Replacement of Fish Meal with a Combination of Fermented Fish and Soybean Meal in the Diet of Juvenile Asian Seabass, *Lates calcarifer* (Bloch, 1790)

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ABSTRACT

Fish meal is commonly used as a protein source in fish feeds due to its high protein content. However, the supply of fish meal is becoming uncertain due to decreased stocks caused by climate change and overexploitation. Consequently, researchers are exploring less expensive alternatives to replace fish meal in fish feeds. This experiment aimed to determine the effects of replacing fish meal with a combination of soybean meal (SBM) and fermented fish (FF) in the diet on the growth performance and feed utilization of juvenile Asian seabass (*Lates calcarifer* Bloch, 1790). Six isonitrogenous (40%) and isolipidic (5%) experimental diets were formulated. Fish meal was replaced with 0% and 10% of SBM in diet 1 (D1) and diet 2 (D2), respectively. In the other four diets, FM was replaced by gradual levels of SBM and FF as follows (%SBM/%FF): 20/10, 20/20, 30/10, and 30/20 (designated as diet 3-diet 6; D3-D6). A commercial feed was used as a comparative diet (D7). Fish, with an initial weight of 3.32–3.33 g, were fed twice daily until satiation for 90 days. The final body weight (FBW), specific growth rate (SGR), and feed intake (FI) of fish fed D1 were significantly difference ($p<0.05$) but did not significantly differ from those of fish fed D2 and D3. Similarly, the FBW, SGR, and FI of fish fed D3 were not significantly different from those of fish fed D7. In conclusion, fish meal can be replaced by a combination of 20% SBM and 10% FF in the diet for juvenile Asian seabass without any adverse effects on growth performance, body indices and feed utilization.

Keywords: Fish hydrolysate, Fish waste, Plant protein, Seabass, Silage

INTRODUCTION

Recently, aquafeed research has been directed towards the utilization of less expensive renewable ingredients in fish feeds due to concerns about the future availability of fish meal (FM). Soybean meal (SBM) is considered the most ideal replacement for FM. However, some studies have reported that high inclusion levels of SBM can reduce weight gain and feed efficiency, especially in carnivorous fish and shrimp (Tantikitti *et al*., 2005; Phumee *et al*., 2011; Wangsoontorn *et al*., 2018; Cherdkeattipol *et al*., 2021). Therefore, combinations of SBM with other protein sources have been considered (Kader *et al*., 2012; Bulbul *et al*., 2016; Sánchez-Muros *et al*., 2020)

Fish hydrolysate either as fish silage, or produced from by-products from canned fish processing, is a relevant product as a feed ingredient. In Thailand, canned processing in 2023 used 907,713 t of fish and produced 226,678 t of solid waste (Fisheries Development Policy and Planning Division, 2021). It generally shows a beneficial effect on growth performance and feed utilization (Srichanun *et al*., 2014; Li *et al*., 2018). Several

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Received 17 July 2023 / Accepted 27 March 2024

reports have evaluated its efficacy as a protein source in fish feed, including for Indian major carp, rohu, *Labdkieo rohita* (Mondal *et al*., 2007); African sharptooth catfish, *Clarias gariepinus* (Fagbenro and Jauncey, 1995); coho salmon, *Oncorhynchus kisutch* (Murray *et al*., 2003); Nile tilapia, *Oreochromis niloticus* (Oliveira Cavalheiro *et al*., 2007); pacu, *Piaractus mesopotamicus* (Wicki *et al*., 2012); Asian seabass, *Lates calcarifer* (Srichanun *et al*., 2014); and Pacific white shrimp, *Litopenaeus vannamei* (Li *et al*., 2018). Utilization of a combination of SBM and fish hydrolysate integrated into fish feed can significantly benefit in terms of minimizing environmental pollution, reducing the recurring cost of fish production, and improving fish health (Mondal *et al*., 2007; Chataigner *et al*., 2021).

Asian seabass, known as barramundi in Australia or kapong in Thailand, is widely distributed in the Indo-West Pacific region from the Arabian Gulf to China, Taiwan, Papua New Guinea, and Northern Australia. Aquaculture of this species commenced in the 1970s in Thailand (Joerakate *et al*., 2018), and rapidly spread throughout Southeast Asia, (Boonyaratpalin and Williams, 2002). The current worldwide annual production of Asian seabass is around 24,363 t per year, providing approximately 100 billion USD in income (Fisheries Development Policy and Planning Division, 2023). Fish meal is the main protein source in the commercial diet for Asian seabass. Over the past decades, there have been studied on alternative sources of protein. Soybean meal had been wildly used as alternative protein source in aquafeed. Glencross *et al*. (2016) reported that soybean meal and poultry meal have the potential to replace fish meal in the diet for juvenile Asian seabass. An experiment on the replacement of fish meal by soybean meal in Japanese seabass (*Lateolabrax japonicus*) found that fish meal can be replaced by soybean meal up to 160 $g \cdot kg^{-1}$ (Zhang *et al*., 2016).

