

Research

Evaluation of Anchovy By-Products as An Ingredient in The Diets Developed For Red Hybrid Tilapia (*Oreochromis* spp.)

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ABSTRACT

The main objective of the present study was to investigate the possibility of anchovy by-product meal (ABPM) as a protein source in the diet of red hybrid tilapia (*Oreochromis* spp.). Five formulated feeds were produced to contain different percentages of ABPM and soybean meal (SBM): Diet contained 100% SBM with the addition of 1% methionine; Diet contained 25% SBM and 75% ABP25 (ABP25). Diet 3 contained 50% SBM and 50% ABP (ABP50); Diet 4 contained 25% SBM and 75% ABP (ABP75), and Diet 5 contained 100% ABP (ABP100). A commercial tilapia feed was used as a control diet (CF). Fish were fed close to apparent satiation, twice a day to triplicate groups of the tilapia fingerlings (1.07 ± 0.28 g) for 10 weeks. Specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) improved with the increase of ABP inclusion in the diets. Among the ABP-based diets, the highest growth performance and feed utilization were obtained by fish fed with ABP100 (SGR: 3.1%/day; FCR: 1.9) while the least was ABP0 (SGR: 1.5%/day; FCR: 2.6). Hepatosomatic index (HSI) and viscerasomatic index (VSI) of ABP meal-based diets were slightly higher compared to ABP0 and CF (0.5 to 1.5 & 7 to 12.8 respectively). There was no significant difference in fish survival rate and condition factor among all treatment groups. Protein apparent digestibility coefficient (ADC) showed an increasing pattern with increasing ABP meal in the diet and no significant difference in crude lipid ADC among all treatments. Whole-body moisture and crude lipid were not affected by the inclusion of ABPM in the diet, while crude protein and ash parallelly increased with the increase in the inclusion level of ABPM in the diet. Findings from this study indicated that ABPM is a good protein source and could replace SBM as the dietary protein ingredient for better growth performance and feed utilization.

Key words: Fish waste, fish nutrition, fish feed, tilapia

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INTRODUCTION

Around 20 million tons of fish processing and aquaculture by-products were value-added to be fishmeal (FM) and fish oil to feed the aquaculture species, livestock farming, and other animal food (Gabriella, 2016). Fishery by-products are defined as a variety of fish species or by-catch products with no or low commercial value and undersized or damaged commercial species. The by-products are usually removed from the edible part of the body, thus reducing the weight of the valuable part and causing low profitability for the seller. This causes significant economic loss and environmental impacts on the aquatic ecosystem by releasing organic wastes that change the community structure and biodiversity of the benthic assemblages (Fodelianakis *et al.*, 2015; Coppola *et al.*, 2021; Mozumder *et al.*, 2022). Several studies have included fishery by-products as the alternative dietary protein to FM, requiring other protein sources to complement better fish growth and utilization performance (He *et al.*, 2011; Fum *et al.*, 2017; Gowsalya & Kumar, 2018; Kim *et al.*, 2018; Kim *et al.*, 2022)

As feed cost contributes more than 50% of the operational cost, the development of cost-effective feeds is critical in the tilapia aquaculture industry. Protein being the most expensive component in fish feed formulation, must be

supplied from an inexpensive source. Many aquaculture operators still prefer fish meal-based feeds because they perform better than those based on alternative ingredients. Unfortunately, the price of FM has steadily increased in the past years.

Soybean meal is one of the most promising candidates to replace FM. It has a relatively high effective protein content compared to other plant protein sources, a balanced amino acid profile, a consistent supply, and a fair price (Meng *et al.*, 2020; Pervin *et al.*, 2020). However, the effects of substituting SBM for dietary FM on fish growth and health largely depend on the type of fish and feeding habits. It is commonly acknowledged that herbivores and omnivorous fish have better utilization of SBM compared to carnivores' fish (Zhao *et al.*, 2016; Zhang *et al.*, 2018). Various studies proved that SBM could partially replace fishmeal as the dietary protein source in aquafeed (Dessouki, 2016; Yaghoubi *et al.*, 2016; Miao *et al.*, 2018; Carneiro *et al.*, 2020; Obirikorang *et al.*, 2020). In commercial tilapia feed formulation, SBM has been extensively used as a main protein source. However, it has some anti-nutritional factors such as protease and trypsin inhibitors, lectins, phytic acids, saponins, phytoestrogens, anti-vitamins, anti-allergens phytates, tannins, and oligosaccharides that caused low digestibility and growth rate which reduce feed utilization due to its low palatability (Liu *et al.*, 2021; Howlader *et al.*, 2023). In addition, for many countries including Malaysia, SBM has to be imported which increases the carbon footprint of the local aquaculture industry.

