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Original Article

Apparent digestibility coefficients of selected feed ingredients for Asian seabass, *Lates calcarifer* reared in recirculatory aquaculture system

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Abstract
Digestible nutrient-based feed formulations have become more prevalent in aquaculture, especially in intensive recirculatory aquaculture systems (RAS). In order to maximize growth performance and feed efficiency in Asian seabass (*Lates calcarifer*) raised in RAS while minimizing waste output, it is imperative to assess the apparent digestibility of nutrients from different feed ingredients used in the feed formulations. Accordingly, an eight-week study was conducted to analyse the apparent digestibility coefficients of nutrients in selected protein sources for Asian seabass, *Lates calcarifer*, reared in RAS. The reference diet consisted of commercial seabass feed with 47% crude protein. The test diets were formulated based on a 70:30 ratio approach for ingredient digestibility evaluation using fishmeal (FM), squid meal (SQM), soybean meal (SBM), corn gluten meal (CGM), wheat flour (WF), and broken rice (BR). The reference and test diets were finely ground for homogeneity, mixed with 0.5% commercial binder for stability, digestibility indicator (0.5% chromic oxide), and 2 mm pellets were produced. Growth of fish fed different diets was significantly affected owing to large variations in the inclusion levels of each ingredient. ADC of protein, lipid and energy varied from 81 to 93%, 62.8 to 90.1% and 81 to 90.3% respectively. FM and SQM exhibited significantly higher nutrient digestibility values, as fish are carnivorous. The higher nutrient digestibility of major plant-based protein ingredients suggests a higher quality of source materials. Overall, the present study provides information on the bioavailability of nutrients in animal and plant feedstuffs for Asian seabass reared in RAS.

Keywords: Asian seabass; feed ingredients; nutrient digestibility; RAS

1 | INTRODUCTION

The global aquaculture production is expanding rapidly, driven by both the exponential growth of the world population and increasing per capita consumption of seafood. According to the FAO's 2024 report, global seafood demand is projected to rise significantly due to population growth and dietary shifts towards more protein-rich diets, with aquaculture expected to contribute nearly 60% of the total aquatic animal production by 2030 (FAO 2024). As feed accounts for 50–60% of the total cost of aquaculture production, this immediately increases the pressure on the aquafeed industry. Consequently, there is an urgent need for high-quality formulated feed (Hua *et al*. 2019; Prabhu *et al*. 2019). Advanced technologies, such as recirculatory aquaculture technology (RAS), counterbalance the industry's rapid growth without substantial expansion of land area for aquaculture. These are becoming more significant because of their higher biosecurity, decreased water consumption, and increased output (Badiola *et al*. 2012), but they have certain drawbacks, such as high energy requirements and operational expertise (Meriac *et al*. 2014). One of the key components of RAS that affects fish productivity and the nutrient load from the operations' effluents is the formulated feed. To reduce the accumulation of waste in the system, effluents must be promptly and effectively eliminated because RAS provides increased fish culture through water reuse by filters.

Dietary modifications affect not only fish growth but also water quality and effluent load in the system (Prabhu *et al*. 2019). To improve effluent management in such systems, the diets should be composed of highly palatable, digestible, and nutrient-rich ingredients for efficient digestion and nutrient retention (Riche 2015; Kokou and Fountoulaki 2018; Turchini *et al*. 2019). Furthermore, the use of feed ingredients is affected by several factors, including the raw material (Kokou and Fountoulaki 2018), target species (Refstie *et al*. 2000), freshness of the ingredients (Aksnes and Mundheim 1997), manufacturing and processing of the ingredients (Opstvedt *et al*. 2000; Drew *et al*. 2007), and storage conditions (Camacho-Rodríguez *et al*. 2018). The type and digestibility of protein sources can significantly affect nitrogenous waste output, which in turn influences system performance, biofilter efficiency, and overall environmental sustainability. Therefore, knowledge of the nutrient digestibility of feed ingredients is crucial for formulating feed for animal cultured in higher stocking density systems, such as RAS. One of the most important phases in choosing appropriate feed ingredients to create nutritionally effective diets is determining the apparent digestibility coefficient (ADC) values of feedstuffs (Irvin and Tabrett 2005).

