The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
21(2): 1632-1646, 2026 www.thebioscan.com

Rearing Performance of Glass Eels (*Anguilla marmorata*) Fed Diets with Different Inclusion Levels of Blended Squid (*Dosidicus gigas*) and Acetes (*Acetes sebogae*)

Isagani P. Angeles Jr., Ph.D.*

Professor VI, Institute of Fisheries, Freshwater Fisheries Center, Isabela State University, Echague Isabela 3309 Philippines
*Corresponding author: Email: isagani.p.angeles@isu.edu.ph

DOI: https://doi.org/10.63001/tbs.2026.v21.i02.pp1632-1646

KEYWORDS

Anguilla marmorata,
Aquafeed,
Formulated diet,
Nursery rearing,
Feed conversion ratio

Received on:
30-05-2026
Accepted on:
19-06-2026
Published on:
29-06-2026

Abstract

This study evaluated the effects of dietary inclusion levels of blended squid (BS) and blended acetes (BA) on the growth performance, survival, and feed utilization of glass eels (Anguilla marmorata) during an eight-week nursery rearing period. Seven dietary treatments were evaluated in a completely randomized design with two replicate tanks per treatment.: a commercial diet (control), diets containing 25%, 50%, and 75% BS, and diets containing 25%, 50%, and 75% BA. Growth performance, survival, feed utilization, and the proximate composition of the experimental diets were assessed to determine the potential of blended squid and blended acetes as alternative dietary ingredients for glass eel culture.

Dietary inclusion levels significantly affected the final weight, weight gain, and specific growth rate of glass eels. Fish fed the 25% BA diet exhibited the highest values for these growth parameters, while the 50% BS diet also showed favorable growth performance. Although feed conversion ratio and survival did not differ significantly among treatments, the 25% BA diet produced the lowest numerical feed conversion ratio. Proximate composition analysis showed that the 25% BA and 50% BS diets contained relatively higher crude protein and balanced lipid levels than most other dietary formulations. Overall, the findings suggest that moderate dietary inclusion levels of blended acetes, particularly at 25%, and blended squid at 50% may enhance the growth performance and feed utilization of glass eels under nursery rearing conditions. Further studies with increased replication and longer culture periods are recommended to validate these findings for broader aquaculture applications.

Introduction

Freshwater eels (*Anguilla* spp.) are among the most valuable aquaculture species worldwide because of their high commercial demand, particularly in East Asian markets where they command premium prices. However, the expansion of eel aquaculture remains constrained by the limited availability of hatchery-produced seed, making wild-caught glass eels the principal source of juveniles for commercial production **(Arai, 2016).** Consequently, improving nursery culture practices has become a priority for enhancing the sustainability and productivity of eel farming.

The Philippines is one of the few countries where high-value freshwater eels occur naturally, placing it in a strategic position to support the development of a sustainable eel aquaculture industry. Freshwater eels, locally known as *kasili*, *igat*, or *palos*, are widely distributed in rivers and estuaries throughout the country **(Shiao, 2003)**. Their high market demand, favorable environmental conditions, and availability of natural glass eel resources provide significant opportunities for commercial production **(Van Herwaarden, 2003)**. Despite these advantages, commercial eel culture continues to face biological and technical constraints that limit production efficiency. Among these, nutritional management remains one of the primary challenges to achieving efficient nursery production.

One of the major challenges in eel culture is optimizing nutrition to achieve efficient growth and feed utilization. Freshwater eels exhibit relatively slow growth and inefficient feed utilization, partly because of their unique feeding behavior and comparatively low digestibility of some dietary ingredients, resulting in poor feed conversion efficiency and prolonged production cycles **(Nawir *et al.*, 2015; Chilmawati *et al.*, 2017).** These constraints highlight the need to develop nutritionally balanced diets that improve growth performance and maximize feed utilization during the nursery stage, a critical phase in eel production.

Marine-derived ingredients are widely recognized as valuable protein sources in aquaculture because of their high digestibility, balanced amino acid composition, and excellent palatability. These characteristics are particularly important for freshwater eels, which exhibit selective feeding behavior and require highly digestible diets to maximize nutrient utilization and growth. Squid (*Dosidicus gigas*) and acetes (*Acetes sibogae*) are rich sources of high-quality protein, essential amino acids, long-chain polyunsaturated fatty acids (EPA and DHA), fat-soluble vitamins (A, D, E, and K), and essential minerals that support growth, metabolism, and feed acceptance **(NRC, 2011; Torrinha *et al.*, 2014).** Consequently, these marine-derived ingredients represent promising alternative dietary protein sources for nursery feeding of *Anguilla marmorata*.

