixture was pelleted using a meat mincer (Orimas TBS200, Malaysia) to produce long strands of feed with a diameter of 3 mm before drying in a ventilating oven at 40°C for 6 hours. The dried pellets were kept refrigerated in ziplock plastic bags at 4°C prior to the feeding trial. The experimental diets were subjected to proximate analysis and their composition is presented in Table 1.

2.2. Feeding trial

The feeding trial was done at the Fish Hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah, Malaysia. Hybrid grouper juveniles were obtained from a local hatchery based in Tawau, Sabah, Malaysia. The fish were acclimatized for two weeks prior to the feeding trial.

Fish with an average body weight of 11.50 ± 0.6 g were randomly distributed into 21 of 100 L (61 ×40 x 61 cm) fibreglass tanks with a density of 16 fish per tank. Experimental tanks were fully equipped with a flow-through seawater system with constant gentle aeration. The experimental groups were performed in triplicates and hand-fed with respective diets twice a day (0800 h and 1600 h) until apparent satiation for ten weeks. Tanks were bottom cleaned daily and feed intake was recorded. The water quality of the tanks was maintained at 6.0 – 6.5 mg/L for dissolved oxygen, 28 – 30 °C for temperature, 30 – 32 ppt for salinity and a pH of 6.0 – 7.0.

During the feeding trial, the growth of the fish was monitored closely, and body measurement was done fortnightly in bulk. Individual body weight of fish was only measured during the initial and upon terminating the experiment. Fish were starved for 24 h prior to measurements and anaesthetized with Transmore® (Nika, Malaysia) to reduce handling stress.

At the end of the feeding trial, fish from each treatment (n = 6) were sacrificed. Three fish were sacrificed for whole body composition analysis, and another three fish for body condition analysis. Growth performance, feed utilization and body indices of the fish were estimated using the following formula:

$$\text{Weight gain (\%)} = \frac{\textit{Final body weight}(g) - \textit{Initial body weight}(g)}{\textit{Initial body weight}(g)} \text{ x } 100$$

$$\text{Specific growth rate (SGR) } (\%/\text{day}) = \frac{\textit{Ln Final body weight}(g) - \textit{Ln Initial body weight}(g)}{\textit{Time}(days)} \text{ x } 100$$

Feed intake (g) = Total feed intake for 10 weeks

$$\text{Feed conversion ratio (FCR)} = \frac{\textit{Feed intake}}{\textit{Wet weight gain}(g)} \text{x} 100$$

$$\text{Protein efficiency ratio (PER)} = \frac{\textit{Wet weight gain}(g)}{\textit{Total protein intake}(g)} \text{ x } 100$$

$$\text{Condition factor (K)} = \frac{\textit{Final body weight}(g)}{\textit{Total length}(cm)3} \text{ x } 100$$

$$\text{Hepatosomatic index (HSI)} = \frac{\textit{Liver weight}(g)}{\textit{Body weight}(g)} \text{ x } 100$$

$$\text{Viscerosomatic index (VSI)} = \frac{\textit{Visceral weight}(g)}{\textit{Body weight}(g)} \text{ x } 100$$

2.3. Apparent Digestibility Coefficient (ADC)

Faeces were collected during and after the feeding trial to determine the apparent digestibility coefficient (ADC) (Mohd Faudzi et al., 2017). When faeces were collected after the feeding trial, the remaining fish were pooled according to their respective treatments and were also hand-fed experimental diets twice daily (0800 h and 1600 h) until apparent satiation level. After each feeding period, fresh faeces were collected by gently siphoning into a small basin using a tube. The faeces were trapped using a sieve, separated from other detritus and cleaned with distilled water. Faeces were dried using filter paper before storing them in a −80 °C ultra-freezer prior to analysis (Mohd Faudzi et al., 2017).

Chromic oxide in both diets and faeces was determined using the acid digestion method by Furukawa and Tsukahara (1966). The ADC was then calculated using the following formula:

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

2.4. Proximate composition

The proximate analysis of both experimental diets and fish samples was done following the methods by Association of Official Analytical Chemists International AOAC International (1995) in determining the moisture, ash, crude protein and crude lipid. Moisture and ash contents were determined after oven drying the samples at 105 °C for 24 hours and incinerated in a muffle furnace for 5 hours at 550 °C. Crude protein was determined using the automatic protein analyzer (Gerhardt Protein Analyzer, Vapodest 500) and crude lipid using Soxtec Lipid Analyzer (Soxtec™ 2043, Foss, Sweden) with petroleum ether as its solvent (40 – 60 °C boiling point).

