Songklanakarin J. Sci. Technol.
43 (4), 976-986, Jul. - Aug. 2021

http://www.sjst.psu.ac.th

Original Article

Effects of alternative lipid sources and levels for fish oil replacement in Asian seabass (*Lates calcarifer*) diets on growth, digestive enzyme activity and immune parameters

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Received: 22 February 2020; Revised: 28 May 2020; Accepted: 11 July 2020

Abstract

An experiment was conducted using factorial (3×4) in completely randomized design to examine incorporation of vegetable oils in diets on seabass growth, fatty acid profile, digestive enzyme activity and immune responses. Ten iso-nitrogenous and iso-lipidic diets were formulated to have soybean, palm and sunflower oil replacing fish oil (FO) at 0%, 25%, 50% and 75%, respectively. Each diet was fed to quadruplicate fish groups (3.29±0.30g initial weight) for eight weeks. Results revealed interaction effects between oil types and replacement levels on diet consumption among the feeding groups. Fish growth and immune parameters were not affected by oil types ($p>0.05$), but final weight, specific growth rate, white blood cell and serum protein were altered by replacement levels ($p<0.05$). Carcass protein and lipid were influenced by oil types and replacement levels. The highest and lowest carcass protein content was observed in fish fed diets having 75% and 25% soybean oil, respectively while the rest of the feeding groups were similar to the control group. Fatty acid profile of fish carcass reflected oil sources. The ratio of α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6) was similar to those fed 100% FO containing diet and 25% palm oil replaced diet. Specific trypsin and lipase activity were affected by oil types and replacement levels in intestine and pyloric caeca, respectively. Diets replaced with soybean and palm oil showed similar results in intestinal specific trypsin activity. The highest specific lipase activity was detected in fish fed 100% FO containing diets followed by 25%, 50% and 75% FO replaced diets, respectively. Therefore, soybean or palm oils can replace FO at 25% in Asian seabass diet without compromising growth and health status.

Keywords: alternative lipid sources, fish oil, *Lates calcarifer*, growth, immune responses

1. Introduction

Fish oil has been the most important ingredient for aquafeed industry to supply essential fatty acids (FA) particularly those belong to the highly and poly unsaturated fatty acids (HUFA and PUFA). In the past few years, FO production has been static due to stable supply of raw material which derived from capture fisheries while its demand has been increased for animal feed and human consumption resulting in increased prices (Turchini, Torstensen, & Ng, 2009). To overcome such an obstacle for aquaculture industry,

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alternative lipid sources are a solution. Over the past two decades, studies have shown that vegetable oils (VO) appeared suitable as FO replacer (Turchini, Torstensen, & Ng, 2009). VOs are rich in C18-PUFA and monounsaturated fatty acids (MUFA) but, lack of HUFA particularly, eicosapentaenoic acid, (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) that play vital role in maintaining proper physiological process and immune system of fish (Turchini, Torstensen, & Ng, 2009). EPA and DHA are essential for marine fish species because of their limited capacity in synthesizing these FA from C18-PUFA (Tocher, 2010). Therefore, sufficient supply of HUFA should be seriously considered when using VOs as an alternative for FO in marine fish diets. Nasopoulou & Zabetakis (2012) and Sales & Glencross (2011) mentioned that higher use of VOs has a negative effect on growth of Atlantic salmon, European seabass and gilthead seabream when partial substitution has no negative effect on growth but few disorders in immune parameters (Jiang *et al*., 2013; Montero *et al*., 2008). As responses to dietary FA may be species specific and depend upon their natural habitats, assessment of FO replacement should not only include fish growth performance but also health parameters.

Asian seabass is an important economic fish in the Indo-West Pacific region and production has increased three times during 2000-2015 (Food and Agriculture Organization [FAO], 2019). Being carnivorous and highly voracious feeder, Asian seabass needs a high protein and high energy diet. Nowadays, culture of this fish relies on commercial diet which is expensive due to high raw material price including fish meal and oil. Few studies that were carried out in Asian seabass on fish oil substitution obtained variable responses which led to questions about the upper limits of FO replacement (Glencross *et al.*, 2016; Salini *et al.*, 2015). This study, thus, aimed to investigate effects of replacing FO by soybean, palm, and sunflower oil at varying levels on growth performance, FA profile, digestive enzyme activity and innate immunity of Asian seabass.

