Effects of perilla (*Perilla frutescens*) seed on growth performance, feed efficiency, and carcass quality of Asian seabass (*Lates calcarifer*) reared in freshwater

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Abstract. Perilla seed is a superfood because it contains high crude protein, crude lipid, and n-3 polyunsaturated fatty acids (n-3 PUFA). This study aimed to investigate the effects of dietary supplementation with 2.5% and 5.0% perilla seed (PS) flour on growth performance, nutrient utilization, and carcass composition of Asian seabass (*Lates calcarifer*) fed with a soybean oil-based diet. Four experimental diets were prepared: a basal diet containing 12% fish oil was formulated as a control (FO diet). A free-fish-oil soybean oil-based diet containing 12% soybean oil without PS supplementation (0% PS diet), a 2.5% PS diet, and a 5.0% PS diet containing 12% soybean oil were supplemented with 2.5% and 5.0% perilla seed flour, respectively. Asian seabass (an initial body weight of 67.2±2.5 g fish^{-1}) was fed twice daily diets for 68 days. The results showed that survival rate, feed intake, growth rate (body weight gain, specific growth rate, average daily gain), feed utilization (feed conversion ratio, protein efficiency ratio, protein intake, protein retention, and lipid retention), hepatosomatic index, and intraperitoneal index of fish were not significantly affected by dietary supplementation of perilla seed flour ($p>0.05$). The high dietary lipid intake of fish fed a 5.0% PS diet was significantly higher than that fed a control diet and a 0% PS diet ($p<0.05$). A significant difference in the chemical composition of fish carcasses was not observed in this study ($p>0.05$). It was concluded that perilla seed flour could be supplemented in Asian seabass's feed at 5% without negatively impacting the growth performance and feed utilization. However, the fatty acid profile in fish fillets should be further investigated to determine the nutritional value of fillet quality for consumers.

Key Words: growth performance, feed utilization, nutrition retention.

Introduction. Aquaculture products are high-quality protein sources for human food and animal feeds. In aquaculture, fish meal and fish oil are the main animal protein and lipid sources in fish feeds, respectively. Fish meals contain balanced amino acids and are palatable, while fish oil has balanced fatty acid profiles for fish requirements (Tacon et al 2009). However, their availability as fish feed ingredients is limited. Therefore, alternative plant ingredients, such as soybean, palm, sunflower, sesame, olive, rapeseed, flaxseed, and cottonseed, have been researched to replace animal protein and lipids in fish feeds due to their availability and sustainability (Alhazaa et al 2019; Tacon et al 2009; Sánchez-Moya et al 2020).

Among those alternative plant ingredients, soybean meal and oil are generally used as alternative sources of protein and lipids, respectively. Soybean oil is rich in n-6 polyunsaturated fatty acids (n-6 PUFA), especially linoleic acid (18:2n-6). Nevertheless, some studies showed that a high intake of soybean oil results in poor growth and feed utilization observed in fish (Williams et al 2006; Rahman et al 2021), which was associated with an exceeded intake level of n-6 PUFA from soybean oil. Further, feeding the soybean oil-based diet could significantly increase the n-6 PUFA level in fish fillets, affecting fish fillet quality (Guan et al 2023; Menoyo et al 2004).

Plant oilseeds, namely linseed, chia seed, perilla seed, and rapeseed, are rich in n-3 PUFA and have been previously studied in feeds for tilapia and common carp (Fatima et al 2021; Aguiar et al 2011; Silva et al 2014; Dos Santos et al 2014). According to the study by Fatima et al (2021), supplementing a soy-based diet with linseed flour could improve the condition and flesh quality of grass carp. Similarly, feeding the dietary linseed to Nile tilapia could improve the fatty acid composition of tilapia by-products (Aguiar et al 2011). Previous studies have improved the flesh quality of fish fed diets supplemented with omega-3 fatty acid-rich oilseeds such as chia seeds and perilla seeds. These plant oilseeds are considered a superfood because they contain high omega-3 fatty acids. Dietary supplementation of 5% chia seeds as a source of dietary lipid improves the flesh quality of Nile tilapia fed the experimental diet for 45 days, compared to fish fed the diet containing soybean oil (Silva et al 2014). Besides, perilla seed contains 25.38% crude protein and 44.24% crude lipid (Sargi et al 2013). It is well-known as a superfood because of its abundance in the n-3 PUFA level, primarily linolenic acid (18:3n-3), which ranges from 52 to 55.5% of total fatty acids (Sargi et al 2013). Dos Santos et al (2014) showed that feeding perilla seed flour enhanced the n-3 PUFA in tilapia fillets within 45 days in fish fed with a soybean oil-based diet (Dos Santos et al 2014). This study indicated that perilla seed had the potential to enhance the omega-3 in tilapia fillets in 45 days. Unfortunately, this study did not investigate the growth performance of tilapia fed with a diet supplemented with perilla seed flour.