2.5. Statistical analysis

All data on growth, survival, feed utilization, body condition, body composition and apparent digestibility coefficient of nutrients in hybrid grouper juveniles fed experimental diets were analyzed using one-way analysis of variance (ANOVA) in Statistical Package of Social Sciences Version 27.0. Duncan's multiple-range test was used to determine the statistical difference between treatment means and statistical significance was set at the 5 % probability level ($p < 0.05$).

3. Results

3.1. Growth performance

Hybrid grouper juveniles fed SBM30 +CA+BA showed significantly the highest weight gain (WG) and specific growth rate (SGR) while fish fed SBM40 showed the lowest ($p < 0.05$) (Table 2). However, no significant differences were observed compared to FM and SBM30 ($p > 0.05$). Meanwhile, among the SBM40 treatments, SBM40 +CA showed the highest growth followed by SBM40 +CA+BA and SBM40 +BA. The growth of fish fed SBM40 +CA was comparable to fish fed SBM30 +CA+BA ($p > 0.05$).

3.2. Feed utilization

Feed utilization in the present study showed a similar trend to the growth. The total feed intake (TFI) increased with the increase of plant protein replacement. However, when organic acids were added, the TFI decreased. This was observed in SBM30 and SBM40, where the TFI of soybean meal replacement was higher than FM and decreased when organic acids were added. TFI of SBM30 was the highest. while SBM40 +CA was the lowest ($p < 0.05$). Among the SBM40 treatments, SBM40 +CA+BA showed the highest TFI followed by SBM40 +BA and SBM+CA however they were comparable ($p > 0.05$).

The feed conversion ratio (FCR) of fish fed SBM30 +CA+BA was the lowest compared to FM and SBM30 however not significant (Table 3). Meanwhile, the FCR of fish fed SBM40 was the highest ($p < 0.05$). Among the SBM40 treatment groups, the FCR of fish fed SBM40 +CA was significantly lower than SBM40, SBM40 +BA and SBM40 +CA+BA ($p < 0.05$).

The protein efficiency ratio (PER) of fish fed SBM30 +CA+BA showed the highest compared to FM and SBM30, while the PER of fish fed SBM40 showed the lowest values ($p < 0.05$). Among the SBM40 treatment groups, fish-fed SBM40 +CA showed the highest PER ($p < 0.05$) followed by SBM40 +CA+BA and SBM40 +BA.

3.3. Body condition

The condition factor (K) of SBM30 +CA+BA was higher compared to FM and SBM30 however there were no significant differences between the treatments (Table 4). Among the SBM40 treatments, SBM40 +CA showed the highest value, while SBM40 +BA showed the lowest ($p < 0.05$). However, the values of fish fed SBM40 +CA were comparable to all other treatments.

For the hepatosomatic index (HSI), FM had the highest value compared to SBM30 and SBM30 +CA+BA; however, there were no significant differences ($p > 0.05$). Among the SBM40 treatments, SBM40 showed the highest HSI value ($p < 0.05$) followed by SBM40 +CA, SBM40 +CA and SBM40 +CA+BA.

Meanwhile, for the viscerosomatic index (VSI), fish-fed SBM30 +CA+BA had a lower value than fish-fed FM but was comparable to fish-fed SBM30 ($p > 0.05$). Among the SBM40 treatment groups, fish fed SBM40 +CA showed the highest VSI values followed by SBM40, SBM40 +CA+BA with SBM40 +BA being significantly the lowest ($p < 0.05$).

3.4. Whole body proximate composition

For the whole-body proximate composition of the fish, the crude protein of fish fed SBM30 +CA+BA was the highest among all treatments ($p > 0.05$) (Table 5). Among the SBM40 treatments, diets with organic acid supplementation showed significantly higher crude protein compared to SBM40 ($p < 0.05$). For crude lipid, diets with SBM replacement generally had lower crude lipid levels compared to FM, although not significantly different ($p > 0.05$). The ash contents of fish-fed SBM-based diets were higher compared to fish fed the FM diet.

3.5. Apparent Digestibility Coefficient (ADC) of nutrients

The ADC of protein and dry matter of fish fed SBM30 +CA+BA was significantly the highest, while SBM40 showed the lowest ($p < 0.05$) (Table 6). Among the SBM40 treatments, SBM40 +CA showed significantly higher ADC of protein and dry matter, followed by SBM40 +BA, SBM40 +CA+BA and SBM40 being the lowest ($p < 0.05$). For ADCs of lipids, FM was significantly highest while SBM40 was the lowest ($p < 0.05$). From Table 6, it can also be seen that SBM-based diets generally had lower ADCs of lipids.