Previous studies have demonstrated the potential of squid-derived ingredients in eel nutrition. **Ndobe *et al.* (2022)** reported that squid meal, incorporated as a feed attractant in diets for *A. marmorata* elvers, improved feed palatability, growth, and feed conversion at an optimal inclusion level of 3%. Likewise, **Huervana *et al.* (2025)** identified moist dough-type diets as the standard feeding strategy for nursery rearing of *A. marmorata*. However, these studies primarily evaluated squid meal as a low-level feed attractant (1–5%) and did not investigate blended squid or blended acetes as major dietary protein ingredients. Moreover, information on the effects of higher dietary inclusion levels of these marine-derived ingredients on the growth performance and feed utilization of glass eels remains limited.

Addressing this knowledge gap is important for developing nutritionally efficient diets for eel aquaculture. Evaluating blended squid and blended acetes at different dietary inclusion levels may provide practical information for improving feed utilization and growth performance while expanding the range of alternative marine-derived protein ingredients available for nursery feeds. Such information is needed to support the development of nutritionally balanced nursery diets for *A. marmorata*.

Therefore, this study evaluated the effects of dietary inclusion levels of blended squid and blended acetes on the growth performance and feed utilization of glass eels (*A. marmorata*) during nursery rearing. Specifically, the study aimed to (1) evaluate the growth performance, survival, and feed utilization of glass eels fed diets containing different inclusion levels of blended squid and blended acetes and (2) determine the proximate composition of the experimental diets. The findings are expected to provide baseline information for developing nutritionally balanced diets that support efficient nursery production of *A. marmorata*.

Material and Methods

Experimental Design

Experimental Fish and Acclimation

Approximately 15,000 glass eels (*A. marmorata*), with a total biomass of approximately 3 kg, were obtained from a private eel consolidator in Aparri, Cagayan, Philippines, and transported to the Aquaculture Experimental Laboratory of the Freshwater Fisheries Center (FFC), Isabela State University, Echague, Isabela.

Upon arrival, the sealed transport bags containing the glass eels were floated in the holding tanks to gradually equalize the water temperature before the fish were released into freshwater. The glass eels were then subjected to a prophylactic salt bath by immersing them in 35 ppt seawater for 30–60 s under continuous aeration following the nursery management protocol for tropical anguillid glass eels described by **Cuvin-Aralar *et al.* (2019).** After treatment, the fish were immediately transferred to freshwater recovery tanks supplied with continuous aeration. No mortality was observed during or immediately after the treatment.

The glass eels were acclimated under laboratory conditions for two weeks before the start of the feeding trial. During this period, the fish were maintained under the same environmental conditions used during the experiment and were fed the commercial eel diet to allow adaptation to the culture system.

The feeding trial was conducted in the Aquaculture Experimental Laboratory of the

Freshwater Fisheries Center, Isabela State University. Fourteen fiberglass tanks (1.0 × 1.0 × 1.0 m) served as the experimental units. Each tank was filled with freshwater to a depth of approximately 0.30 m and continuously aerated throughout the study.

A completely randomized design (CRD) consisting of seven dietary treatments with two replicate tanks per treatment was employed. Each tank was stocked with 1,500 glass eels, corresponding to a stocking density of approximately five individuals L^{-1}. The feeding experiment was conducted for eight weeks.

Table 1. Experimental treatment with blended squid (*D. gigas*) and acetes (*A. sibogae*).

Treatments	Description
1	Control group (Commercial eel diet only)
2	Commercial eel diet + 25% Blended Squid (25BS)
3	Commercial eel diet + 50% Blended Squid (50BS)
4	Commercial eel diet + 75% Blended Squid (75BS)
5	Commercial eel diet + 25% Blended Acetes (25BA)
6	Commercial eel diet +50% Blended Acetes (50BA)
7	Commercial eel diet +75% Blended Acetes (75BA)

Experimental Diets

Seven experimental diets were evaluated. The control treatment consisted of a commercial eel diet, whereas the remaining treatments contained blended squid (BS) or blended acetes (BA) incorporated into the commercial diet at dietary inclusion levels of 25%, 50%, and 75%.