Asian seabass, or Barramundi (*Lates calcarifer*), is an economically significant aquatic species in Southeast Asia and Australia. It is a rich protein source in human diets. The production of this species has increased over the past decades due to the rapid expansion of the global market in different countries (Lim et al 2019). Based on previous studies, it is reported that *L. calcarifer* requires high contents of protein (45-50% of crude protein) and lipid (15-17% of crude lipid) in diets (Williams et al 2003; Glencross 2006; Glencross et al 2013). Thus, alternative plant ingredients to replace fish meal and fish oil in diets have also been intensively studied for *L. calcarifer* diets (Alhazaa et al 2019; Glencross et al 2016). It is necessary to investigate the effect of dietary supplementation of perilla seed on the growth performance, nutrient utilization, and carcass composition of *L. calcarifer* fed with a soybean oil-based diet.

Material and Method

Experimental diet. Perilla seeds used in this study were purchased from Royal Project Doi Kam (Chiang Mai province, Thailand). Perilla seeds (*Perilla frutescens*) were dried at 60ºC for 24 hours and ground as perilla seed flour (PS) before supplementing to the experimental diets at 2.5 and 5.0%. Four experimental diets were prepared: a basal diet containing 12% fish oil was formulated as a control diet (FO diet). A free-fish oil soybean oil-based diet containing 12% soybean oil without PS supplementation (0% PS diet) was also prepared. The 2.5% PS and 5.0% PS diets containing 12% soybean oil were supplemented with 2.5 and 5.0% perilla seed flour, respectively. As presented in Table 1, all powdered ingredients were thoroughly mixed with fish oil or soybean oil, and water was subsequently added to produce stiff dough. The dough was pelleted using a laboratory pellet mill. The diets were stored at −20ºC until use. The samples of perilla seed flour and the experimental diets were analyzed for their chemical composition, including dry matter, crude protein, crude lipid, and ash.

Experimental animal. Three hundred juvenile *L. calcarifer* used in this study were purchased from a commercial farm (Petchburi province, Thailand). Fish were reared in a freshwater recirculating system with an external biofilter with a water refreshing capacity of 10 L min^{-1}. Before the experiment, fish were fed with commercial diets for two weeks to acclimatize. Tanks were scrubbed weekly, and the solid waste was removed from the sump tank daily. The values of temperature, dissolved oxygen, and pH of the water were all measured during the feeding experiment: temperature = 28.0±3.2°C, dissolved oxygen = 6.0±0.8 mg L^{-1}, and pH = 7.9 - 8.2.

Table 1
Ingredient and proximate composition (on wet basis) of experimental diet fed to *Lates calcarifer*

Ingredients (g kg^{-1})	*Dietary treatments*			
	Control	*0% PS*	*2.5% PS*	*5% PS*
Fish oil	120	0	0	0
Soybean oil	0	120	120	120
Perilla seed flour	0	0	25	50
Fish meal	430	430	430	430
Soybean meal	188	188	188	188
Poultry offal meal	82	82	82	82
Corn starch	50	50	50	50
Wheat flour	55	55	55	55
Carboxymethyl cellulose	5	5	5	5
Guar gum	5	5	5	5
Calcium phosphate	5	5	5	5
Vitamin premix	5	5	5	5
Mineral premix	5	5	5	5
Cellulose	50	50	25	0
Total	1000	1000	1000	1000
Analyzed proximate composition (%)				
Dry matter	65.4	66.3	66.2	67.9
Crude protein	24.2	25.4	25.7	26.4
Ether extract	9.9	10.5	11.4	11.9
Total ash	11.3	11.9	12.0	12.5