The objective of this study was to evaluate the effects of replacing fish meal with a combination of soybean meal and fermented fish on growth performance and feed utilization of juvenile Asian seabass.

MATERIALS AND METHODS

Preparation of fermented fish

Fish waste comprising the viscera and head of yellow stripe trevally (*Selaroides leptopis*), was purchased from a canned fish factory in Trang province, Thailand. The fish waste was chopped and mixed with effective microorganisms (EM KYUSEI, EM Kyusei Co,. Ltd. Japan), molasses and water at a ratio of 1: 0.15: 0.5: 0.1 (w/v/w/v). The mixture was then incubated at 40 ºC for four weeks in sealed plastic buckets, after which autolysis was halted by using potassium metabisulfite. The silage was dehydrated in hot air oven at 60 ºC for 24 h. and then ground.

Preparation of experimental diets

Six isonitrogenous (40 % crude protein) and isolipidic (5 % crude lipid) experimental diets were formulated to contain different level of SBM and FF as FM replacement, namely: 0% of SBM (D1), 10% of SBM (D2), 20% of SBM and 10% of FF (D3), 20% of SBM and 20% of FF (D4), 30% of SBM and 10% of FF (D5), 30% of SBM and 20% of FF (D6). A commercial feed was used as a comparative diet (D7). Feed ingredients (Table 1) were mixed in a feed mixer and made into pellets with a diameter of 3 mm with a pelleting machine. The pellets were dried in a hot air oven at 60 °C for 20 h and stored in plastic bags at 4 °C until used. The nutritional composition of the experimental diets was analyzed according to the protocol described by the official methods of analysis of the Association of Official Analytical Chemists (AOAC, 1990), and shown in Table 1.

Fish feeding trial and experimental conditions

Juvenile Asian seabass were purchased from Krabi Coastal Fisheries Research and Development Center, Krabi Province, Thailand. Prior to the experiment, the fish were acclimatized to laboratory conditions in a 1×2 m^2 cement tank and fed a commercial sea bass diet for two weeks. Four hundred and twenty fish (initial body weight = 3.32–3.33 g) were randomly distributed among twenty-one 70 L aquaria (60×30×35 cm^3). Each tank was supplied with seawater (25 ppt) and continuously aerated. The fish were fed twice daily, at 08.00 am and 5.00 pm, until satiation for a duration of 90 days. During the feeding trial, 30% of the water was changed every other day. Dissolved oxygen (DO) levels ranged between 4.10 and 5.50 $mg \cdot L^{-1}$. Individual fish in each tank were weighed at the beginning and end of the experiment, while group weighing was conducted fortnightly to monitor fish growth. Fish were starved for 24 h before weighing. Feed intake was an accumulative feed throughout feed trial.

The following parameters were calculated using the formula mentioned below:

Specific growth rate (SGR, $\% \cdot d^{-1}$)
$= [(\ln Wt - \ln Wi) / T] \times 100$

Relative growth rate (RGR, %)
$= [(Wt - Wi) / Wi] \times 100$

Feed conversion ratio (FCR)
= total feed intake (g) / total wet weight gain (g)

Total feed intake per fish (FI, gf^{-1})
= total feed intake / number of fish

Hepatosomatic index (HSI, %)
= (liver weight / body weight) × 100

Where Wt refers to the mean final weight, Wi is the mean initial weight and T is the feeding trial period in days.

Table 1. Ingredients and nutritional composition of experimental diets.

Ingredients (%)	Experimental diets						
	D1	D2	D3	D4	D5	D6	D7[1]
Fish meal	61.75	55.58	43.24	37.05	37.05	30.88	-
Soybean meal	-	7.92	15.85	15.85	23.78	23.78	-
Fermented fish	-	-	10.59	21.20	10.59	21.20	-
Soybean oil	5.57	5.98	6.57	6.76	6.99	7.17	-
Wheat flour	26.68	24.52	17.75	13.14	15.59	10.97	-
Vitamin mix.[2]	2.00	2.00	2.00	2.00	2.00	2.00	-
Mineral mix.[3]	2.00	2.00	2.00	2.00	2.00	2.00	-
CMC	2.00	2.00	2.00	2.00	2.00	2.00	-
Nutritional composition (% dry matter basis)							
Crude protein	41.08	40.86	40.36	40.68	40.75	40.51	45.24
Crude lipid	5.41	5.40	5.82	5.33	5.61	5.54	4.32
Ash	17.26	16.40	16.54	16.05	14.34	14.80	10.64
Moisture	10.94	10.71	10.32	11.04	10.69	10.33	10.86
GE ($MJ \cdot kg^{-1}$)	17.66	18.37	18.43	18.30	18.82	19.09	18.68
P/E ratio ($g \cdot MJ^{-1}$)	23.26	22.24	21.90	22.23	21.65	21.22	24.22