4. Discussion

The replacement of FM with SBM in a formulated diet at its 30 % suitable level (Mohd Faudzi et al., 2017) with the supplementation of

Table 2
Growth performance of hybrid grouper TGGG juveniles fed with experimental diets for 10 weeks.

Parameter	Experimental diets						
	FM	**SBM30**	**SBM30 +CA+BA**	**SBM40**	**SBM40 +CA**	**SBM40 +BA**	**SBM40 +CA+BA**
Final weight (g)	70.72 ± 0.74^{b}	69.95 ± 9.07^{b}	86.27 ± 3.68^{c}	55.95 ± 4.74^{a}	70.95 ± 5.84^{b}	65.40 ± 1.31^{ab}	64.61 ± 3.76^{ab}
Weight gain (%)	524.31 ± 22.8^{abc}	589.42 ± 45.60^{abc}	596.17 ± 39.15^{c}	412.73 ± 67.18^{a}	548.28 ± 65.53^{bc}	423.70 ± 18.28^{a}	476.10 ± 43.00^{ab}
Specific growth rate (%/day)	3.27 ± 0.07^{bc}	3.26 ± 0.33^{bc}	3.46 ± 0.10^{c}	2.91 ± 0.23^{a}	3.33 ± 0.18^{c}	2.96 ± 0.06^{ab}	3.12 ± 0.13^{abc}

Different superscripts within rows indicate significant differences between treatments ($p < 0.05$). Values are expressed as means ± standard deviations (n = 3).

Table 3
Feed utilization of hybrid grouper TGGG juveniles fed with experimental diets for 10 weeks.

Feed utilization	Experimental diets						
	FM	**SBM30**	**SBM30 +CA+BA**	**SBM40**	**SBM40 +CA**	**SBM40 +BA**	**SBM40 +CA+BA**
TFI[1] (g/fish)	56.57 ± 2.27^{b}	57.53 ± 7.54^{b}	56.00 ± 7.50^{b}	57.31 ± 4.54^{b}	43.33 ± 2.71^{a}	49.81 ± 4.59^{ab}	52.28 ± 5.88^{ab}
FCR[2]	0.95 ± 0.04^{ab}	0.94 ± 0.0^{b}	0.76 ± 0.08^{a}	1.28 ± 0.15^{c}	0.78 ± 0.07^{ab}	0.99 ± 0.09^{ab}	0.99 ± 0.19^{b}
PER[3]	2.02 ± 0.08^{b}	2.08 ± 0.15^{b}	2.57 ± 0.26^{c}	1.51 ± 0.17^{a}	2.51 ± 0.22^{c}	1.94 ± 0.18^{b}	2.00 ± 0.40^{b}

Different superscripts within rows indicate significant differences between treatments ($p < 0.05$). Values are expressed as means ± standard deviations (n = 3).
[1] TFI: total feed intake;
[2]FCR: feed conversion ratio;
[3]PER: protein efficiency ratio

Table 4
Body condition of hybrid grouper TGGG juveniles fed with experimental diets for 10 weeks.

Body condition	Experimental diets						
	FM	**SBM30**	**SBM30 +CA+BA**	**SBM40**	**SBM40 +CA**	**SBM40 +BA**	**SBM40 +CA+BA**
Condition factor (K)	1.77 ± 0.09^{ab}	1.77 ± 0.03^{ab}	1.81 ± 0.04^{ab}	1.75 ± 0.05^{ab}	1.94 ± 0.13^{b}	1.71 ± 0.07^{a}	1.87 ± 0.15^{ab}
Hepatosomatic index (HSI)	1.69 ± 0.16^{abc}	1.53 ± 0.23^{abc}	1.58 ± 0.25^{abc}	1.94 ± 0.17^{c}	1.78 ± 0.02^{bc}	1.45 ± 0.11^{ab}	1.26 ± 0.22^{a}
Viscerosomatic index (VSI)	11.08 ± 0.24^{bc}	8.30 ± 0.75^{ab}	8.30 ± 1.48^{ab}	10.73 ± 2.32^{bc}	11.24 ± 1.38^{c}	6.98 ± 0.22^{a}	9.06 ± 0.30^{abc}

Different superscripts within rows indicate significant differences between treatments ($p < 0.05$). Values are expressed as means ± standard deviations (n = 3).