Feed Preparation

Fresh squid and fresh acetes were thoroughly washed with clean water before processing. The raw materials were weighed according to the required dietary inclusion level and steamed whole for approximately 10 min. After steaming, each ingredient was blended separately into a homogeneous paste.

The blended squid or blended acetes was mixed thoroughly with the commercial eel feed and vitamin–mineral premix according to the experimental formulation (Table 2). Water was gradually added while mixing until a uniform dough consistency was obtained. No synthetic binder was added because the natural moisture and protein content of the blended ingredients provided sufficient binding properties. The prepared diets were stored under refrigeration (approximately 2 °C) until use during the feeding trial.

Feeding Management and Water Quality Monitoring

Glass eels were hand-fed twice daily, in the morning and afternoon, using the respective experimental diets. The initial daily feeding rate was equivalent to 20% of the total biomass and was adjusted every two weeks based on the average body weight determined during periodic sampling.

Approximately 1–2 h after each feeding, uneaten feed was removed from the feeding trays, and feeding rates were adjusted according to feed consumption to minimize feed wastage and maintain water quality.

Approximately 50% of the culture water was exchanged 2 h after each feeding using a 1-hp submersible pump. Water temperature (°C), dissolved oxygen (mg/L), and pH were monitored daily before the morning feeding using a portable multiparameter water quality meter. Total ammonia concentration (mg/L) was measured weekly throughout the experiment. Old fishing nets, plastic mesh, and PVC pipes were placed in each tank to provide shelter for the glass eels.

Growth Performance and Feed Utilization

Fish growth was monitored every two weeks by randomly collecting 100 glass eels from each tank and determining their collective body weight. The average body weight obtained during each sampling period was used to adjust the daily feeding rate.

At the end of the eight-week feeding trial, the following growth performance and feed utilization parameters were determined:

Weight gain (WG, g)

WG = Final body weight − Initial body weight

Specific growth rate (SGR, % day^{-1})

SGR = [(ln Final body weight − ln Initial body weight) / Culture period (days)] × 100

Feed conversion ratio (FCR)

FCR = Total feed consumed / Total weight gain

Survival (%)

Survival = (Final number of fish / Initial number of fish) × 100

Proximate Composition Analysis

Samples of the experimental diets were submitted to the Department of Agriculture Regional Field Office II for proximate composition analysis. Moisture content, crude protein, crude fat, crude fiber, and ash were determined following the appropriate AOAC Official Methods. Moisture and ash contents were determined using gravimetric procedures, crude protein by the semi-automatic Kjeldahl method, and crude fat and crude fiber using the ANKOM filter bag technique. The analytical results were expressed on an as-fed basis.

Statistical Analysis

The effects of dietary inclusion levels of blended squid and blended acetes on growth performance, feed utilization, survival, and proximate composition were analyzed using one-way analysis of variance (ANOVA). When significant differences among treatment means were detected, Tukey's Honestly Significant Difference (HSD) test was used for multiple comparisons. All statistical analyses were performed using SAS software version 9.0 (SAS Institute Inc., Cary, NC, USA), and differences were considered statistically significant at $P \leq 0.05$.

Results

1. Growth Performance and Feed Utilization

The effects of dietary inclusion levels of blended squid (BS) and blended acetes (BA) on the growth performance and feed utilization of *A. marmorata* glass eels after eight weeks of nursery rearing are presented in **Table 3**. Initial body weight did not differ significantly among treatments ($P \geq 0.05$), indicating uniformity of the experimental fish at the start of the feeding trial.

Dietary treatment significantly affected final body weight (Wf), weight gain (WG), and specific growth rate (SGR) ($P \leq 0.05$). Fish fed the 25BA diet attained the greatest final body weight (1.132 ± 0.04 g), recorded the greatest weight gain (0.990 ± 0.04 g), and exhibited the highest specific growth rate (3.7 ± 0.06% day^{-1}). These values were significantly higher than those of the control and 25BS treatments but were statistically comparable with those of the 50BS, 75BS, 50BA, and 75BA treatments. The 50BS treatment also showed improved growth performance, with final body weight (1.057 ± 0.01 g), weight gain (0.915 ± 0.00 g), and specific growth rate (3.6 ± 0.01% day^{-1}), although these values were not significantly different from those of the other intermediate dietary treatments.