Note: [1]D1 = solely FM; D2 = FM replaced by 10%SBM; D3 = FM replaced by 20%SBM and 10%FF; D4 = FM replaced by 20%SBM and 20% FF; D5 = FM replaced by 30%SBM and 10% FF; D6 = FM replaced by 30%SBM and 20% FF; D7 = commercial feed; CMC = Carboxy methyl cellulose; GE = Gross energy measured by bomb calorimeter;
[2]Vitamin mix·kg^{-1} (V-MIX): vit A 10,000,000 IU, vit D3 2,000,000 IU, vit E 1,500 IU, thiamine 2 g, riboflavin 2.5 g, pantothenic acid 14 g, pyridoxine 2 g, cyanocobalamin 10 mg, folic 0.5 g, niacin 12 g, vit K3 2 g, vit C 20 g
[3]Mineral mix·kg^{-1} (Good-Minner): Ca 100,000 mg, P 80,000 mg, Cu 2,500 mg, Fe 1,200 mg, Mn 1,200 mg, Zn 1,540 mg, K 260 mg, I 740 mg, Mg 2,160 mg, Se 10 mg, Co 240 mg

Data analysis

Data on growth performance, feed intake, and feed utilization were analyzed using one-way analysis of variance. Significant differences between treatments were evaluated using Duncan Multiple Range Tests (DMRTs). All tests were considered significant at $p < 0.05$. All statistical analysis was performed using SPSS program version 25.

RESULTS

Growth performance

The growth performance, feed intake, and feed utilization of the experimental fish at the end of the feeding trial are summarized in Table 2. Fish fed the diet containing FM as the sole protein source (D1) attained the highest final body weight, although this was not significantly different (p>0.05) from those fed diets containing 10% of SBM (D2), and a combination of 20% of SBM and 10% fish flour (FF) (D3). However, fish fed diets containing 20–30% SBM (D4–D6) exhibited the lowest final body weight. The highest relative growth rate (RGR) was observed in fish fed D1, but it was not significantly different from those fed D2, followedby D3 and the commercial diet (D7). Conversely, the lowest RGR was found in fish fed on D4, D5, and D6. Superior SGR was found in fish fed D1, D2, D3, and D7, while fish fed on D4, D5 and D6 showed inferior SRG.

Feed utilization

The feed conversion ratio (FCR) differed significantly (p<0.05) among treatments. The lowest FCR was observed in fish fed the diet containing 30% of SBM and 10% of FF (D5), which was not insignificantly different from the FCR of fish fed on D2 and D3. Conversely, fish fed D1, D4, D6, and D7 presented higher FCR compared to the others (Table 2).

Additionally, feed intake decreased gradually with increasing SBM levels and showed significant differences (p<0.05) among treatments. Fish fed diet D1 and D2 had the highest feed intake, followed by those fed D3 and D7, while fish fed on D4 D6 showed the lowest feed intake.

Table 2. Growth performance, hepatosomatic index and feed utilization of fish fed on experimental diets for 90 days.

Parameters	Experimental diets						
	D1	D2	D3	D4	D5	D6	D7[1]
IBW (g)	3.33±0.02^{a}	3.33±0.01^{a}	3.33±0.01^{a}	3.32±0.01^{a}	3.32±0.01^{a}	3.33±0.01^{a}	3.33±0.01^{a}
FBW (g)	10.29±0.70^{a}	9.95±0.98^{ab}	8.95±0.55^{ab}	5.50±0.22^{cd}	5.84±0.21^{cd}	5.68±0.33^{cd}	7.78±0.04^{bc}
RGR (%)	209.24±2.48^{a}	199.42±13.10^{ab}	168.39±3.78^{bc}	65.43±6.92^{d}	75.88±6.22^{d}	70.33±10.09^{d}	133.60±1.50^{c}
SGR (% d^{-1})	1.25±0.07^{a}	1.21±0.10^{a}	1.10±0.07^{a}	0.56±0.05^{b}	0.62±0.04^{b}	0.59±0.07^{b}	0.94±0.01^{a}
FCR	1.57±0.16^{b}	1.34±0.17a^{b}	1.11±0.01^{ab}	1.36±0.05^{b}	0.71±0.13^{a}	1.36±0.21^{b}	1.46±0.06^{b}
FI (gf^{-1})	7.09±0.76^{a}	6.12±0.64^{a}	3.58±0.38^{bc}	1.23±0.28^{d}	1.01±0.21^{d}	1.71±0.11^{cd}	3.93±0.17^{b}
HSI (%)	1.17±0.09^{ab}	1.29±0.12^{ab}	1.07±0.08^{b}	1.51±0.10^{a}	1.10±0.03^{b}	1.11±0.07^{b}	0.97±0.02^{b}