In Sabah, dried anchovy trade is considered an essential economic activity, with supply available all year round in almost all fish markets. Dried anchovies are sold in the form of whole-body (including head & viscera) and peeled (after removing head & viscera) at varying prices according to the anchovy sizes. Some broken bodies may also be included during the head and viscera removal process as the dried anchovies are brittle and easily broken. The head, viscera, and broken bodies typically end up with no specific use and reduce the profit of the anchovy suppliers. Anchovy is normally traded in dried form, with supply available all year round in almost all fish markets, either with or without the waste part. Hence, to fully utilize the anchovy and thus optimize the supplier's profit, anchovy could potentially be incorporated into the fish diet as the replacement for FM.

Red hybrid tilapia (*Oreochromis* spp.) is an omnivorous species and highly preferred cultured fish due to its high growth rate, hardiness, and disease resistance. For years, tilapia has been the top three freshwater fish species that are most cultured after grass and silver carp. In 2021, the species produced 4.5 billion metric tonnes, 8.3% of the total world production (FAO, 2022). In the previous study, the fish meal can be replaced by tuna by-products, mackerel by-products, and a mix of Pomfret, stingray, and skipjack tuna waste fed to *Oreochromis niloticus* (~2.21 g to ~5.4 g) at the optimum level of 30% and 50% respectively (Fum *et al.*, 2017; Anizah *et al.*, 2021). The application of anchovy by-products to replace soybean meal at 50% fed to *Osteochilus vittatus* showed the highest growth and feed utilization performance (Zulfahmi *et al.*, 2022)

Anchovy by-products can be easily obtained in Sabah. However, the utilization of anchovy by-products as a dietary feed ingredient as an alternative to SBM has not been explored. Hence, the present study aims to evaluate the possibility of ABPM as an ingredient in the diets developed for red hybrid tilapia (*Oreochromis* sp.) juveniles.

MATERIALS AND METHODS

Diet preparations and proximate analysis

Dried anchovy by-products were obtained from the dry market main anchovy collection station in Papar, Tawau, and Pitas districts of Sabah, Malaysia. The samples separated from foreign objects and unwanted species such as squid paralarvae, juvenile mackerel, krill, and other small marine shrimp species. Each sample source was dried in the oven at 60 °C for 2 h before being ground and sieved with a 250 µm mesh size laboratory sieve. The combination sample from the three districts was prepared by mixing at a 1:1:1 ratio from each source. The anchovy by-product meal (ABPM) was analyzed for proximate composition following AOAC (2003). Five diets were formulated to contain 35% crude protein and 10% crude lipid, by replacing soybean meal with ABPM at 0% (ABP0 + 1% Met), 25% (ABP25), 50% (ABP50), 75% (ABP75), and 100% (ABP100). A diet with no ABPM was added with 1% methionine (ABP0 + 1% Met), considering the low methionine content in SBM used in the present study. Tilapia commercial feed (CF) containing fish meal (CF-crude 32%, crude lipid 4%) was used as a control to compare the commercially available tilapia feed in the market with the experimental diets. Chromic oxide at 1% was added to the formulation as an inert marker to determine the diets' apparent digestibility coefficient (ADC). The ingredients were weighed, ground, and mixed homogeneously. The mixtures were then transferred into a meat grinder to form pellets. The ingredient composition of the experimental diets is shown in Table 1.

Table 1. Ingredient compositions (g/kg) of the experimental diets

Ingredients	Diets (%)				
	ABP0 +Met	ABP25	ABP50	ABP75	ABP100
ABPM	-	16.88	33.76	50.64	67.52
SBM	81.53	54.22	36.15	18.07	-
Tapioca starch	1.66	13.28	15.18	17.07	18.97
CMC	1.50	1.50	1.50	1.50	1.50
Vitamin Premix	3.00	3.00	3.00	3.00	3.00
Mineral Premix	2.00	2.00	2.00	2.00	2.00
Palm Oil	8.81	8.61	7.91	7.22	6.52
Chromic oxide	0.50	0.50	0.50	0.50	0.50
L- Methionine (1%)	1.00	-	-	-	-

* Olein Palm Oil from Lam Soon Group

* Carboxymethyl cellulose (CMC) (Golden Fish, Bake with Me, Malaysia).

* Mineral mixture (g/kg mixture): Calcium phosphate monobasic, 270.98; Calcium lactate, 327.0; Ferrous sulfate, 25.0; Magnesium sulfate, 132.0; Potassium chloride, 50.0; Sodium chloride, 60.0; Potassium iodide, 0.15; Copper sulfate, 0.785; Manganese oxide, 0.8; Cobalt carbonate, 1.0; Zinc oxide, 3.0; Sodium salenite, 0.011; Calcium carbonate, 129.274.

* Vitamin mixture (g/kg mixture): Inositol, 5.0; choline chloride, 75.0; niacin, 4.5; riboflavin, 1.0; pyridoxine HCl, 1.0; thiamine HCl, 0.92; d-calcium pantothenate, 3.0; retinyl acetate, 0.60; vitamin D3, 0.083; Menadione, 1.67; DL alpha-tocopherol, 8.0; d-biotin, 0.02; folic acid, 0.09; vitamin B12, 0.00135. All ingredients were diluted with alpha-cellulose to 1 kg.