2. Materials and Methods

2.1. Experimental design and diet preparation

Three human food grade vegetable oils (soybean oil from Thai Vegetable Oil Public Company Limited, palm oil and sunflower oil from P.K. Trading (Thailand) Company Limited) and four replacement levels (0%, 25%, 50% and 75%) with four replicates were employed in the study. Therefore, nine iso-nitrogenous and iso-lipidic diets and a control diet containing 100% FO were prepared. Diet formulation and proximate composition, and FA profile of the diets are shown in Tables 1 and 2, respectively.

2.2. Fish and feeding trial

The trial was conducted at Kidchakan Supamattaya Aquatic Animal Health Research Center, Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Thailand. Asian seabass fingerlings were obtained from Coastal Aquaculture Technology and Innovation Research and Development Center, Songkhla, Thailand and nursed in 1,000 L circular tanks with nursery diet for four weeks. The fish was gradually acclimatized to freshwater environment by gradual salinity adjustment from 20 to 0 ppt (5 ppt/day). After reaching an average initial weight of 3.29±0.30 g, they were distributed into 40 aquaria (100 L, 60 cm×40 cm×50 cm) at 12 fish/ aquarium. Aeration was supplied by an air blower, water was changed at 80% daily and quality parameters were monitored accordingly. Fish were fed daily at 8:30 and 17:00 to apparent satiation for eight weeks and daily feed consumption was recorded. Dry matter loss of the diets was estimated and tabulated according to Tantikitti, Chookird, & Phongdara (2016).

2.3. Growth performance and feed utilization assessment

At the end of eight-week feeding, fish were starved for the last two meals in order to prepare them for weight measurement and sample collection. On the next day, fish from each aquarium were individually weighed. Survival rate (SR, %), weight gain (WG, g/fish), diet consumption (g/fish), feed conversion ratio (FCR) and specific growth rate (SGR, %/day) were calculated. Two fish per replication were anesthetized and dissected to take the weight of viscera, liver and abdominal fat to assess viscerosomatic index (VSI) hepatosomatic index (HSI), and intraperitoneal fat (IPF), respectively. Protein efficiency ratio (PER), protein retention efficiency (PRE, %), lipid efficiency ratio (LER) and lipid retention efficiency (LRE, %) were calculated according to Martino, Cyrino, Portz, & Trugo (2005).

2.4. Proximate composition and fatty acid profile analysis

Proximate composition, moisture, ash, crude protein, and crude lipid of initial and final fish were determined according to standard methods of AOAC (1995). Two fish per replication were used for FA profile determination. They were freeze-dried (LABCONCO, FreeZone 6) and ground by a grinder (Philips HR2115). Freeze-dried fish samples and diets were analyzed for FA profile at Food Science and Technology Department, Faculty of Agro-industry, Kasetstart University, Thailand by gas chromatography (GC) using a 6890N GC equipped with a flame ionization detector and a 6890 GC equipped with a HP5973 mass selective detector (Agilent Technologies, Palo Alto, CA, USA) as described by Mahisanunt, Na Jom, Matsukawa, & Klinkesorn, (2017).

2.5. Digestive enzyme activity determination

On the same sampling day, intestine, and pyloric caeca were collected (4 fish/treatment) for analysis of specific trypsin, lipase and α-amylase activity as described by Srichanun, Tantikitti, Utarabhand, & Kortner (2013). The enzymatic activity measurement was performed under optimum pH and controlled room temperature (25 °C). The specific enzyme activity was expressed as U/mg protein/min.

Table 1. Ingredients and proximate composition (% as-fed basis) of the experimental diets