Asian seabass (*Lates calcarifer*) is a fast-growing, euryhaline, carnivorous species of high commercial value in aquaculture (Katya *et al*. 2017; Hong *et al*. 2021). Its ability to tolerate a wide range of salinities and adapt to diverse cultural environments has led to its rapid expansion across many Asian countries to satisfy increasing market demand. Consequently, considerable research attention has been directed toward optimizing feed formulations to enhance growth performance while reducing production costs (Katya *et al*. 2017; Van Vo *et al*. 2020; Hong *et al*. 2021; Madhubabu *et al*. 2021). Although such nutritional strategies have demonstrated promising outcomes in terms of growth and feed utilization, they have largely overlooked the influence of diet composition on culture system dynamics. The specific objective of this study was to evaluate the nutrient digestibility of selected feed ingredients for Asian seabass reared in RAS, using apparent digestibility coefficients (ADC).

2 | METHODOLOGY

2.1 Experimental diet and preparation

A reference diet was formulated using finely ground commercial seabass feed (Uni-President Vietnam Co. Ltd., Vietnam) 2.0 mm pellets containing 47% protein and 10% lipid with 0.5% commercial binder for stability and 0.5% chromic oxide was added as an inert indicator. Six test diets were formulated using 70% of reference diet and 30% each of the test ingredients (Cho and Slinger 1979; Bureau and Hua 2006). Test ingredients comprised fishmeal (FM), squid meal (SQM), soybean meal (SBM), corn gluten meal (CGM), corn gluten meal (CGM), a concentrated nutrient source derived from corn, wheat flour (WF), and broken rice (BR), which were classified as protein and carbohydrate sources respectively. The experimental diets were named as Ref. diet, FM, SQM, SBM, CGM, WF, BR respectively. Proximate composition of the test ingredients is shown in Table 1 and the proximate composition of the experimental diets are presented in Table 2. The diets were prepared in the Aquafeed extrusion mill, Directorate of Incubation and Vocational training in Aquaculture (DIVA), Tamil Nadu Dr. J. Jayalalithaa Fisheries University (TNJFU), Chennai, India. In brief, all the ingredients were finely ground, sieved in a 100-micron mesh and mixed as per the respective formulations in a horizontal mixer (Jinan Sunpring Machinery Co. Ltd.) at 960 rpm for 5 minutes along with the required level of water. The mixture was then processed through twin-screw extruder (Jinan Sunpring Machinery Co. Ltd.) to get 2 mm slow sinking pellets maintaining the steam at five bars and screw temperature at 90–105°C. The pellets were then air-dried and stored in airtight containers.

2.2 Experimental fish and trial

A total of 2,500 Asian seabass (*Lates calcarifer*) fry (0.8±0.2 g) were procured from the Rajiv Gandhi Centre for Aquaculture (RGCA), Sirkazhi, Tamil Nadu, India, and acclimatized in a RAS facility at DIVA, Chennai, India. The fry was reared for one month to attain an initial stocking weight of approximately 5 g. During the nursery phase, fish were graded at seven-day intervals to maintain size uniformity. Initially, the fish were fed with a commercial diet (Uni-President Vietnam Co. Ltd., Vietnam; 0.8 mm sinking pellets containing 520 g kg^{-1} crude protein and 120 g kg^{-1} lipid), and gradually transitioned to a larger pellet size (2.0 mm) with 470 g kg^{-1} protein and 100 g kg^{-1} lipid. After the nursery phase, uniformly sized fingerlings (5.16±0.10 g) were selected, graded to reduce size variation, and randomly stocked into 21 polypropylene tanks