Experimental design

The feeding trial was conducted at the Fish Hatchery of Borneo Marine Research Institute, University Malaysia Sabah (UMS) for 10 weeks. Experimental fish was obtained from a local tilapia supplier. The fish were acclimatized in the experimental tanks and fed twice daily with CF at apparent satiation for two weeks before the experiment started. A total of 15 fiberglass tanks (cylindro-conical bottom) with a capacity of 160 L each with a static water system was set up. *Oreochromis* spp. juveniles were obtained from the Borneo Marine Research Institute (UMS), Kota Kinabalu, Sabah.

After the acclimatization period ended, the juveniles (1.07 ± 0.28 g) were randomly assigned to triplicate groups of *Oreochromis* spp. juveniles with 25 fish per cage. Experimental fish were fed twice daily (0800 & 1630 h) at apparent satiation, and the amount of feed consumed was recorded daily for each tank.

Growth and digestibility measurement

Experimental fish were bulk-weighed fortnightly. Two hours after feeding, fecal samples were collected daily by siphoning (Shiau & Liang, 1995) to allow the feces accumulated in the sediment column to be harvested. The collected feces were rinsed with distilled water before being stored in a freezer at -20 °C until enough feces for apparent digestibility coefficient (ADC) analysis. Chromic oxide concentration in each treatment tank will be determined using the acid digestion method (Furukawa & Tsukahara, 1966). Water temperature was maintained at the range of 27 to 30 °C, dissolved oxygen at 6.0 to 9.0 ppm, and pH at 7.0 to 7.5. At the end of the experiment, the juveniles were starved for 24 h before sampling. The total number of fish counted, and individual body weight were measured using a ruler and digital balance (WANT Balance Instrument Co. Ltd, Jiangsu, China).

Proximate composition analysis and body indices

Moisture, crude ash, crude protein, and crude lipid of ABPM, SBM, experimental diets, and fish whole-body were determined using methods described by the Association of Official Analytic Chemistry (AOAC, 2003) on a dry basis. Before the feeding trial started, 10 fish were collected and frozen at -80 °C before proximate analysis. At the end of the feeding trial, fish was starved for 24 h before being harvested to empty the digestive tract. Fish viscera and liver from each treatment tank were excised and weighed to measure the viscerasomatic index (VSI) and hepatosomatic index (HSI). Five fish were sacrificed and kept at -80 °C before whole-body proximate analysis.

Amino acid composition analysis

Amino acids of SBM, ABPM, and experimental diets were determined using the methods described by the Association of Official Analytic Chemistry (AOAC, 1997). Hydrolyzed with 6N HCl at 110 °C for 24 h in AccQ-fluor reagent (Waters, Milford, MA) before chromatographic separation using AccQ-Tag (Merck, Germany) with analytical column (dimension: 150 × 3.9mm). Alliance e2695 (Waters Corporation, Milford, MA) liquid chromatography (LC) with an autosampler and integrated to 2475 Multi-Fluorescence Detector was used to examine derivatized amino acids (Waters Corporation, Milford, MA). Data collection and analysis were carried out using Waters Empower 2 for Microsoft Windows.

Methionine was determined from the same method of acid hydrolysis after treatment with performic acid hydrolysis.

Statistical analysis

IBM Statistical Packages of Social Sciences (SPSS) Version 28.0 was used for statistical analysis. One-way ANOVA was applied to compare the growth performance, feed utilization efficiency, body indices, whole-body composition, and apparent digestibility coefficient. Levene's test was used to test the homogeneity of variances and multiple comparisons among the diet treatments were performed with the Tukey HSD post-hoc analysis test. The significance level was set at $p < 0.05$.

RESULTS

Proximate composition, the amino acid of dietary ingredients (%), and experimental diets

Proximate composition and amino acid profiles of dietary ingredients and experimental diets are shown in Table 2. Generally, nutrient composition in ABPM was in the range of *Oreochromis* spp. nutrient requirement (Santiago & Lovell, 1988). Anchovy by-product meal had a slightly higher crude protein compared to SBM and a moderately higher level of crude lipid. On the other hand, SBM contained much lower crude lipid as it was defatted SBM. Ash content in ABPM was higher compared to SBM. The crude fiber was not detected in ABPM while it was moderately higher in SBM. Essential amino acids found in ABPM were found to be balanced while Methionine was found to be the limiting factor in SBM. Therefore, 1% of Methionine was complementarily included in ABP0 ABP0 (+1% Methionine) to form a balanced amino acid control diet.