Feed conversion ratio (FCR) and survival were not significantly affected by dietary treatment ($P \geq 0.05$) (Table 3). The lowest numerical FCR was recorded in the 25BA treatment (2.3 ± 0.18), followed by 75BS (2.5 ± 0.10), whereas the control exhibited the highest FCR (2.9 ± 0.21). Survival ranged from **77.0 ± 0.5%** to **84.0 ± 2.5%** across treatments. The highest survival was observed in the 50BS treatment (84.0 ± 2.5%), followed by 25BS and 75BS (82.0 ± 5.0% and 82.0 ± 6.0%, respectively), while the lowest survival was recorded in the 25BA treatment (77.0 ± 0.5%).

Table 3. Mean (±SE) values of initial weight (Wi), final weight (Wf), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and survival (%) of *A. marmorata* glass eels fed diets containing different dietary inclusion levels of blended squid (BS) and blended acetes (BA) for eight weeks.

Trt[1]	Wi (g)[2]	Wf (g)[3]	WG (g)[4]	SGR[5]	FCR[6]	SUR[7]
C	0.142^{a} (±0.00)	0.896^{b} (±0.01)	0.754^{b} (±0.01)	3.3^{b} (±0.03)	2.9^{a} (±0.21)	80.00^{a} (±3.50)
25BS	0.142^{a} (±0.00)	0.927^{b} (±0.05)	0.785^{b} (±0.05)	3.4^{b} (±0.10)	2.7^{a} (±0.08)	82.00^{a} (±5.00)
50BS	0.142^{a} (±0.00)	1.057^{ab} (±0.01)	0.915^{ab} (±0.00)	3.6^{ab} (±0.01)	2.6^{a} (±0.07)	84.00^{a} (±2.50)
75BS	0.142^{a} (±0.00)	1.015^{ab} (±0.02)	0.873^{ab} (±0.02)	3.5^{ab} (±0.03)	2.5^{a} (±0.10)	82.00^{a} (±6.00)
25BA	0.142^{a} (±0.00)	1.132^{a} (±0.04)	0.990^{a} (±0.04)	3.7^{a} (±0.06)	2.3^{a} (±0.18)	77.00^{a} (±0.50)
50BA	0.142^{a} (±0.00)	1.019^{ab} (±0.04)	0.877^{ab} (±0.04)	3.5^{ab} (±0.07)	2.60^{a} (±0.01)	80.00^{a} (±1.50)
75BA	0.142^{a} (±0.00)	1.004^{ab} (±0.02)	0.862^{ab} (±0.02)	3.5^{ab} (±0.04)	2.6^{a} (±0.12)	80^{a} (±1.50)

Mean (±SE) without a common superscript is significantly different at $p \leq 0.05$
n= 2 replicates per treatment; values are expressed as mean ± SE per tank
[1]Treatments: C= Control (commercial feeds); 25BS = 250 g/kg blended squid, 50BS=500 g/kg blended squid, 75BS=750 g/kg blended squid, 25BA=250 g/kg blended acetes, 50BA=500 g/kg blended acetes, and 75BA=750 g/kg blended acetes
[2]Wi: Initial weight
[3]Wf: Final weight
[4]WG: Weight gain
[5]SGR: Specific growth rate
[6]FCR: Feed conversion ratio
[7]SUR: Survival rate

2. Water Quality

Water quality parameters recorded during the eight-week feeding trial are presented in **Table 4**. Water temperature ranged from **19.3 to 19.6°C**, pH ranged from **7.9 to 8.2**, dissolved oxygen ranged from **4.1 to 4.6** mg/L, and total ammonia remained at **0** mg/L throughout the experimental period. No marked variation in the measured water quality parameters was observed among the dietary treatments.

Table 4. Water quality parameters recorded during the eight-week feeding trial.