Ingredients (% of diet)	Diets (Source, % of FO replacement)									
	1 (Control)	2 (SO, 25%)	3 (SO, 50%)	4 (SO, 75%)	5 (PO, 25%)	6 (PO, 50%)	7 (PO, 75%)	8 (SFO, 25%)	9 (SFO, 50%)	10 (SFO, 75%)
Poultry by product meal (66.22% protein)	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00
Soybean meal[1] (49.41% protein)	15.19	15.19	15.19	15.19	15.19	15.19	15.19	15.19	15.19	15.19
Soy protein concentrate[1] (63.48% protein)	11.82	11.82	11.82	11.82	11.82	11.82	11.82	11.82	11.82	11.82
Wheat flour	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00
Ground rice husk	4.08	4.08	4.08	4.08	4.08	4.08	4.08	4.08	4.08	4.08
Fish oil	6.67	5.00	3.34	1.67	5.00	3.34	1.67	5.00	3.34	1.67
Soybean oil	-	1.67	3.34	5.00	-	-	-	-	-	-
Palm oil	-	-	-	-	1.67	3.34	5.00	-	-	-
Sunflower oil	-	-	-	-	-	-	-	1.67	3.34	5.00
Vitamin and mineral premix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Carboxymethyl cellulose (CMC)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
DL-Methionine	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Tuna viscera powder	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Proximate composition[2] (%)										
Moisture	9.60	9.41	9.71	9.30	9.39	9.73	9.73	9.64	9.26	9.21
Ash	9.18	9.26	9.08	9.15	9.25	9.10	9.13	9.12	9.09	9.13
Crude protein	47.28	47.07	46.96	47.52	47.23	47.29	47.14	47.78	47.37	47.53
Crude lipid	10.10	10.33	10.70	10.30	10.69	10.33	10.23	10.65	10.40	10.21

[1]Obtained from Thai Union Group, Thailand,
[2]By analysis (AOAC, 1995)

Table 2. Fatty acid profile (% of total fatty acids) of experimental diets for Asian seabass

Diets (Oil type, % of FO replacement)	Nonanoic acid (9:0)	Myristic acid (14:0)	Palmitic acid (16:0)	Stearic acid (18:0)	Nonadecylic acid (19:0)	Oleic acid (18:1n-9)	Gondoic acid (20:1n-9)
1 (100% FO)	1.13±0.06	3.57±0.27	35.52±0.10	15.85±0.25	2.21±0.09	3.18±0.14	9.32±0.33
2 (SO, 25%)	0.79±0.18	2.89±0.11	31.13±0.12	13.07±0.56	2.71±0.01	3.30±0.14	4.06±0.47
3 (SO, 50%)	0.70±0.18	1.98±0.22	29.73±0.79	14.28±1.46	1.36±0.22	3.24±0.10	5.98±1.18
4 (SO, 75%)	1.10±0.08	1.08±0.01	28.17±0.93	14.08±0.29	1.17±0.03	3.02±0.10	3.28±0.09
5 (PO, 25%)	0.78±0.14	2.72±0.26	35.60±1.99	14.02±0.12	2.03±0.05	2.67±0.01	10.43±1.93
6 (PO, 50%)	0.80±0.18	1.97±0.15	35.63±0.42	13.68±0.38	1.41±0.26	2.67±0.09	5.43±0.19
7 (PO, 75%)	0.92±0.18	1.32±0.13	37.93±0.84	12.59±0.54	0.84±0.22	2.54±0.17	3.52±0.18
8 (SFO, 25%)	0.81±0.04	2.69±0.01	29.83±2.60	13.78±1.24	1.86±0.13	3.03±0.03	10.48±2.48
9 (SFO, 50%)	1.15±0.02	1.55±0.07	26.81±0.55	14.71±0.76	1.51±0.11	3.00±0.03	6.77±1.87
10 (SFO, 75%)	1.04±0.21	1.11±0.04	25.12±0.40	13.13±0.73	0.97±0.16	2.83±0.16	3.45±0.17

Diets (Oil type, % of FO replacement)	Linoleic acid (18:2n-6)	α-Linolenic acid (18:3n-3)	SFA [1]	UFA [2]	MUFA[3]	PUFA[4]	18:3n-3 / 18:2n-6
1 (100% FO)	23.75±0.15	5.47±0.87	58.28±0.24	41.72±0.24	12.50±0.48	29.22±0.72	0.23±0.04
2 (SO, 25%)	28.39±0.28	13.66±0.82	50.59±0.76	49.41±0.76	7.36±0.34	42.05±1.10	0.48±0.02
3 (SO, 50%)	25.99±0.79	16.75±2.29	48.05±1.99	51.95±1.99	9.22±1.09	42.74±3.08	0.64±0.07
4 (SO, 75%)	28.62±0.62	19.48±0.54	45.60±1.17	54.40±1.17	6.31±0.00	48.09±1.16	0.68±0.00
5 (PO, 25%)	25.11±0.71	6.62±0.48	55.16±2.18	44.84±2.18	13.11±1.95	31.73±0.23	0.26±0.03
6 (PO, 50%)	30.47±0.31	7.94±0.07	53.49±0.51	46.51±0.51	8.09±0.28	38.41±0.24	0.26±0.01
7 (PO, 75%)	32.53±0.74	7.81±0.82	53.60±1.21	46.40±1.21	6.07±0.35	40.34±1.56	0.24±0.02
8 (SFO, 25%)	26.34±0.75	11.18±0.52	48.97±3.73	51.03±3.73	13.51±2.45	37.52±1.28	0.42±0.01
9 (SFO, 50%)	29.53±0.78	14.97±1.40	45.73±0.27	54.27±0.27	9.77±1.90	44.50±2.17	0.51±0.03
10 (SFO, 75%)	32.15±0.25	20.20±1.70	41.38±1.13	58.62±1.13	6.28±0.32	52.34±1.45	0.63±0.06