Control: Diet containing fish oil at 12%; 0% PS: Diet containing soybean oil at 12%; 2.5% PS: Diet containing soybean oil at 12% supplemented with 2.5% perilla seed flour; 5.0% PS: Diet containing soybean oil at 12% supplemented with 5.0% perilla seed flour. Analyzed chemical composition (% as dry matter) of *P. frutescens*: dry matter = 90.95%, ash = 2.77%, crude protein = 17.82%, and ether extract = 42.49%

Feeding protocol, sample collection, and analysis. A group of 20 fish with an initial body weight of 67.2±2.5 g fish^{-1} was randomly allocated to each of the twelve tanks. The initial and final body weights of all the fish were measured individually before the start of the feeding experiment and after it ended. Each tank was randomly assigned to one of four dietary treatments in triplicates. Fish were hand-fed to satiation two times daily for 68 days. Feed intake and mortality were recorded daily. Five fish were randomly collected and stored at −20°C for analysis of the initial chemical composition in the whole body. The fish were starved for 24 hours and euthanized by clove oil following the feeding trial. The weight gain, other growth parameters, and survival rate of each tank's fish were subsequently ascertained by counting and weighing the fish. Two fish per tank at the end of the experiment were randomly collected and stored at -20°C for the analysis of whole-body proximate composition. Proximate compositions were analyzed in experimental diets and in initial and final samples of the fish according to AOAC (1995).

Calculation. At the end of the feeding trial, all the fish were individually weighed and counted for the calculation of final weight gain, body weight gain, average daily gain (ADG), total feed intake, daily feed intake, specific growth rate (SGR), survival rate, hepatosomatic index (HSI), and intraperitoneal fat (IPF), feed conversion ratio (FCR), the protein efficiency ratio (PER), protein retention, and lipid retention. Growth performance parameters, including weight gain, SGR, FCR, and protein and lipid retentions, were calculated as follows:
Body weight gain (g fish^{-1}) = (final weight - initial weight)
Body weight gain (%) = [(final weight - initial weight) / initial weight] x 100
Average daily gain (g day^{-1}) = (final weight - initial weight) / days

Specific growth rate (% day^{-1}) = [(ln final weight - ln initial weight)/days] x 100
Hepatosomatic index (HSI) = 100 x liver weight / body weight
Intraperitoneal fat (IPF) = 100 x intraperitoneal fat weight / body weight
Feed conversion ratio = total feed intake / wet weight gain
Protein efficiency ratio = weight gain / protein intake
Protein retention (%) = 100 x protein gain / protein intake
Lipid retention (%) = 100 x lipid gain / lipid intake.

Statistical analysis. Data were analyzed using GraphPad Software (La Jolla, California, USA), which considered the dietary treatments using one-way ANOVA, followed by Turkey's multiple comparison test. Results were computed as mean and standard deviation. Differences between treatments were considered significant at $p<0.05$.

Results. Perilla seed flour used in this study had 90.95% dry matter. The chemical composition (% as dry matter) was 2.77% ash, 17.82% crude protein, and 42.49% crude lipid. Increasing levels of dietary supplementation of Perilla seed flour resulted in enhancing the crude lipid levels in the experimental diet in this study.

Growth performance. The growth performance of *L. calcarifer* fed the experimental diets for 68 days is presented in Table 2 and Figure 1. Dietary supplementation of perilla seed flour (PS) did not significantly affect the survival rate and feed intake (expressed as both total feed intake (g fish^{-1}) and daily feed intake (% body weight)) of *L. calcarifer* in this study ($p>0.05$). There was no significant difference ($p>0.05$) in the growth rate parameters, namely, the final body weight (g fish^{-1}), body weight gain (g fish^{-1} and %), average daily gain (g day^{-1}), and specific growth rate (% day^{-1}) of *L. calcarifer* fed the different experimental diets ($p>0.05$). The hepatosomatic index of *L. calcarifer* was not significantly different among the dietary treatments ($p>0.05$), besides a significant difference in IPF was not observed in this study ($p>0.05$).