Water Parameters[1]	Treatments[2]							Ideal Range (Santos *et al.*, 2023)[3]
	C	25BS	50BS	75BS	25BA	50BA	75BA	
Temp (°Celsius)	19.3 (±0.03)	19.5 (±0.05)	19.4 (±0.05)	19.4 (±0.15)	19.4 (±0.10)	19.6 (±0.05)	19.4 (±0.05)	18–20°C
pH	8.0 (±0.03)	8.0 (±0.10)	8.0 (±0.10)	8.1 (±0.05)	8.0 (±0.00)	8.2 (±0.05)	7.9 (±0.00)	7.5–8.5
DO (mg/L)	4.6 (±0.17)	4.2 (±0.20)	4.1 (±0.05)	4.1 (±0.00)	4.1 (±0.10)	4.2 (±0.15)	4.1 (±0.10)	> 4.0 mg/L
NH_3 (ppm)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0.00-0.5

[1]*Water parameters: Temp- temperature; pH- pH; DO- dissolved oxygen; NH_3- Ammonia*
[2]*Treatments: C= Control (commercial feeds); 25BS = 250 g/kg blended squid, 50BS=500 g/kg blended squid, 75BS=750 g/kg blended squid, 25BA=250 g/kg blended acetes, 50BA=500 g/kg blended acetes, and 75BA=750 g/kg blended acetes*
[3]*Ideal range: Based from Santos et al., 2023*

3. Proximate Composition of Experimental Diets

The proximate composition of the formulated diets containing blended squid (BS) and blended acetes (BA) is presented in **Table 5**. Variations were observed among the dietary treatments in crude protein, crude fiber, crude fat, moisture, and ash contents.

Among the blended squid diets, crude protein ranged from 20.40% to 25.05%**,** with the highest value recorded in the 50BS treatment. Crude fiber ranged from 0.65% to 12.59%, crude fat from 5.40% to 12.53%**,** moisture from 7.90% to 25.65%**,** and ash from 9.49% to 21.10%. The highest crude fiber and ash contents were observed in the 25BS treatment, whereas the highest crude fat content was recorded in the 75BS treatment.

Among the blended acetes diets, crude protein ranged from 22.35% to 30.59%, with the highest value recorded in the 25BA treatment. Crude fiber ranged from 0.26% to 6.03%, crude fat from 1.17% to 8.04%, moisture from 8.14% to 61.00%**,** and ash from 5.40% to 19.43%**.** The 75BA treatment recorded the highest moisture content, whereas the 25BA treatment exhibited the highest crude protein and crude fat contents.

Table 5. Proximate composition (wet basis) of the experimental diets containing blended squid (BS) and blended acetes (BA).

Trt [1]	Proximate Composition[2]				
	Crude Protein %	**Crude Fiber %**	**Crude Fat %**	**Moisture %**	**Ash %**
Ideal range (Cuvin-Aralar *et al.*, 2019 / UF IFAS)[3]	27.0 – 30.0%	<4.8	3.6 – 9.0	40-70	4.2 – 7.2
Control	29.33	0.80	2.00	40.67	11.00
25BS	20.40	12.59	5.40	8.92	21.10
50BS	25.05	3.24	7.59	7.90	11.18
75BS	20.65	0.65	12.53	25.65	9.49
25BA	30.59	4.38	8.04	10.00	19.43
50BA	28.26	6.03	7.05	8.14	18.36
75BA	22.35	0.26	1.17	61.00	5.40

[1]*Treatments: C= Control (commercial feeds); 25BS = 250 g/kg blended squid, 50BS=500 g/kg blended squid, 75BS=750 g/kg blended squid, 25BA=250 g/kg blended acetes, 50BA=500 g/kg blended acetes, and 75BA=750 g/kg blended acetes*
[2]*Proximate composition: Crude protein, Crude fiber, Crude fat, Moisture, and Ash*
[3]*Ideal range: The reference values for crude protein, crude fiber, crude fat, and ash were originally reported on a dry matter basis and were converted to wet basis using the formula: Wet basis (%) = Dry matter basis (%) × [(100 − moisture percentage)/100] to allow direct comparison with the proximate composition values of the formulated diets. Moisture values represent the actual measured moisture content of the diets and were not subjected to conversion.*

Discussion

Growth Performance and Feed Utilization

The present study demonstrated that dietary inclusion of blended squid (BS) and blended acetes (BA) influenced the growth performance of *A. marmorata* glass eels during the eight-week nursery rearing period. Among the dietary treatments, the 25BA diet produced the highest final body weight, weight gain, and specific growth rate, whereas the 50BS diet also supported improved growth compared with the control. In contrast, feed conversion ratio and survival did not differ significantly among treatments. These findings indicate that moderate inclusion levels of marine-derived ingredients can enhance growth performance without adversely affecting feed utilization efficiency or fish survival.