Table 2. Nutrient composition of dietary ingredients (% dry matter basis)

Nutrient	ABPM	SBM
Moisture	9.9 ± 0.1	10.51 ± 0.4
Crude Protein	51.8 ± 0.2	48.4 ± 0.1
Crude Lipid	5.2 ± 0.0	1.0 ± 0.0
Crude Ash	31.7 ± 0.1	7.7 ± 0.3
Crude Fiber	n.d	5.7 ± 0.2
NFE	11.4 ± 0.1	37.2 ± 0.1
Essential amino acid		
Histidine	5.9 ± 4.6	2.8 ± 0.0
Arginine	2.1 ± 1.8	7.1 ± 0.0
Threonine	4.5 ± 4.7	4.2 ± 0.0
Valine	5.0 ± 5.1	5.1 ± 0.0
Methionine	3.3 ± 3.7	1.3 ± 0.2
Lysine	6.4 ± 0.8	7.5 ± 0.0
Isoleucine	4.3 ± 4.9	5.2 ± 0.0
Leucine	7.1 ± 8.4	7.9 ± 0.0
Phenylalanine	3.9 ± 5.0	5.4 ± 0.0

* Mean ± standard deviation

* n.d- Not detected

* NFE- Nitrogen-free extract = 100 – (% protein + % lipid + % ash)

There was no significant difference ($P < 0.05$) of moisture, crude protein, and crude lipid in all ABPM-based diets, and ABP0 (+1% Methionine) (Table 3). Crude lipid content in CF do not fulfill the lipid requirement in tilapia juvenile and has a significant difference with all ABPM-based diets and control diet ($P > 0.05$). Ash content increased with the increase of ABPM in the diets and was significantly different with all dietary treatments ($P > 0.05$). The crude fiber was highest in the control treatment. Amino acid profiling analysis of the experimental diets shows a similar trend where the most abundant essential amino acids in the experimental diets were lysine, leucine, and arginine. Methionine content increases with the increase of ABP inclusion in the diet while glutamic acid shows the opposite.

Table 3. Proximate composition and essential amino acids of experimental diets (% dry matter basis)

	Dietary Treatments					
	ABP0 + 1% Met	ABP25	ABP50	ABP75	ABP100	CF
Moisture	8.54 ± 0.3	9.65±0.2	9.52±0.4	9.35±0.3	9.26±0.5	8.46±0.3
Crude Protein	37.5 ± 0.1	35.6 ± 0.3	34.3 ± 0.2	36.7 ± 0.2	35.7 ± 0.3	35.0 ± 0.1
Crude Lipid	9.7± 0.2 ^b	9.7 ± 0.3 ^b	8.9 ± 0.2 ^b	9.1 ± 0.8 ^b	9.1 ± 0.1 ^b	4.3 ± 0.0 ^a
Ash	7.9 ± 0.1 ^a	12.0 ± 0.5 ^c	16.6 ± 0.6 ^d	22.4 ± 0.6 ^e	28.2 ± 0.0 ^f	9.8 ± 0.1 ^b
Crude Fiber	14.7 ± 0.2 ^d	12.5 ± 0.3 ^{cd}	10.6 ± 0.2 ^{bc}	7.2 ± 0.7 ^{ab}	7.0 ± 0.7 ^a	7.1 ± 0.9 ^{ab}
Dry Matter	91.5± 0.1 ^b	89.3± 0.9 ^{ab}	89.6 ± 0.3 ^a	90.7 ± 0.4 ^{ab}	90.7 ± 0.1 ^{ab}	93.5 ± 0.5 ^c
NFE	30.2± 0.9 ^c	30.2 ± 0.0 ^c	29.6 ± 0.1 ^{bc}	19.9 ± 0.7 ^a	23.9 ± 0.8 ^b	43.8 ± 0.1 ^d
Essential amino acids						
Histidine	6.6 ± 0.2	7.1 ± 0.1	6.8 ± 0.1	6.7 ± 0.0	7.9 ± 0.3	6.8 ± 0.3
Arginine	2.4 ± 0.0	2.6 ± 0.1	2.4 ± 0.0	2.5 ± 0.0	3.0 ± 0.2	2.3 ± 0.1
Threonine	4.2 ± 0.1	4.9 ± 0.2	4.8 ± 0.0	5.0 ± 0.2	6.2 ± 0.4	4.4 ± 0.4
Valine	5.5 ± 0.2	5.5 ± 0.2	5.5 ± 0.1	5.8 ± 0.1	6.2 ± 0.5	5.7 ± 0.5
Methionine	3.6 ± 0.1	3.6 ± 0.2	3.7 ± 0.0	3.8 ± 0.0	4.2 ± 0.1	1.7 ± 0.1
Lysine	6.9 ± 0.2	6.4 ± 0.6	7.0 ± 0.3	7.2 ± 0.4	7.4 ± 0.5	6.4 ± 0.5
Isoleucine	5.3 ± 0.2	5.2 ± 0.3	5.0 ± 0.2	5.1 ± 0.1	5.2 ± 0.4	4.8 ± 0.4
Leucine	8.3 ± 0.3	8.2 ± 0.4	8.0 ± 0.2	8.3 ± 0.2	7.8 ± 0.4	8.0 ± 0.4
Phenylalanine	4.8 ± 0.1	5.1 ± 0.4	4.6 ± 0.1	4.5 ± 0.1	5.4 ± 0.4	4.4 ± 0.4

* Values with different superscripts within a row are significantly different ($P < 0.05$)

¹CF, Commercial feed: fish meal-based dietary protein source (Leong Hup International Berhad)

* Essential amino acids: *Mean ± standard deviation

Effect of different inclusion levels of ABP meal on the growth parameters and body indices of *Oreochromis* spp.