Table 2

Growth performance and feed utilization of *Lates calcarifer* fed the experimental diets for 68 days

Growth parameters	*Dietary treatments*			
	Control	*0% PS*	*2.5% PS*	*5% PS*
Initial body weight (g fish^{-1})	68.4±7.3	66.3±1.3	67.4±1.2	66.8±0.3
Final body weight (g fish^{-1})	134.2±12.7	129.4±2.3	127.5±2.5	133.2±3.5
Body weight gain (g fish^{-1})	65.8±5.4	63.1±2.9	60.1±3.6	66.4±3.6
Body weight gain (%)	96.5±3.0	95.3±5.7	89.2±6.8	99.3±5.7
ADG (g fish^{-1} day^{-1})	1.01±0.08	0.97±0.04	0.92±0.06	1.02±0.06
SGR (% day^{-1})	1.04±0.02	1.03±0.04	0.98±0.06	1.06±0.04
Daily feed intake (%BW)	2.89±0.05	2.99±0.03	2.98±0.06	2.99±0.08
Total feed intake (g fish^{-1})	123.0±10.9	121.8±1.2	121.9±1.1	127.0±4.4
Survival rate (%)	96.7±2.4	81.7±12.5	75.0±10.8	85.0±8.2

ADG-Average daily gain; SGR-Specific growth rate; IPF-Intraperitoneal fat; Control: Diet containing fish oil at 12%; 0% PS: Diet containing soybean oil at 12%; 2.5% PS: Diet containing soybean oil at 12% supplemented with 2.5% perilla seed flour; 5.0% PS: Diet containing soybean oil at 12% supplemented with 5.0% perilla seed flour. Values are the means and standard deviation of three replicates of each treatment. Values in the same row with different superscripts are significantly different ($p<0.05$).

Feed utilization and chemical composition. Table 3 and Figure 2 show the results of feed utilization parameters. Feeding the diets supplemented with Perilla seed flour did not negatively impact the feed conversion ratio, protein efficiency ratio, protein intake, protein retention, and lipid retention of *L. calcarifer* fed the experimental diets for 68 days ($p>0.05$), but lipid intake (Figure A2) of fish fed a 5.0% PS diet was significantly higher than fish fed a control diet and 0% PS diet ($p<0.05$). The chemical composition of the carcass of *L. calcarifer* fed the experimental diet for 68 days was not affected by dietary supplementation of Perilla seed flour (Table 4).

Table 3

Feed utilization of *Lates calcarifer* fed the experimental diets for 68 days

Feed utilization	*Dietary treatments*			
	Control	*0% PS*	*2.5% PS*	*5% PS*
Feed conversion ratio	1.87±0.05	1.93±0.10	2.04±0.13	1.92±0.15
Protein efficiency ratio (%)	2.72±0.22	2.48±0.11	2.34±0.14	2.52±0.14
Protein retention (%)	37.7±0.9	35.2±2.5	34.3±2.0	33.2±1.2
Lipid retention (%)	52.3±6.9	48.6±5.0	50.5±6.1	46.7±3.3

Control: Diet containing fish oil at 12%; 0% PS: Diet containing soybean oil at 12%; 2.5% PS: Diet containing soybean oil at 12% supplemented with 2.5% perilla seed flour; 5.0% PS: Diet containing soybean oil at 12% supplemented with 5.0% perilla seed flour. Values are the means and standard deviation of three replicates of each treatment. Values in the same row with different superscripts are significantly different ($p<0.05$).

Table 4

Proximate composition of carcass (wet basis) of *Lates calcarifer* fed experimental diets for 68 days

Parameters (%)	*Dietary treatments*				
	Initial	*Control*	*0% PS*	*2.5% PS*	*5% PS*
Dry matter	29.6	32.3±0.5	32.4±0.5	33.6±0.6	33.4±0.4
Ash	5.0	5.0±0.5	5.70±0.3	5.8±0.6	5.3±0.5
Protein	16.1	16.6±0.3	16.7±0.1	17.0±0.2	16.4±0.7
Ether extract	7.0	8.3±0.6	8.4±0.3	9.2±0.4	8.8±0.5

Control: Diet containing fish oil at 12%; 0% PS: Diet containing soybean oil at 12%; 2.5% PS: Diet containing soybean oil at 12% supplemented with 2.5% perilla seed flour; 5.0% PS: Diet containing soybean oil at 12% supplemented with 5.0% perilla seed flour. Values are the means and standard deviation of three replicates of each treatment. Values in the same row with different superscripts are significantly different ($p<0.05$).