Table 5
Whole body composition (dry weight basis) of hybrid grouper TGGG juveniles fed with experimental diets for 10 weeks.

Proximate composition	Experimental diets						
	FM	**SBM30**	**SBM30 +CA+BA**	**SBM40**	**SBM40 +CA**	**SBM40 +BA**	**SBM40 +CA+BA**
Crude Protein	66.24 ± 0.87^{c}	63.64 ± 1.55^{b}	71.84 ± 2.04^{e}	61.08 ± 0.93^{a}	67.32 ± 0.42^{cd}	67.33 ± 1.24^{cd}	69.28 ± 1.03^{d}
Crude Lipid	18.32 ± 5.50	16.51 ± 1.29	13.96 ± 2.16	15.65 ± 1.16	16.10 ± 5.15	13.96 ± 1.68	11.20 ± 5.95
Ash	16.14 ± 0.32	18.52 ± 3.2	15.84 ± 3.02	19.83 ± 1.11	18.14 ± 1.81	17.41 ± 2.20	19.62 ± 0.29

Different superscripts within rows indicate a significant difference between treatments ($p < 0.05$). Values are expressed as means ± standard deviations (n = 3).

Table 6
Apparent Digestibility Coefficient of hybrid grouper TGGG juveniles fed with experimental diets for 10 weeks.

Apparent Digestibility Coefficient	Experimental diets						
	FM	**SBM30**	**SBM30 +CA+BA**	**SBM40**	**SBM40 +CA**	**SBM40 +BA**	**SBM40 +CA+BA**
Dry matter (%)	77.88 ± 0.50^{c}	71.68 ± 0.92^{b}	82.17 ± 0.71^{d}	64.68 ± 0.97^{a}	76.70 ± 1.85^{c}	76.18 ± 0.74^{c}	70.28 ± 1.51^{b}
Crude protein (%)	92.01 ± 0.16^{d}	88.65 ± 0.46^{ab}	93.88 ± 0.27^{e}	87.99 ± 0.34^{a}	93.15 ± 0.42^{e}	90.80 ± 0.43^{c}	88.82 ± 0.76^{b}
Crude lipid (%)	97.1 ± 0.17^{g}	87.25 ± 0.24^{b}	94.87 ± 0.11^{f}	82.47 ± 0.15^{a}	92.79 ± 0.37^{d}	93.33 ± 0.23^{e}	91.22 ± 0.24^{c}

Different superscripts within rows indicate a significant difference between treatments ($p < 0.05$). Values are expressed as means ± standard deviations (n = 3).

the combination of citric and butyric acids enhanced the highest growth of hybrid grouper, although not significantly higher than fish fed FM and SBM30 ($p > 0.05$) in the present study. The present study also showed that the inclusion of SBM at 40 % (SBM40) which is a higher level than the recommended level (30 %), significantly decreased the growth performance of hybrid grouper juveniles. This finding is in agreement with previous studies that had been done on the hybrid grouper (*E. fuscoguttatus* x *E. lanceolatus*) (Firdaus et al., 2016; Mohd Faudzi et al., 2017; Zhang et al., 2021), giant grouper (*E. lanceolatus*) (Lin and Cheng, 2017; Yong et al., 2020) and tiger grouper (*E. fuscoguttatus*) (Shapawi et al., 2013) where growth was observed to deteriorate with the increase of SBM above the suitable replacement level.

However, the supplementation of the organic acids in the 40 % SBM feed improved the growth of hybrid grouper, resulting in comparable growth performance with fish fed the control FM feed. Among the 40 % SBM feeds, hybrid grouper fed with citric acid-supplemented feed (SBM40 +CA) exhibited the highest growth and was significantly higher than those fed SBM40. Hybrid grouper fed with a diet supplemented with the combination of citric and butyric acids (SBM40 +CA+BA) and those fed with butyric acid alone (SBM40 +BA) also demonstrated better growth compared to those fed with SBM40. However, the differences were not statistically significant. The improved growth performance of the hybrid grouper with citric acid supplementation observed in the present study is similar to other previous studies. Anthonious *et al.* (2018) reported that 3 % citric acid supplementation in a feed partially replacing FM with duckweed meal (*Spirodela polyrrhiza*) enhanced the growth, feed utilization, and digestibility of hybrid grouper. Similarly, the supplementation of 1.5 % and 3 % citric acid in the diets of juvenile turbot (*Scophthalmus maximus* L.) with 40 % SBM increased the growth of the fish (Chen et al., 2018). A study on beluga (*Huso huso*) also reported that the supplementation of 3 % citric acid in a 40 % SBM feed improved the growth of the fish compared to the control (Khajepour and Hosseini, 2012). In the present study, citric acid supplementation significantly improved the growth of fish-fed diet with 40 % of SBM, indicating that citric acid can increase the acceptance level of SBM in hybrid grouper from 30 % to 40 %.