This study revealed that the inclusion of ABPM in the feed formulation of *Oreochromis* spp. had better growth and feed utilization performances. The highest percentage weight gain (863.3%) and specific growth rate (3.1%/ day) were attained by fish fed with ABP100 (Table 4). Weight gain and specific growth rate of ABP0 (+ 1% methionine) were significantly lower than other dietary treatments. A similar trend was also shown in PER of all ABPM-based diets (1.4 to 1.5) and CF (2.2). Fish fed with ABP0 +1% methionine and CF were significantly different in PER from the ABP-based diets which were 2.2 and 1.1, respectively. There was a trend of increased feed intake with the increase in ABPM inclusion in the diets. The feed conversion ratio (FCR) ranged from 1.3 to 2.6. The FCR of fish fed with CF performed the best result (1.3) which was significantly different from other treatments ($P > 0.05$). All diets with ABPM inclusion had no significant difference in FCR (1.9) ($P < 0.05$), while ABP0 (+ 1% Methionine) yielded the poorest FCR that was significantly different from other dietary treatments (2.6) ($P > 0.05$). Overall, fish were observed to be in good health conditions. The survival rate of fish was generally high (77.3 to 93.3%) and was not significantly influenced by dietary treatments.

$${}^1\text{Weight gain} = \frac{\text{final weight gain (g)} - \text{initial weight gain (g)}}{\text{initial weight gain (g)}} \times 100$$

$${}^2\text{Specific growth rate (SGR)} = \frac{\text{final weight} - \text{ln initial weight}}{\text{days}} \times 100$$

$${}^3\text{Feed conversion ratio (FCR)} = \frac{\text{feed fed (g)}}{\text{weight gain (g)}}$$

$${}^4\text{Protein efficiency ratio (PER)} = \frac{\text{wet weight gain (g)}}{\text{total protein intake (g)}}$$

$${}^5\text{Survival rate (SR)} = \frac{\text{final fish number}}{\text{initial fish number}} \times 100$$

$${}^6\text{Hepatosomatic index (HSI)} = \frac{\text{liver weight (g)}}{\text{body weight (g)}} \times 100$$

$${}^7\text{Viscerasomatic index (VSI)} = \frac{\text{visceral weight (g)}}{\text{body weight (g)}} \times 100$$

$${}^8\text{Condition factor (CF)} = \frac{\text{fish weight (g)}}{\text{total length (cm}^3\text{)}} \times 100$$

Table 4. Effect of different inclusion levels of ABP meals on the growth parameters and body indices of the experimental diets

Parameters	Dietary Treatments					
	ABP0 + 1% Methionine	ABP25	ABP50	ABP75	ABP100	CF
Initial fish weight (g)	1.1 ± 0.1	1.1 ± 0.10	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.0	1.1 ± 0.1
Final fish weight (g)	3.9 ± 0.4 ^a	8.5 ± 1.8 ^b	9.4 ± 0.9 ^b	9.2 ± 1.3 ^b	10.0 ± 1.8 ^b	8.3 ± 0.2 ^b
¹ Weight gain (%)	261.1 ± 40.3 ^a	682.9 ± 137.8 ^b	791.0 ± 124.0 ^b	757.4 ± 72.4 ^b	863.3 ± 264.5 ^b	676.6 ± 94.1 ^b
² Specific Growth Rate (%/day)	1.5 ± 0.2 ^a	2.8 ± 0.3 ^b	3.0 ± 0.1 ^b	2.9 ± 0.2 ^b	3.1 ± 0.3 ^b	2.8 ± 0.0 ^b
Total Feed Intake (g)	7.1 ± 1.1 ^a	14.0 ± 0.6 ^b	15.8 ± 0.6 ^b	16.2 ± 1.1 ^b	16.7 ± 1.9 ^b	9.5 ± 0.8 ^a
³ Feed Conversion Ratio (FCR)	2.6 ± 0.2 ^b	2.0 ± 0.4 ^{ab}	1.9 ± 0.2 ^{ab}	1.9 ± 0.2 ^{ab}	1.9 ± 0.3 ^{ab}	1.3 ± 0.1 ^a
⁴ Protein Efficiency Ratio (PER)	1.1 ± 0.1 ^a	1.5 ± 0.4 ^b	1.5 ± 0.1 ^b	1.4 ± 0.1 ^b	1.5 ± 0.2 ^b	2.2 ± 0.2 ^c
⁵ Survival Rate (%)	85.3 ± 4.6	77.3 ± 22.0	86.7 ± 8.3	86.7 ± 6.1	93.3 ± 6.2	86.67 ± 11.6
⁶ Hepatosomatic Indices (HSI)	0.6 ± 0.2 ^a	0.9 ± 0.4 ^{ab}	1.2 ± 0.6 ^b	1.5 ± 0.4 ^c	1.5 ± 0.3 ^c	0.5 ± 0.1 ^a
⁷ Viscerasomatic Index (VSI)	9.5 ± 3.2 ^{ab}	9.7 ± 2.9 ^{ab}	10.6 ± 2.1 ^b	11.9 ± 2.5 ^b	12.8 ± 2.6 ^b	7.0 ± 1.2 ^a
⁸ Condition factor (K)	1.0 ± 0.0	1.3 ± 0.2	1.0 ± 0.0	1.0 ± 0.0	1.2 ± 0.2	1.0 ± 0.0