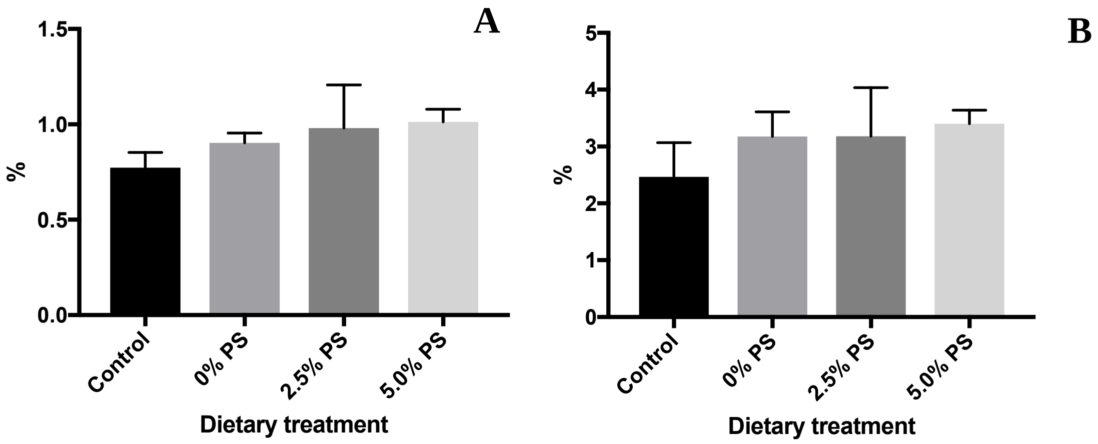

Figure 1. (A) Hepatosomatic index (HSI, % body weight) and (B) intraperitoneal fat index (IPF, body weight) of *Lates calcarifer* fed the experimental diets for 68 days. Values are the means and standard deviation bar of three replicates of each treatment. Abbreviation: Control: Diet containing fish oil at 12%; 0% PS: Diet containing soybean oil at 12%; 2.5% PS: Diet containing soybean oil at 12% supplemented with 2.5% perilla seed flour; 5.0% PS: Diet containing soybean oil at 12% supplemented with 5.0% perilla seed flour.