(500 L capacity) at a density of 50 fish per tank. Each treatment was maintained in triplicate and connected to a common RAS filtration unit comprising a sediment filter, drum filter, biofilter, protein skimmer, and UV sterilizer. The feeding trial was conducted for 8 weeks, during which fish were fed at 5% of their body weight per day. Sampling was carried out at fortnightly intervals to adjust feeding rates based on biomass. For digestibility assessment, faecal samples were collected daily by careful siphoning from the tank bottom approximately 2–3 h after feeding to minimize nutrient leaching. Feeding was temporarily suspended prior to collection to avoid contamination from uneaten feed. Care was taken to collect intact fecal strands with minimal disturbance to prevent disintegration. The collected samples were immediately transferred to fine-mesh sieves and gently rinsed with distilled water to remove adhering debris and feed particles without excessive washing. The samples were then blotted dry, pooled by tank, oven-dried at 60°C to constant weight, and stored at −18°C until further chemical analysis.

TABLE 1 Proximate and amino acid composition of feed ingredients (g/100g).

Nutrients	FM	SQM	SBM	CGM	WF	BR
Dry matter	82.58	89.9	92.66	92.68	88.33	90.34
Crude Protein	65.44	59.12	50.62	63.65	12.21	10.06
Crude lipid	9.1	6.54	1.99	2.46	5.05	1.82
Crude fiber	<1	<1	4.77	1.1	2.75	2.1
Ash	12.84	10.39	6.31	1.25	1.62	0.58
GE (MJ/kg)	18.84	17.69	18.41	20.27	16.42	16.14
Essential amino acids						
Arginine	4.2	4.2	5.1	2.0	0.6	0.6
Histidine	1.9	1.0	1.0	1.3	0.2	0.2
Isoleucine	2.8	2.8	1.6	2.5	0.3	0.3
Leucine	6.5	5.4	2.9	11.4	0.6	0.6
Lysine	6.3	6.7	1.1	0.9	0.3	0.3
Methionine	2.4	1.6	1.2	1.4	0.1	0.2
Phenylalanine	2.4	2.3	1.9	3.6	0.5	0.4
Threonine	3.1	2.1	1.5	2.2	0.3	0.3
Tryptophan	1.1	1.3	0.6	0.4	0.1	0.1
Valine	3.1	2.6	2.1	3.1	0.4	0.5
Non-essential amino acids						
Alanine	4.6	4.3	2.0	5.8	0.3	0.5
Aspartic acid	5.8	4.6	5.4	3.8	0.4	0.8
Cysteine	0.8	1.1	0.7	1.1	0.3	0.1
Glutamic acid	8.9	8.6	8.3	12.2	3.0	1.3
Glycine	5.9	4.4	2.0	1.7	0.3	0.4
Serine	2.3	1.9	2.3	3.3	0.4	0.4
Tyrosine	2.8	1.9	7.8	3.4	0.3	0.4

BR = broken rice, CGM = corn gluten meal, FM = fish meal, SBM = soybean meal, SQM = squid meal, WF = wheat flour.

TABLE 2 Ingredient composition of the experimental diets (g/kg of diet).

Ingredients	Ref. diet	FM	SQM	SBM	CGM	WF	BR
Commercial feed	99.0	69.3	69.3	69.3	69.3	69.3	69.3
Fishmeal[1]		30.0					
Squid meal[1]			30.0				
Hi-pro soy[2]				30.0			
Corn gluten[2]					30.0		
Wheat flour						30.0	
Broken rice							30.0
Binder[3]	0.5	0.35	0.35	0.35	0.35	0.35	0.35
Chromic oxide	0.5	0.35	0.35	0.35	0.35	0.35	0.35

[1]Bismi Fisheries Pvt. Ltd., Mayiladuthurai, Tamil Nadu, India; [2]Sakthi soya, Marchinaickenpalayam, Tamil Nadu, India; [3]Pegabind, Bentoli Agri Nutrition India Pvt. Ltd. BR = broken rice, CGM = corn gluten meal, FM = fish meal, SBM = soybean meal, SQM = squid meal, WF = wheat flour.