Note: Values are mean±SD, obtained from three replications;
Values in the same column with different superscript letters are significantly different (p<0.05);
D1 = FM only; D2 = FM replaced with 10% SBM; D3 = FM replaced with 20% SBM and 10% FF;
D4 = FM replaced with 20% SBM and 20% FF; D5 = FM replaced with 30% SBM and 10% FF;
D6 = FM replaced with 30% SBM and 20% FF; D7 = commercial feed; IBW = initial body weight;
FBW = final body weight; RGR = relative growth rate; SGR = specific growth rate;
FCR = feed conversion ratio; FI = feed intake; HSI = hepatosomatic index

Hepatosomatic index (HSI)

At the conclusion of the feeding trial, the hepatosomatic index (HSI) of the experimental fish exhibited significant differences ($p<0.05$) among treatments (Table 2). Fish fed with D4 demonstrated the highest his, although this difference was not significant ($p>0.05$) compared to fish fed with D1 and D2. Conversely, the lowest HSI was observed in fish fed with D7, with no significant difference compared to the other groups.

DISCUSSION

The present study demonstrates that high levels of SBM inclusion in diet retard the growth of juvenile Asian seabass. Additionally, the result from this study showed low feed intake in fish fed diets containing high SBM content. These findings align with those of Phumee *et al*. (2011), who observed similar effects in striped catfish (*Pangasianodon hypophthalmus*, Sauvage 1878) fry, and Song *et al*. (2014), who reported that a high SBM-based diet resulted in low growth performance in juvenile starry flounder (*Platichthys stellatus*). Tantikitti *et al*. (2005) also noted a decreasing growth performance of Asian seabass with increasing SBM inclusion. This may be attributed to the presence of anti-nutritional factors leading to deficiencies in certain amino acids, low palatability, or high fiber content (García-Rebollar *et al*., 2016).

Incorporating fermented fish into the high SBM-based diet improved the growth and feed intake of fish (Tantikitti, 2014). The present study found that fish fed a diet comprising 20% SBM and 10 % FF diet (D3) had similar final body weight and SGR compared to fish fed on 10 % SBM diet (D2). Additionally, the feed intake of fish fed D3 was equivalent to that of fish fed a commercial diet (D7). Other studies have reported similar results. Sae-alee and Tantikitti (2008) reported that protein hydrolysate produced from tuna viscera improved the attractability and palatability of the diet of giant freshwater prawn (*Macrobrachium rosenbergii*). Tropea *et al*. (2021) reported that fermented fish waste has a high concentration of protein and crude lipids, but low in ash percentage. Fermentation did not affect the concentration of saturated fatty acids, but increased monounsaturated fatty acids. Furthermore, fermentation led to the hydrolysis of substrate protein into low molecular weight compound (less than 10 KD), resulting in immunomodulatory effects and increased palatability and digestibility (Samaddar, 2018). This experiment indicates that incorporating FF can increase fish weight due to the fermentation of fish protein, resulting in low molecular weight proteins that stimulate feed consumption, thus improving feed intake and growth of Asian seabass. Some studies have shown that fermented fish or fish silage serves as an effective feeding stimulant. Refstie *et al*. (2004) found that Atlantic salmon (*Salmo salar*) fed diets containing 10% and 15% of fish protein hydrolysate showed higher feed consumption and growth compared to those without fish protein hydrolysate.

CONCLUSION

The present study demonstrated that fish meal can be replaced by 20% soybean meal and 10% fermented fish in the diet for juvenile Asian seabass. Furthermore, incorporating fermented fish into the diet can enhance feed intake and growth performance of juvenile Asian sea bass.

ACKNOWLEDGEMENTS

This work was funded by the National Research Council of Thailand (NRCT) (current name: National Research Agency). The author wishes to thank the Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya for providing the instruments.

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