The supplementation of butyric acid in the feed in the present study did not improve the fish growth as effectively as the citric acid. Contrary to previous reports, butyric acid has shown positive results in promoting the growth of fishes such as European seabass (*Dicentrarchus labrax*) (Abdel-Mohsen et al., 2018)(Abdel-Mohsen *et al.*, 2018), Asian seabass (*Lates calcarifer*) (Aalamifar et al., 2020), common carp (*Cyprinus carpio*) (Liu et al., 2014), Nile tilapia (*Oreochromis niloticus*) (Dawood et al., 2020) and juvenile turbot (*Scopthalmus maximus* L.) (Liu et al., 2019).

However, some studies reported no significant beneficial effects of butyric acid, particularly on growth when compared to the control. These studies include European seabass (*Dicentrarchus labrax*) (Terova et al., 2016), giant grouper (*E. lanceolatus*) (Lin and Cheng, 2017; Yong et al., 2020), grass carp (*Ctenopharyngodon idella*) (Zhou et al., 2020). These contradictory results obtained among studies cannot simply be compared as the differences could be due to the different fish species, supplementation rates, experimental setup, and plant protein sources (Abdel-Latif et al., 2020; Ye et al., 2019).

In animal nutrition, organic acids and their salts function as growth promoters by influencing both feed quality and gastrointestinal health. In aquafeeds, they are commonly used as preservatives, preventing microbial spoilage and nutrient loss during storage by reducing feed pH and improving hygienic quality (Ng and Koh, 2016; Ricke, 2003; Skrivanova et al., 2006; van Dam, 2006). Additionally, organic acids may directly stimulate the proliferation of gastrointestinal mucosal cells, particularly butyric acid, which supports the growth of intestinal epithelial cells and enhances nutrient absorption (Topping and Clifton, 2001). The enhanced growth with the organic acids particularly citric acid supplementation may be attributed to its ability to improve gastrointestinal tract function by lowering gastric pH through the deposition of H^+ ions which leads to increased pepsin activation. Organic acids also act as chelating agents, binding cations in the intestine to improve mineral absorption, and inhibit harmful microbial colonization, ensuring nutrients are available to the host rather than being utilized by microbes (Ng and Koh, 2016).

Among the factors that lead to decreasing growth when FM is replaced with plant protein are the existence of anti-nutritional factors (ANF) (Lim et al., 2011), deficiency of essential amino acids (Tantikitti et al., 2005), poor palatability (Deng et al., 2006; Lee et al., 2016) and low protein and energy digestibility (Zhang et al., 2014). Also, different fish have different abilities to utilize SBM as a protein source. Many fish species exhibit poor growth and nutrient utilization when fed plant protein diets even at levels less than 50 % replacement (Ahmad et al., 2020; He et al., 2020; Kumar et al., 2017; Ye et al., 2019; Yanti et al., 2019). In the present study, the inclusion of 40 % SBM significantly reduced the growth of the fish.

The growth performance of hybrid grouper in the present study, however, was not affected by varying amounts of fish oil in the SBM-based feed compared to the control FM feed. The fish oil in SBM-based feeds was adjusted to maintain a dietary lipid level of 12 % for hybrid groupers (Yong et al., 2019). Since fishmeal, which was partially replaced by SBM, contains higher lipid levels, adjusting the lipid content in SBM-based feed is necessary to compensate for the lower lipid content (2 %, data not shown) of SBM (Arriaga-Hernandez et al., 2021; Wang et al., 2022). In feeds with 40 % SBM, which contained the same level of fish oil, growth was significantly higher in fish fed SBM40 +CA compared to SBM40. However, there was no significant difference in growth between fish fed SBM40 +CA and those fed the control FM feed or the feed with 30 % SBM that contained lower levels of fish oil. The adjustments in fish oil in the SBM30 and SBM40-based diets did not result in any significant growth trend, indicating that the adjustment of fish oil did not affect the overall growth of the fish.