The superior growth observed in the 25BA treatment may be attributed to the nutritional characteristics of acetes. Acetes shrimp are rich in highly digestible protein, essential amino acids, phospholipids, and minerals that support tissue development and normal physiological functions in aquatic animals. Marine-derived ingredients also contain naturally occurring free amino acids and other chemoattractants that enhance feed palatability and stimulate feeding behavior, thereby improving nutrient intake and utilization **(Tacon & Metian, 2015; Glencross *et al.*, 2020; Jiang *et al.*, 2019).** These nutritional characteristics likely contributed to the superior growth performance observed in glass eels fed the 25BA diet.

Similarly, the improved growth obtained with the 50BS diet may be associated with the nutritional quality of squid-derived ingredients. Squid contains highly digestible protein and favorable amino acid profiles together with naturally occurring compounds such as glycine, alanine, and betaine that function as feeding stimulants in many aquatic species **(Torrinha *et al.*, 2014; Tacon & Metian, 2015).** Previous work by **Ndobe *et al.* (2022)** demonstrated that squid meal improved feed acceptance, growth, and feed conversion when incorporated as a feed attractant in *A. marmorata* elvers. Although squid was incorporated at substantially higher inclusion levels in the present study, the results suggest that moderate dietary inclusion (50BS) may provide an appropriate balance between nutrient supply and feed palatability.

The reduced growth response observed at the highest inclusion levels of both marine ingredients indicates that increasing the proportion of a single ingredient beyond an apparent optimum level does not necessarily improve growth performance. Feed quality depends not only on crude protein concentration but also on amino acid balance, nutrient digestibility, dietary energy, and interactions among nutrients **(Glencross *et al.*, 2007; Oliva-Teles *et al.*, 2015).** Excessive inclusion of individual protein sources may alter dietary nutrient balance and reduce the efficiency with which nutrients are utilized for tissue accretion, emphasizing the importance of optimizing ingredient inclusion rather than maximizing dietary concentration.

Although feed conversion ratio did not differ significantly among treatments, the lowest numerical FCR was observed in the 25BA diet, indicating efficient feed utilization. The absence of significant differences may partly reflect the limited statistical power associated with two experimental replicates per treatment. Similar findings have been reported in eel feeding studies where improvements in growth were accompanied by only minor differences in feed conversion efficiency **(Chilmawati *et al.*, 2017; Ndobe *et al.*, 2022).** Likewise, survival remained consistently high across dietary treatments, indicating that the inclusion of blended squid or blended acetes did not adversely affect fish health during nursery culture. Comparable survival has been reported for tropical anguillid eels reared under suitable nutritional and environmental conditions **(Arai, 2016; Cuvin-Aralar *et al.*, 2019).**

Overall, the present findings indicate that moderate dietary inclusion of blended acetes (25BA) and blended squid (50BS) may improve the nursery rearing performance of *A. marmorata*. Nevertheless, additional studies incorporating greater experimental replication, longer feeding periods, and commercial-scale validation would provide stronger evidence for the practical application of these alternative marine-derived ingredients in eel aquaculture.

Water Quality

Water quality remained stable throughout the eight-week feeding trial, with temperature, pH, dissolved oxygen, and total ammonia remaining within acceptable ranges for anguillid eel culture. Maintaining stable environmental conditions is essential because fluctuations in these parameters can influence feeding activity, metabolism, physiological stress, and growth performance **(Arai, 2016; Randall & Tsui, 2002).** The absence of detectable ammonia throughout the culture period further suggests that water management practices were effective in minimizing the accumulation of nitrogenous wastes that could adversely affect fish health.

Because no marked differences in water quality were observed among treatments, the variation in growth performance was more likely attributable to dietary composition than to environmental conditions. Similar observations have been reported in nutritional studies where maintaining uniform water quality minimizes environmental variation and enables more reliable evaluation of dietary treatments **(Arai, 2016).** These findings indicate that the improved performance of the 25BA and 50BS diets was achieved under comparable culture conditions, supporting the interpretation that dietary formulation was the principal factor influencing growth.