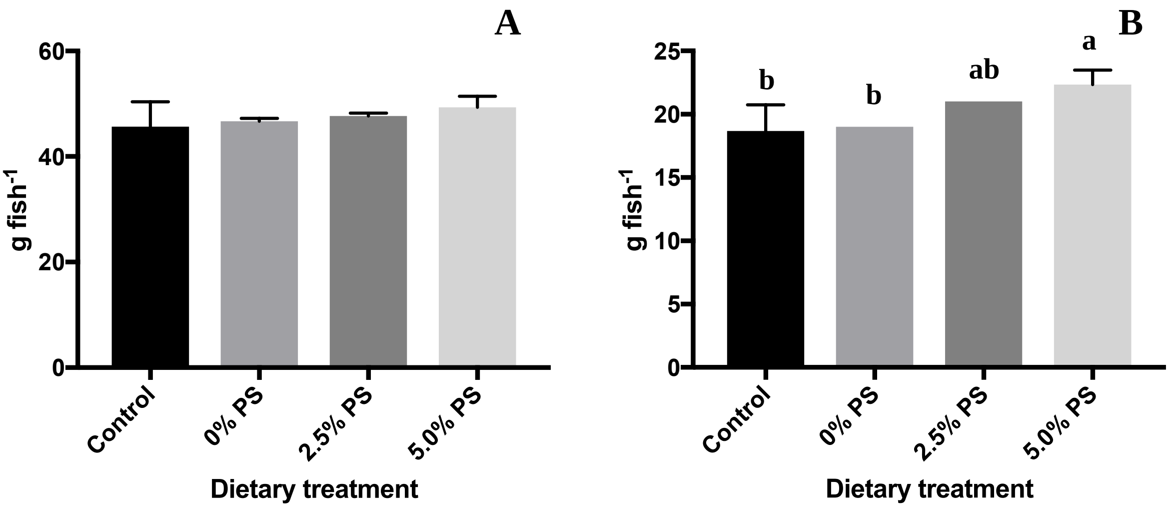

Figure 2. (A) Protein intake (g fish^{-1}) and (B) lipid intake (g fish^{-1}) of *Lates calcarifer* fed the experimental diets for 68 days. Values are the means and standard deviation bar of three replicates of each treatment. Values with different superscript letters are significantly different ($p<0.05$). Abbreviation: Control: Diet containing fish oil at 12%; 0% PS: Diet containing soybean oil at 12%; 2.5% PS: Diet containing soybean oil at 12% supplemented with 2.5% perilla seed flour; 5.0% PS: Diet containing soybean oil at 12% supplemented with 5.0% perilla seed flour.

Discussion. Fish oil is a source of n-3 fatty acids in fish feed, particularly EPA and DHA, as n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA). Unfortunately, fish oil is an unsustainable product; an alternative source of n-3 fatty acids has been seeked intensively. Oilseeds, such as soybean, palm, sunflower, sesame, olive, rapeseed, flaxseed, and cottonseed are commonly used as alternative ingredients in fish feed because of their sustainability and availability. Among those alternative oilseeds, the flaxseed, chia seed, rapeseed, and perilla seed are high in n-3 fatty acids, especially linolenic acid (18:3n-3), which is beneficial in health and anti-inflammatory (Sargi et al 2013). The perilla seed used in our study consisted predominantly of crude lipid (42.94%), surpassed by crude protein (17.82%). Therefore, supplementing the diet with perilla seed flour enhanced the crude lipid content in the 2.5% PS diet and 5.0% PS diet. Soltan (2005) also discovered that crude lipid increased when linseed flour was supplemented in the diet. Oilseeds (perilla seed and flaxseed) contained a high crude lipid content, ranging from 37 to 42% (Sargi et al 2013).

The study of perilla seed flour incorporation in fish feed is very limited (Dos Santos et al 2014). Our study is the first report on the effect of dietary supplementation of perilla seed flour on growth performance, feed utilization, and chemical composition in the carcass of *L. calcarifer*. As a result of our study, *L. calcarifer* fed with the experimental diets for 68 days showed a regular growth rate, with SGR ranging from 1.04 to 1.06 % day^{-1}. Further, there was no significant difference in the survival rate, which ranged from 75 to 96.7% in this study. This signifies that the dietary supplementation of perilla seed flour in *L. calcarifer* diets did not negatively impact the growth and survival rate of the fish. Moreover, dietary supplementation of 5% perilla seed flour did not significantly affect the feed utilization, such as FCR, PER, lipid intake, lipid retention, and protein retention, of *L. calcarifer* fed with the experimental diet for 68 days in our study. Our study is in line with chia seed flour; Silva et al (2014) found that feeding a diet containing 5% chai seed flour for 45 days did not statistically affect the growth rate of Nile tilapia (initial body weight of 7.4 g fish^{-1}, *Oreochromis niloticus*). Soltan (2005) investigated the effects of dietary inclusion of linseed flour at 11.4%, 22.8%, 34.2%, and 45.6% in diets in replacement of soybean meal protein at levels of 25, 50, 75, and 100%, on growth performance of juvenile Nile tilapia (initial body weight of 2.76-2.91 g fish^{-1}). They found that the growth performance of fish was significantly depressed when feeding up to 22.8% dietary linseed flour in diets. This study suggested that linseed could be included in the diet of juvenile Nile tilapia at 11.4% without detrimental impact on the growth performance. Mazurkiewicz et al (2011) demonstrated that the growth performance and feed conversion ratio of common carp (initial body weight of 500 g fish^{-1}) was not significantly affected by feeding diets containing graded levels of cold-pressed rapeseed cake ranging from 11-33% in diets. Although our study did not detect a significant difference in HSI and IPF of *L. calcarifer* fed a 2.5% PS diet and a 5.0% PS diet, the increased tendency of HSI and IPF of *L. calcarifer* fed a 2.5% PS diet and a 5.0% PS diet might be associated with a high intake of dietary lipid of fish fed a 2.5% PS diet and 5.0% PS diet (Figure 2A and 2B), which resulted from Perilla seed flour supplementation.