2.3 Water source

The experimental water was sourced from a nearby creek with a salinity of 25±3 ppt. The water was initially pumped into a reservoir tank and disinfected using bleaching powder containing 33% active chlorine. Subsequently, it was dechlorinated, thoroughly aerated to eliminate residual chlorine, and stored for use. Water exchange was minimal, with replenishment occurring only to compensate for losses due to evaporation and filter backwashing. Water quality parameters were monitored daily and maintained within optimal ranges throughout the experimental period. The recorded values were as follows: temperature (27±1°C), pH (8.0±0.2), dissolved oxygen (6.0±0.5 mg L^{-1}), salinity (25±1 ppt), total ammonia nitrogen (0.32±0.2 mg L^{-1}), nitrite-N (0.05±0.03 mg L^{-1}), and nitrate-N (10.0±5.0 mg L^{-1}).

2.4 Sampling and chemical analyses

At the beginning and end of the feeding trial, the fish were weighed to record the initial and final body weights. Mortality was monitored daily throughout the experimental period to estimate the survival rate and accurately adjust feed intake.

2.5 Growth parameters

The following growth parameters and bioindices were calculated from the obtained data:

Weight gain (g/fish) = Final weight (g) – Initial weight (g)

Survival (%) = (Final No. of fish / Initial No. of fish) × 100

Feed conversion ratio (FCR) = Total feed consumed (g) / Total weight gain (g)

Thermal-unit growth coefficient (TGC) = {[(Final weight)$^{1/3}$ – (Initial weight)$^{1/3}$] / [∑ (water temperature × duration in days)]} × 100

2.6 Proximate analyses

Dry matter, crude protein, lipid, ash, fiber, energy, calcium, and phosphorus were estimated in the prepared diets following the standard protocols of the AOAC (2010) in the Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institute, Namakkal, India.

2.7 Apparent digestibility coefficients

The proximate composition of the faecal samples was determined according to the standard protocols of the AOAC (2010). Chromic oxide was used as an inert marker at 0.5% of diet. The chromium (Cr) content of all the experimental diets and the feces were analysed by inductively coupled plasma atomic emission spectrometer (Arcos-SOP, Spectro Analytical Instruments GmbH, Kleve, DE). The apparent digestibility coefficients (ADC) for the diets were estimated by the following equation:

$$\text{ADC diet} = 100 - \{100 \times [(Cd / Nd) \times (Nf / Cf)]\}$$

where Cd is the concentration of chromic oxide in the diet, Cf is the concentration of chromic oxide in fish feces, Nd is the concentration of nutrients in the diet, and Nf is the concentration of nutrients in fish feces.

The ADCs of nutrients in the test ingredient were calculated from their respective ADCs of the reference and test diets based on a 70:30 substitution of the test ingredient in the RF diet (Cho and Slinger 1979; Bureau and Hua 2006).

$$\text{ADC test ingredient} = \text{ADC test diet} + [(\text{ADC test diet} - \text{ADC ref. diet}) \times (0.7 \times D\ \text{ref.} / 0.3 \times D\ \text{ingredient})]$$

where $ADC_{test\ diet}$ = apparent digestibility coefficient of nutrient in test diet; $ADC_{ref.\ diet}$ = apparent digestibility coefficient of nutrient in reference diet; $D_{ref.}$ = concentration of nutrient in reference diet mash; and $D_{ingr.}$ = concentration of nutrient in test ingredient.

2.8 Data analysis

All collected and calculated data were analyzed using the statistical software package, Statistical Package for the Social Sciences (version 26.0; SPSS, Chicago, IL, USA), and are presented as mean ± standard deviation (SD) of three replicates. Prior to analysis, data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. One-way analysis of variance (ANOVA) was performed, followed by Tukey's post hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

3 | RESULTS

3.1 Growth and feed utilization

The growth and feed utilization of Asian seabass fed different experimental diets are presented in Table 3. These parameters were significantly influenced by dietary treatments, as indicated by the statistical analysis ($p < 0.05$; Table 3), reflecting differences in nutrient composition among the experimental diets formulated using a 70:30 reference-to-test ingredient ratio.