Proximate Composition of the Experimental Diets

The differences in growth performance among dietary treatments appear to be closely associated with the nutritional characteristics of the formulated diets. Although all diets contained marine-derived ingredients, the superior growth observed in the 25BA and 50BS treatments suggests that fish performance may have been influenced not only by the inclusion level of squid or acetes but also by differences in the proximate composition of the experimental diets. Marine ingredients are recognized as valuable protein sources because they provide highly digestible proteins, essential amino acids, long-chain polyunsaturated fatty acids, and bioactive compounds that improve nutrient utilization and growth in cultured fish **(Tacon & Metian, 2015; Glencross *et al.*, 2020).**

The 25BA diet produced the greatest growth response and was characterized by relatively high crude protein together with moderate crude fat and crude fiber levels. These nutritional characteristics may have contributed to the observed growth response. A balanced dietary protein-to-energy ratio is essential for efficient protein utilization because sufficient non-protein energy allows amino acids to be directed toward tissue synthesis rather than energy metabolism **(Kaushik & Seiliez, 2010).** The combination of relatively high protein availability and appropriate lipid concentration in the 25BA diet may therefore have enhanced nutrient retention and supported the superior growth performance observed in the present study. Likewise, the 50BS diet, although containing slightly lower crude protein, exhibited moderate lipid and relatively low fiber contents, suggesting a more balanced nutrient profile that was capable of supporting efficient growth. These findings demonstrate that dietary quality depends not solely on crude protein concentration but also on the balance among nutrients that influence digestibility, energy utilization, and protein retention **(Glencross *et al.*, 2007; Oliva-Teles *et al.*, 2015).**

In contrast, increasing the inclusion level of either marine ingredient beyond the apparent optimum did not further improve growth. The comparatively poorer performance of the 75BS diet may be associated with its elevated crude fat content, whereas the 75BA diet contained substantially higher moisture and lower concentrations of crude protein and crude fat. Excess dietary lipid may reduce nutrient balance and limit feed efficiency, while excessive moisture decreases nutrient density by increasing the proportion of water relative to available nutrients **(Tocher, 2015; Hertrampf & Piedad-Pascual, 2009).** These nutritional characteristics may partly explain why higher inclusion levels of squid or acetes did not translate into improved growth despite their marine origin.

The elevated ash content observed in several squid- and acetes-based diets also reflects the mineral-rich composition of marine-derived ingredients, particularly whole acetes containing exoskeletal materials. Although minerals are indispensable for normal physiological processes, excessive ash may reduce the proportion of digestible organic nutrients within the diet and consequently lower nutrient density **(Percot *et al.*, 2008; Glencross *et al.*, 2020).** Optimizing ingredient processing, such as reducing shell fractions or improving formulation strategies, may therefore enhance the nutritional quality of acetes-based feeds without compromising their value as alternative protein sources.

Overall, the results indicate that the superior performance of the 25BA and 50BS diets was associated with a more favorable balance of dietary protein, lipid, fiber, moisture, and mineral contents rather than with crude protein concentration alone. These findings suggest that optimizing the overall balance of dietary nutrients may be more important than maximizing the inclusion level of an individual marine ingredient in the formulation of practical diets for nursery-reared *A. marmorata*. Future studies should further evaluate amino acid composition, fatty acid profiles, nutrient digestibility, and longer feeding periods to better understand the nutritional mechanisms underlying the improved growth responses observed in this study.

Conclusions

The present study demonstrated that dietary inclusion of blended squid and blended acetes influenced the growth performance of *A. marmorata* glass eels during the nursery rearing period. Among the dietary treatments evaluated, the 25BA diet produced the highest final body weight, weight gain, and specific growth rate, while the 50BS diet also supported improved growth compared with the control. Feed conversion ratio and survival were not significantly affected by dietary treatment, indicating that moderate inclusion of these marine-derived ingredients did not adversely influence feed utilization efficiency or fish survival under the conditions of this study.

The findings suggest that moderate inclusion levels of blended acetes and blended squid may support improved growth performance when incorporated into practical diets for nursery-reared *A. marmorata*. However, because the study was conducted over a relatively short culture period with limited replication, further research involving larger sample sizes, longer feeding trials, and evaluation under commercial production conditions is warranted to confirm the consistency and broader applicability of these results.

Conflict of Interest

The authors declare that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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