* Values with different superscripts within a row are significantly different ($P < 0.05$)

* Mean ± standard deviation

* Formula below referred to (Kim *et al.*, 2022)

Body indices and proximate composition

All nutrient composition of the whole-body experimental fishes improved compared to the initial fish (Table 5). There was no significant difference ($P < 0.05$) between moisture and crude lipid among the fish. Crude ash was highest in fish-fed ABP75 and ABP100 (6.3%) and the lowest was in CF (4.2%). The crude protein content of ABP25 and ABP50 were significantly different ($P < 0.05$) from ABP75, ABP100, and CF. Crude protein content in the commercial feed, ABP75, and ABP50 were comparable to CF (14.5 to 15.2%).

Table 5. Whole-body proximate composition of experimental fish (% wet weight basis)

Proximate Composition (%)	Dietary Treatments					
	ABP0 + 1% Methionine	ABP25	ABP50	ABP75	ABP100	CF
Moisture	78.9 ± 2.6	76.7 ± 1.8	76.3 ± 1.5	78.4 ± 1.2	77.0 ± 0.7	79.4 ± 2.1
Crude Ash	5.0 ± 0.80 ^{ab}	5.6 ± 0.8 ^{ab}	5.8 ± 1.1 ^{ab}	6.3 ± 1.5 ^b	6.3 ± 1.3 ^b	4.2 ± 0.3 ^a
Crude Protein	11.2 ± 1.6 ^a	13.5 ± 0.5 ^b	13.5 ± 0.8 ^b	15.2 ± 0.9 ^c	14.8 ± 1.1 ^c	14.5 ± 1.1 ^c
Crude Lipid	8.40 ± 0.70	8.3 ± 0.1	8.8 ± 0.4	8.4 ± 0.8	9.5 ± 0.5	9.3 ± 0.6

* Initial whole-body proximate: Moisture: 77.6 ± 0.3; crude ash: 2.6 ± 0.2; crude protein: 9.5 ± 0.5; crude lipid: 6.6 ± 0.5

* Values are the mean of triplicate groups of 25 juveniles.

* Values with different superscripts within a row are significantly different ($P < 0.05$)

Table 6. Apparent Digestibility Coefficient of Experimental Diets

ADC (%)	¹ Dietary Treatments					
	ABP0 + 1% Methionine	ABP25	ABP50	ABP75	ABP100	CF
² Dry Matter	86.3 ± 0.5 ^a	86.9 ± 0.2 ^a	88.1 ± 0.4 ^b	87.6 ± 0.1 ^b	89.7 ± 0.5 ^b	88.7 ± 0.2 ^b
³ Crude Protein	82.2 ± 0.2 ^a	84.7 ± 0.4 ^a	87.6 ± 0.8 ^b	85.3 ± 0.3 ^b	88.5 ± 0.4 ^b	90.8 ± 0.7 ^c
³ Crude Lipid	85.9 ± 0.9 ^a	90.4 ± 0.6 ^b	93.7 ± 1.2 ^b	93.0 ± 0.4 ^b	94.7 ± 1.0 ^b	96.7 ± 0.2 ^c

* Values with different superscripts within a row are significantly different ($P < 0.05$)

¹ Refer to Table 1 for diet designation.

$$^2 \text{ADC of dry matter (\%)} = 100 \times \left[\frac{1 - (\% \text{dietary chromic oxide})}{\text{fecal chromic oxide}} \right]$$

$$^3 \text{ADC of nutrient (protein and lipid) (\%)} = 100 \times \left[1 - \left(\frac{\text{fecal nutrient (\%)}}{\text{dietary nutrient (\%)}} \right) \times \left(\frac{\text{dietary chromic oxide (\%)}}{\text{fecal chromic oxide (\%)}} \right) \right]$$

There was a trend in increasing hepatosomatic index (HSI) and viscerasomatic index (VSI) with the increase of ABPM inclusion in the diet (Table 4). Fish VSI fed with ABP75 and ABP100 significantly differed from other treatments, while fish fed with CF had the lowest VSI and HSI. There was no significant difference in the K factor between all treatments. This indicated that fish in all treatments are in good health condition and grow isometrically.