3.2 Apparent digestibility coefficients of reference and test diets

The apparent digestibility coefficients (ADCs) of dry matter ranged from 75% to 78%, with significant differences observed among dietary treatments ($p < 0.05$; Table 4). Similarly, the ADCs of protein (87.7–91.3%) and lipid (83.1–91.9%) varied significantly across treatments ($p < 0.05$), with the highest values recorded in the fishmeal (FM) diet and the lowest in the broken rice (BR) diet (Table 4). Detailed mean values along with standard deviations are presented in Table 4.

3.3 Apparent digestibility coefficients of test ingredients

The apparent digestibility coefficients of the test ingredients are presented in Table 5. The digestibility of nutrients in different ingredients varied based on the source and quality of the ingredients. Asian seabass, being a carnivore, had higher digestibility coefficients for fishmeal and squid meal. Hi-Pro soy and corn gluten meals had higher digestibility coefficient values than carbohydrate sources, wheat flour and broken rice, for which the ADC values were lower.

TABLE 3 Growth and feed utilisation of Asian seabass fed experimental diets.

Parameters	Ref. diet	FM	SQM	SBM	CGM	WF	BR	*p*-value
IBW (g/fish)	5.10±0.05	5.26±0.17	5.16±0.10	5.10±0.02	5.25±0.08	5.15±0.12	5.19±0.09	0.460
FW (g/fish)	45.43±2.19^{a}	44.42±3.16^{ab}	37.35±0.66^{c}	40.28±1.54^{bc}	37.28±0.45^{c}	23.80±1.00^{d}	23.68±0.94^{d}	<0.001
WG (g/fish)	40.33±2.23^{a}	39.17±3.33^{ab}	32.19±0.75^{c}	35.18±1.55^{bc}	32.03±0.45^{c}	18.65±0.11^{d}	18.49±0.90^{d}	<0.001
Survival (%)	98.67±1.15^{a}	98.67±1.15^{a}	98.00±0.00^{a}	95.33±1.15^{a}	98.00±2.00^{a}	98.00±2.00^{a}	94.67±4.16^{a}	<0.001
Feed intake (g/fish)	47.33±1.81^{a}	47.07±2.54^{a}	43.13±0.82^{ab}	43.51±2.25^{ab}	39.48±1.11^{b}	46.08±2.76^{a}	45.58±0.43^{a}	0.001
FCR	1.17±0.03^{d}	1.20±0.05^{d}	1.34±0.04^{cd}	1.24±0.04^{d}	1.23±0.02^{b}	2.48±0.29^{ab}	2.47±0.11^{ab}	<0.001
TGC	0.12±0.004^{a}	0.12±0.01^{a}	0.10±0.002^{a}	0.11±0.003^{a}	0.10±0.001^{a}	0.07±0.004^{b}	0.07±0.003^{b}	<0.001

Values are expressed as means ± SD of three replicate tanks per treatment (n=3). Values within a row with different superscript values indicate significant differences ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's test. IBW, Initial body weight; FW, Final weight; WG, Weight gain; FCR, Feed conversion ratio; TGC, Thermal growth coefficient. BR = broken rice, CGM = corn gluten meal, FM = fish meal, SBM = soybean meal, SQM = squid meal, WF = wheat flour.

TABLE 4 Nutrient digestibility of Asian seabass fed experimental diets.