Feeding the dietary oilseed could affect the chemical composition of fish carcasses. It was reported that feeding diets containing 22% and 33% cold-pressed rapeseed cake in diets significantly enhanced dry matter and crude lipid of common carp (Mazurkiewicz et al 2011). Additionally, feeding the diets 22.8% and 45.6% rapeseed meal significantly reduced crude lipid and crude protein in the carcass of Nile tilapia, respectively. Meanwhile, the ash content was increased in the carcass of Nile tilapia fed with a diet containing 45.6% linseed meal (Soltan 2005). In contrast, our study did not observe a significant difference in the chemical composition, namely dry matter, crude protein, crude lipid, and ash of the carcass of *L. calcarifer*. Our result is in accordance with Dos Santos et al (2014), who reported that feeding a diet containing 5% perilla seed flour did not alter the crude protein and crude lipid in the fillets of Nile tilapia during the 60-day feeding period. However, feeding low levels of dietary perilla seed flour did not improve the growth performance of *L. calcarifer* in our study. Nonetheless, there was obvious evidence demonstrating that small amounts of 5% dietary supplementation of perilla seed flour, chai seed flour, and linseed flour significantly improved the nutritional quality of fish fillets, fish carcasses, and fish by-products (Dos Santos et al 2014; Nishiyama et al 2014; Silva et al 2014; Zanqui et al 2016). Dos Santos et al (2014) compared the muscle quality of tilapia fed with the soybean oil-based diet to that fed a diet containing 5% perilla seed flour, during the 60 day-feeding period. They observed that perilla seed flour significantly increased the content of the n-3 polyunsaturated fatty acids (n-3 PUFA) such as linolenic acid (18:3n-3), arachidonic acid (20:4n-3), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) in the fillets of tilapia. Moreover, Nishiyama et al (2014) reported that dietary supplementation of 5% linseed flour significantly enhanced the muscle quality of tilapia. The linolenic acid content was significantly increased at 50% in the muscle. Further LC-PUFA, such as EPA and DHA, also increased in the fillets of the tilapia fed with the diet for 60 days. However, the author suggested that the dietary supplementation of 5% linseed flour for 45 days was sufficient to enhance linolenic acid in fish muscle (Nishiyama et al 2014). Similar to the study on chia seed flour, Silva et al (2014) revealed that dietary supplementation of 5% chia seed flour significantly increased the levels of total PUFA and n-3 fatty acids, namely linolenic acid, EPA and DHA, in fillets of the tilapia fed with the experimental diet for 45 days. Interestingly, the linolenic acid level was five times higher than in the fish fed the control diet. Based on the findings of previous studies (Dos Santos et al 2014; Nishiyama et al 2014; Silva et al 2014), dietary supplementation with 5% n-3 PUFA-rich oilseeds is a suitable strategy to efficiently improve the nutritional quality of fish fillets for consumers. According to the current study, further investigation of the effect of perilla seed flour supplementation on the quality of *L. calcarifer* fillets is necessary.

Conclusions. A dietary supplementation of 2.5 and 5.0% perilla seed flour did not significantly affect the growth performance, nutrient utilization, and carcass composition of *L. calcarifer* fed with a free-fish oil soybean oil-based diet for 68 days. It is recommended that further investigation of perilla seed flour supplementation in diets on the fatty acid profile as fillet quality parameters in *L. calcarifer* should be conducted.

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Conflict of interest. The authors declare no conflict of interest.

Ethical approval. The study was conducted in compliance with the guidelines of the Animal Use Protocol approved by the National Research Council of Thailand, and all methods were in accordance with relevant guidelines and regulations (approval no. U1 08621-2562). The study was approved by the Animal Care and Use Committee of Chiang Mai University; Reference No. AG03005/2567.

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