The treatment diets were highly digestible. There was a significant difference ($P < 0.05$) in the ADC for dry matter (86.3-89.7%), crude protein (82.2-90.8%), and crude lipid (85.9-96.7%) among the experimental diets. Dry matter ADC was highest in ABP100, followed by CF, ABP75, ABP50, ABP25 and ABP0 (+1% methionine). Crude protein and crude lipid feed digestibility had a similar trend, in which CF was the highest followed by ABP100, ABP50, ABP75, ABP25, and finally ABP0 (+1% Methionine).

DISCUSSION

Seasons of the year, life stages, the effect of spawning, migration, and availability of nutrients in the sea contribute to the difference in the nutrient composition of fish, including its by-products (Simat & Bogdanovic, 2012; Putra *et al.*, 2018; Kari *et al.*, 2022). In the present study, anchovy by-products sourced from Sabah had higher content of protein (51.8%) and slightly lower lipid (5.2%) compared to ABPM sourced from Pulau Pasaran, Indonesia (44.43% & 6.35% respectively) and from Pangkor Island, Malaysia (3.19%, 64.88-67.34%, respectively) (Ahmad *et al.*, 2018; Mahrus *et al.*, 2018; Zulfahmi *et al.*, 2022). High ash content in ABPM was consistent with the previous findings found in pollock bones, crab carapace, shrimp by-products and arrow tooth head and viscera, cod, saithe by-products, meager, and gilthead sea bream by-products (Jobling, 2012; Ola *et al.*, 2017; Pateiro *et al.*, 2020). It was expected that ABPM mainly consist of the anchovy head and bones.

All fish fed with ABP-based diets attained good growth and survival performance compared to non-ABPM diets and CF, as the diets contained all essential amino acids. Fish fed with ABP100 yielded the highest growth rate and best FCR, which contained the highest level of essential amino acids. A similar finding was also observed in a study by Ali *et al.* (2015) when the total replacement of SBM with anchovy by-products was evaluated in tilapia (*Oreochromis sp.*).

Lysine and leucine were the most abundant essential amino acids in ABP-based diets which act as feed additives, apart from having an important role in metabolism. Lysine can exclusively enhance protein deposition in the body and fillet content and increase weight gain capacity (Hamid, 2016). Lysine improves muscle growth by stimulating muscle fibers' increasing size and length through hyperplasia and hypertrophy (Michelato *et al.*, 2013). While isoleucine plays a significant role in multiple physiological changes involved in a multitude of physiological processes, including protein synthesis and fatty acid metabolism, and protein synthesis (Du *et al.*, 2012; Ranga Niroshan Appuhamy *et al.*, 2012). Isoleucine also improves growth performance, intestinal immunology, and physical barrier (Zhao *et al.*, 2021). Past studies exhibited that the deficiency of isoleucine affects low feed efficiency to poor growth and low feed efficiency, as reported by common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon Idella*) juveniles (Su *et al.*, 2018), catfish hybrid (Zhao *et al.*, 2021), juvenile olive flounder (*Paralichthys olivaceus*) (Rahimnejad & Lee, 2013). Hence, this explains that the high feed utilization was influenced by the good proportions of leucine and lysine in ABP-based diets and promoted the overall performance of the fish. Therefore, the poor acceptance of ABP0 (+1% Methionine) resulted in low usable nutrients for fish growth. The CF performance was comparable to ABPM-based diets, except for a better FCR. The better FCR in CF is most likely due to the better physical characteristics of the feed as it is produced via the extrusion method. Meanwhile, the experimental diets were produced using a meat mincer which

produced a less stable feed. Although the manufacturer did not disclose the exact inclusion level, CF was labeled to contain FM in the formulation. Based on the current trend in tilapia feed manufacturers, it is anticipated that FM will be included at a minimum level in commercial tilapia feed formulation due to its high cost.

ABPM inclusion in the diets had increased feed palatability and intake. Despite being a fish by-product, ABPM still emits a strong fish smell that acts as an attractant. All diets containing ABPM had a comparable amino acid profile, except for CF, which had slightly lower levels of methionine. Fish require essential amino acids in their diets as they are unable to synthesize them internally. These amino acids are crucial for the proper functioning of cells and tissues and are particularly important for increasing body weight, improving feed efficiency, and increasing fillet yield in tilapia (Li *et al.*, 2021).