[1]SFA: Saturated fatty acids: 9:0, 14:0, 16:0, 18:0 and 19:0; [2]UFA: Unsaturated fatty acids: 18:1n-9, 20:1n-9, 18:2n-6 and 18:3n-3; [3]MUFA: Monounsaturated fatty acid: 18:1n-9 and 20:1n-9; [4]PUFA: Polyunsaturated fatty acid: 18:2n-6 and 18:3n-3

2.6. Determination of haemato-immunological parameters

Two fish per replication were used to determine innate immune parameters as described by Suwannasang, Dangwetngam, Issaro, Phromkunthong & Suanyuk (2014). After being anesthetized by clove oil, blood was collected from caudal vein using 1mL syringe with 25G needle. Blood was immediately processed for total blood cell count (red and white blood cell), hemoglobin (Hb), hematocrit (Ht) and respiratory burst activity through reduction of nitroblue tetrazolium to formazan as a measure of superoxide anion (O^{2-}) production. The remaining blood samples were allowed to coagulate at room temperature for serum collection for total serum protein and lysozyme activity analysis.

2.7. Statistical analysis

Firstly, data were checked for normality and homogeneity of variance by the Shapiro–Wilk W-test and Levene's test, respectively using statistical package SPSS 24 for Windows. Two-way ANOVA was then applied to analyze experimental data. Before analysis the percentage data were transformed using square root and the difference between the treatment means were determined using Tukey's HSD test at 95% confidence level ($p<0.05$).

3. Results

3.1 Diet acceptance

Consumption of diets by fish in different treatments is shown in Table 3. The statistical analysis revealed an influence of oil types and the levels of replacement for FO ($p<0.05$) on dietary intake of fish. Most experimental groups of fish had a similar level of consumed feed to that of the control groups except those fed diet with 75% SO. In comparison with the control 100% FO diet, a reducing trend of feed consumption was observed with the diets containing PO and SFO regardless of replacement levels.

3.2 Growth performance

Growth performance and nutrient utilization parameters are shown in Table 3 and 4, respectively. Survival rate and FCR were unaffected ($p>0.05$) by lipid sources and levels of FO replacement. Considering overall results of VO types on growth parameters, it was found that FW, WG and SGR of fish fed diets having SO, PO and SFO were similar to those fed the control diet. On the contrary, as levels of VOs replacing for FO increased, FW, WG and SGR significantly decreased ($p<0.05$) as compared with those of the control group. FW, WG and SGR of fish fed VO replacement diets at

Table 3. Final weight, weight gain, survival rate, consumed diet, food conversion ratio (FCR), specific growth rate (SGR), viscerosomatic index (VSI), hepatosomatic index (HIS) and intraperitoneal fat (IPF) of Asian seabass after 8 week feeding fish oil replacement diets