Parameters	Ref. diet	FM	SQM	SBM	CGM	WF	BR	*p*-value
Dry matter	77.25±0.38^{a}	76.35±0.82^{abc}	75.91±0.51^{bc}	75.43±0.26^{cd}	77.60±0.14^{a}	76.70±1.68^{cd}	74.92±0.48^{d}	<0.001
Protein	90.59±0.32^{ab}	91.3±0.30^{a}	90.29±0.70^{ab}	90.64±0.35^{ab}	90.37±0.34^{ab}	89.12±1.08^{bc}	87.73±0.60^{c}	<0.001
Lipid	91.90±0.56^{ab}	91.30±0.56^{ab}	90.60±0.96^{b}	90.01±0.65^{b}	90.92±0.91^{ab}	86.46±1.73^{c}	83.13±1.18^{d}	<0.001
Energy	91.02±0.21^{a}	90.90±0.19^{a}	88.48±0.26^{b}	87.35±0.15^{b}	88.48±0.20^{b}	85.51±0.84^{c}	83.73±0.36^{d}	<0.001

Values are expressed as means ± SD of three replicate tanks per treatment ($n = 3$). Values within a row with different superscript values indicate significant differences ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's test. FM = fish meal, SBM = soybean meal, SQM = squid meal, WF = wheat flour.

TABLE 5 Nutrient digestibility of test ingredients.

Parameters	FM	SQM	SBM	CGM	WF	BR	*p*-value
Dry matter	74.24±2.12^{ab}	72.79±1.82^{b}	71.18±1.03^{bc}	78.40±1.30^{a}	69.46±3.08^{bc}	66.64±1.23^{c}	<0.001
Protein	93.44±1.10^{a}	90.01±2.33^{ab}	91.19±1.17^{ab}	90.28± 1.11^{ab}	86.13±3.60^{bc}	81.49±1.98^{c}	<0.001
Lipid	90.04±1.87^{a}	87.72±3.21^{a}	85.76± 2.14^{a}	88.81±3.04^{a}	73.94±5.76 ^{b}	62.81±3.9 3^{c}	<0.001
Energy	90.33±0.64^{a}	82.27±1.80^{b}	78.51±0.49^{b}	82.16±1.67^{b}	72.39±3.30^{c}	66.47±1.28^{d}	<0.001

Values are expressed as means ± SD of three replicate tanks per treatment ($n = 3$). Values within a row with different superscript values indicate significant differences ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's test. BR = broken rice, CGM = corn gluten meal, FM = fish meal, SBM = soybean meal, SQM = squid meal, WF = wheat flour.

4 | DISCUSSION

Knowledge of the impact of feed ingredients and their nutrients in diet composition is essential for effective management of fish growth and waste output in aquaculture systems. Aquaculture diets have moved towards formulations based on digestible nutrient and energy standards (Cho and Kaushik 1990). Therefore, one of the clearest ways of determining the nutritional value of the diet to the fish is by assessing the digestibility of nutrients and energy from diets and ingredients (Glencross *et al*. 2004; Aslaksen *et al*. 2007). This measurement denotes the amount of nutrient or energy that is not excreted by the fish and has been utilised for growth and metabolism (Glencross *et al*. 2007). These digestible nutrients and energy are the drivers for feed intake (Glencross 2020) and their variability is one of the critical points of variabilities in the quality of the feed (Bureau *et al*. 1999; Aslaksen *et al*. 2007; Glencross *et al*. 2017; Booth and Pirozzi 2018; Glencross *et al*. 2018).

FM has been used as an essential protein source in formulated diets for carnivorous species owing to its well-balanced nutrient profile (Tacon and Metian 2008; Samaddar 2018). In the present study, the digestibility of FM was higher than that of all the other ingredients, indicating that Asian seabass had higher animal protein digestibility. Studies on FM of different origins by Booth and

Pirozzi (2021) have shown that even within FM of similar nutritional value, digestibility tends to vary depending on the ash content. Fortunately, the ash content was lower in the FM under study. The protein digestibility of their prime-quality FM was 95.6%, which was equivalent to the results of the present study (93.4%), but the protein digestibility of tuna FM used in their study was only 88.3%. This indicates the quality and nutrient availability of different FM sources. A similar study on low-quality FM (53.6% CP) showed very low protein digestibility (78.2%) in African catfish. The digestibility coefficients of Atlantic cod with high- and low-grade FM varied, with the former having better coefficient values (Albrektsen *et al*. 2006). With the aforementioned studies and the results of the present study, sardine FM used in the present study was highly digestible, similar to most prime-quality FM.