In the present study, the high ash content in the ABP-based diet did not negatively affect fish growth and has considerably high dry matter, crude protein, and crude lipid digestibility. This finding is in line with Goddard *et al.* (2008) that used various fishery by-products such as mixed benthic meal (49.72%), catfish by-product meal (42.08%), and tuna cannery waste meal (45.69%), whereby catfish by-products that contained the highest ash and lower dietary protein showed the best growth and feed utilization in tilapia juveniles. By comparing the ABP meal with the other fish by-products, the ABP meal contains higher and balanced essential amino acids that optimize the muscle development of tilapia. Total replacement of salmon by-products in replacing anchovy meal in Pacific white shrimp (*Penaeus vannamei*) without causing impaired growth performance (Guo *et al.*, 2020). The difference in fish growth performance was due to the difference in the quality and composition of the by-products (Tangendjaja, 2015).

Low PER in ABP0 (+ 1% methionine) was likely due to the reduced growth rate and whole-body protein proximate. The high ash content indicated that the ABP-based diets contained high minerals. It is supported by a proximate study by Gencbay and Turhan (2016) that anchovy by-products contain high calcium, phosphorus, and other minerals such as Ferum, Manganese, Natrium, and zinc that are derived from the head and bones. Based on the growth and digestibility performance, tilapia excrete a large proportion of minerals that allow them to maintain relatively normal concentration levels in their bodies (Lall & Kaushik, 2021). Nevertheless, the minerals contributed to good bioavailability in tilapia. There is limited information on the mineral requirements of *Oreochromis* spp. Studies on tilapia mineral requirements are quite challenging to conduct the total dietary intake of a particular mineral experimentally. The fish can absorb significant levels of minerals from the rearing water. However, the presence of high phosphorus, calcium, iron, sodium, and potassium may have also contributed to the tilapia's growth rates fed on anchovy by-products.

There was a trend in the increase of VSI and HSI paralleled with the level of inclusion of ABP in the diet. Fish often accumulate more lipids in the viscera and liver when dietary fat levels rise. This shows that an excess lipid beyond the utilizable limit stored in the liver and internal organs causes an increase in the body mass as well. A substantial amount of the energy in the protein-sparing effect of lipids is derived from dietary lipids, allowing the protein to be used completely for growth with less unavoidable catabolism, leading to growth development and the utilization of nutrients (Sagada *et al.*, 2017). This result is similar to raw anchovy pellets fed to rainbow trout (Uyan, 2007). All treatments had no significant difference in the condition factor (K). This indicated that fish in all treatments were in good condition and grew isometrically without physical deformation.

The high digestibility value of ABPM-based diets indicated that it was a good nutrient source. The nutrient digestibility of ABP-based diets resulted in high ADC values of above 82%, comparable to the FM-based diet (CF treatment). Even though ABPM contained high ash, it did not significantly reduce the fish digestibility or growth. This trend aligned with a previous study using Nile tilapia fed with catfish by-product meal (Goddard *et al.*, 2008). The finding differs with juvenile rose spotted grouper (*Lutjanus guttatus*) fed with tuna by-products (Hernández *et al.*, 2014). Fish by-product digestibility performance related to the inconsistent quality and composition of the by-product applied therefore contribute to the variability of nutrient (Hua *et al.*, 2019). The slightly lower digestibility of diets with higher SBM contents in the present study was also well documented in other studies of juvenile Chinese sucker (*Myxocyprinus asiaticus*) (Yu *et al.*, 2013), juvenile Nile tilapia (*Oreochromis niloticus*) (Sharda *et al.*, 2017), zebrafish (*Danio rerio*) (Valentine & Kwasek, 2022), stinging catfish (*Heteropneustes fossilis*) (Howlader *et al.*, 2023) mainly because of the nature of SBM as a plant-based ingredient and the presence of trypsin inhibitors and other anti-nutritional factors (Liu *et al.*, 2021; Howlader *et al.*, 2023).

Since the ABP meal-based diet was locally sourced, it was cheaper than SBM, which has to be imported. This finding was in line with diets with the previous studies on the combination of SBM with other fishery by-products such as tuna by-products, shrimp by-products meal, shrimp hydrolysates, and other fish by-products (Kim *et al.*, 2019; Abu-Alya *et al.*, 2021).

CONCLUSION

Anchovy by-product meal is a good source of protein to replace SBM in the diets of tilapia fingerlings. Fish growth was highest when fed with 100% of ABPM, without the inclusion of SBM. The fish performance

was comparable to commonly used commercial tilapia feed. Longer-term feeding experiments at the grow-out stage are suggested to study the full potential of ABP in the production of tilapia feeds. Most importantly, using locally available ingredients will reduce the carbon footprint and support the sustainability of the aquaculture industry.

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ETHICAL STATEMENT

Humane protocol was applied when handling the experimental fish. The methodology has been approved by the Animal Ethic Research Committee, University Malaysia Sabah (AEC002/2022).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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