In the present study, palatability does not seem to be an issue as their feed intake does not differ much among treatments. A previous study stated that in the industrial production of SBM, the heat treatment method that is applied is supposedly to reduce the activities of some ANFs in soybean, particularly trypsin inhibitors and other heat-sensitive ANFs (Liener, 1980). However, this treatment is not effective in removing non-starch polysaccharides, lectins, saponins, and phytic acid (Refstie et al., 1999). SBM generally contains 30 % carbohydrate: approximately 20 % non-starch polysaccharides and 10 % oligosaccharides (Storebakken et al., 2000) and both non-starch polysaccharides and oligosaccharides are known to cause digestive disorders and reduced growth in fish (Hollung et al., 2005). This may be the main reason that substituting FM with high levels of SBM will lead to a decrease in digestibility and growth performance (Liu et al., 2021).

In the present study, the presence of plant proteins influenced the VSI of hybrid grouper juveniles. This could be attributed to the lower fat deposition in the liver which affects the size of the viscera as a whole. This observation is also seen in previous studies on obscure puffer *Takifugu obscurus* (Ye et al., 2019), redlip mullet (*Liza haematocheila*) (Liu et al., 2021) and stinging catfish (*Heteropneustes fossilis*) (Howlader et al., 2023) where increasing replacement of SBM in the diets caused reduction in body lipid, lipid gain and lipid retention of the fish. This decreasing trend involving reduced lipid content as SBM replacement increases in agreement with other previous findings that reported fish fed SBM-based diet normally have hypocholesterolemia due to the presence of the high amount of estrogenic isoflavons and/or inefficient cholesterol in diets (Deng et al., 2014; Taher et al., 2017; Ye et al., 2019).

In the present study, it can also be observed that the diets with the supplementation of citric acid caused a reduction in the size of the liver as can be observed in the HSI values compared to diets without citric acid. As reported in a previous study, high inclusion levels of duckweed showed the appearance of fat changes in the liver which is characterized by the presence of empty spaces in the hepatocytes (Anthonius et al., 2018; Lin and Cheng, 2017; Yong et al., 2020). Dietary acidification has been reported to aid the digestive enzyme activity in fish gut hence optimizing digestion and utilization of nutrients and minerals (Hussain et al., 2011). Carnivorous fish are reported to have a shorter stomach compared to omnivorous and herbivorous species (Day et al., 2014). The supplementation of organic acids which are acidifiers causes absorption and digestion to be more efficient thus stomach of the fish does not need to be big. The presence of organic acids also shortens the absorption and digestion time, and gastric evacuation time and increases metabolic rate (Mirzakhani et al., 2020).

The lower ADC protein of fish fed SBM diet in the present study may be due to the presence of fiber. Dietary plant ingredients can alter the gastrointestinal transit time of feed due to the presence of fibers and sugars which subsequently alters the digestibility of nutrients ingested by the fish (Anthonius et al., 2018). Therefore, with the supplementation of organic acids, protein digestibility was improved as it lowers gastric pH and promotes the rate of pepsinogen to pepsin (Emami et al., 2013). Inclusion of citric acid in the diets for red drum (*Sciaenops ocellatus*) and hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) butyric acid in barramundi (*L. calcarifer*) and yellowfin seabream (*Acanthopagrus latus*) increased the ADC protein of the fish (Aalamifar et al., 2020; Castillo et al., 2014; Li et al., 2009; Sotoudeh et al., 2020).

In conclusion, the results of the present study showed that the supplementation of citric acid and butyric acid in SBM30 (SBM30 +CA+BA) has a positive influence on the growth of grouper. For feed with a higher level of SBM at 40 %, supplementing citric acid in the feed increased the growth of hybrid grouper more efficiently than supplementing butyric acid. With the supplementation of citric acid, the SBM acceptance level by hybrid grouper can be increased to 40 % without compromising the growth.

CRediT authorship contribution statement

Yong Annita Seok Kian: Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Zhuo Li-Chao:** Methodology, Conceptualization. **Lin Yu-Hung:** Writing – review & editing, Supervision, Conceptualization. **Lau Benjamin Yii Chung:** Writing – review & editing. **Yong Christina Seok Yien:** Writing – review & editing. **Shapawi Rossita:** Writing – review & editing. **Abang Zamhari Dayang Nur Jazlyn:** Writing – original draft, Visualization, Investigation, Data curation. **Mohd Faudzi Norfazreena:** Writing – review & editing. **Lim Leong Seng:** Writing – review & editing, Validation.

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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Data availability

Data will be made available on request.

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