Studies on soybean meal digestibility in seabass mostly evaluated the effects at the diet level and not on particular ingredients. In the present study, the protein digestibility was 91%, which is similar to the value reported by Booth and Pirozzi (2021), who recorded the digestibility at 89% in Asian seabass. The authors suggested that the increased protein digestibility might be related to the processing conditions, in which ANF may be deactivated. However, the energy digestibility was higher in the present study (an increase of 7%), which may be due to the increased lipid digestibility. Generally, the factors limiting SBM in the diets of carnivorous fish are the presence of trypsin inhibitors and the phytic acid content of SBM (Venou *et al*. 2006). In addition, the SBM used in the present study was Hi-Pro Soy, in which the protein content was enhanced with processing along with the removal of ANF. The study by Booth and Pirozzi, (2021) also used a soybean meal where the protein content was above 50% stating the lower amount of ANF, thereby confirming the increased digestibility with the processing conditions. The fact that ANF reduces the digestibility of SBM was confirmed in studies conducted with cobia (Suarez *et al*. 2013) and Asian seabass (Glencross *et al*. 2012; Ngo *et al*. 2015; Ma *et al*. 2019), where regular soybean meal was used.

The protein digestibility of CGM in the present study was 90%, which was equivalent to that of SBM. Another study on CGM digestibility in Asian seabass observed a lower digestibility value of only 82%, with an energy digestibility of 71% (Booth and Pirozzi 2021), whereas the energy digestibility in the present study was calculated as 82%. Both experiments were conducted in RAS at similar salinities, with the only difference being the fish size, which might explain the differential digestibility values, as explained by the authors. Further studies are warranted in this section, as the source and processing of CGM could also play a major role in the variation in digestibility values.

The ingredient protein digestibility values for wheat flour and broken rice were similar to those reported by Booth and Pirozzi (2021), whereas dry matter and energy digestibility values were much higher in the present study. The results of the present study were similar to the ingredient digestibility values of wheat reported by Glencross *et al* (2012). The reason for the higher dry matter and energy digestibility is the extrusion process, in which the starch gelatinizes and binds with the ingredients, making it more digestible (Hua and Bureau 2009). This might be the reason for the differences in the digestibility values among the above studies as the diets of the present study and Glencross *et al* (2012) were prepared by twin screw extruder, whereas Booth and Pirozzi (2021) prepared diets by cold pelleting using a meat grinder. Moreover, the dietary inclusions of these ingredients act more like a dietary binder and diluent than a source of protein and energy, as Asian seabass is a carnivore (McMeniman 1996). In addition, they play a vital role in the fecal consistency of fish (Amirkolaie *et al*. 2005; Glencross *et al*. 2012; Booth and Pirozzi 2021). Hence, these ingredients can only be used for pelletizing feeds and not for nutritional requirements of Asian seabass.

In summary, the nutrient digestibility values of the major feed ingredients evaluated in the present study were comparatively high, particularly for fishmeal and squid meal, indicating efficient nutrient utilization by Asian seabass reared in RAS. However, palatability was not directly assessed in this study and therefore cannot be inferred. These findings provide useful insights for the formulation of species- and system-specific feeds for Asian seabass in RAS. It is crucial to take feed composition into account in aquaculture waste management strategies, particularly in recirculation aquaculture systems, because the variations thus produced may have an impact on the efficiency of solid waste collection.

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ETHICAL APPROVAL
All applicable international, national and/or institutional guidelines for the care and use of animals were followed in this study.

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

Kalidoss Manikandan: original draft, data curation, writing – review and editing; Nathan Felix: investigation, conceptualization, methodology, validation; Cheryl Antony: Validation, review and editing; Arumugam Uma: Analysis and Data curation; Elangovan Prabu: supervision, methodology, visualization.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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