Diets (Oil type , % of FO replacement)	Final weight (g/fish)	Weight gain (g/fish)	Survival rate (%)	Consumed diet (g/fish)	FCR	SGR (%/Day)	VSI (%)	HSI (%)	IPF (%)
1 (Control)	27.94±1.10	24.61±1.11	100.00±0.00	33.19±1.75^{ab}	1.35±0.02	3.80±0.07	8.28±1.39	2.09±0.41	2.93±1.14
2 (SO, 25%)	28.15±0.46	24.82±0.45	97.92±4.17	34.42±2.98^{a}	1.39±0.12	3.81±0.03	8.46±0.87	2.67±1.15	3.10±0.89
3 (SO, 50%)	24.56±3.06	21.21±3.05	100.00±0.00	29.40±3.19^{ab}	1.40±0.07	3.55±0.23	7.83±0.34	2.08±0.36	2.63±0.43
4 (SO, 75%)	24.30±2.01	20.97±2.01	100.00±0.00	28.42±2.03^{b}	1.36±0.07	3.55±0.14	8.96±0.86	2.25±0.30	3.45±0.84
5 (PO, 25%)	26.53±1.61	23.20±1.61	100.00±0.00	30.90±1.41^{ab}	1.33±0.04	3.70±0.12	7.50±0.57	2.08±0.33	2.55±0.45
6 (PO, 50%)	26.58±1.73	23.24±1.76	97.92±4.17	32.48±2.12^{ab}	1.40±0.08	3.71±0.12	8.04±1.44	1.92±0.49	2.99±1.10
7 (PO, 75%)	25.20±1.16	21.89±1.16	95.83±4.81	32.66±1.68^{ab}	1.49±0.03	3.61±0.08	7.91±1.55	2.11±0.60	3.01±1.46
8 (SFO, 25%)	25.08±2.65	21.75±2.66	97.92±4.17	29.58±2.33^{ab}	1.37±0.02	3.60±0.20	7.13±0.60	1.83±0.36	2.25±0.36
9 (SFO, 50%)	26.51±1.60	23.19±1.60	97.92±4.17	31.86±2.41^{ab}	1.38±0.11	3.71±0.11	8.45±1.20	2.06±0.36	3.40±1.09
10 (SFO, 75%)	25.98±1.17	22.67±1.17	93.75±12.50	31.28±1.92^{ab}	1.38±0.03	3.66±0.07	8.29±1.12	2.28±0.55	3.25±0.80
Mean of main effects									
SO	26.24±2.54	22.90±2.54	99.48±2.08	31.36±3.46	1.37±0.07	3.67±0.19	8.38±0.99	2.27±0.67	3.03±0.87
PO	26.56±1.61	21.89±1.61	98.44±3.36	32.31±1.80	1.39±0.08	3.70±0.11	7.93±1.27	2.05±0.45	2.87±1.06
SFO	26.38±1.89	23.05±1.89	97.40±6.61	31.48±2.32	1.37±0.07	3.71±0.13	8.04±1.19	2.06±0.44	2.96±0.97
0% (Control)	27.94±1.00^{p}	24.61±1.00^{p}	100±0.00	33.19±1.58	1.35±0.02	3.80±0.07^{p}	8.28±1.33	2.09±0.39	2.93±1.09
25%	26.59±2.10^{pq}	23.26±2.10^{pq}	98.61±3.24	31.63±3.00	1.36±0.08	3.71±0.15^{pq}	7.70±0.87	2.19±0.77	2.63±0.69
50%	25.88±2.25^{q}	22.55±2.25^{q}	98.61±3.24	31.25±2.74	1.39±0.08	3.65±0.17^{q}	8.11±1.08	2.01±0.40	3.00±0.94
75%	25.16±1.54^{q}	21.84±1.54^{q}	96.53±7.50	30.79±2.51	1.41±0.07	3.61±0.11^{q}	8.39±1.24	2.22±0.48	3.24±1.04
p-value									
Oil type	0.865	0.860	0.441	0.412	0.527	0.763	0.256	0.168	0.803
Level	0.003	0.003	0.329	0.056	0.122	0.006	0.162	0.538	0.193
Oil type × Level	0.098	0.099	0.760	0.007	0.188	0.104	0.259	0.238	0.408

Values are mean ± SD of four replicates except for VSI, HSI and IPF where n=8. Means of main effects in the same row with different superscripts (Sources: a, b, c and d; Levels: p, q, r and s) are significantly different ($p<0.05$). Weight gain (g/fish) = final weight (g) – initial weight (g)

Survival rate (%) = 100× (final fish number/initial number)

Food conversion ratio (FCR) = feed intake {corrected leaching lost (g)}/weight gain (g/fish)

Specific growth rate (SGR, % /day) = (lnW2- lnW1/ T2-T1) ×100; W1 = Initial weight, W2 = Final weight, T2-T1 = Cultured period (days)

Viscerosomatic index (VSI, %) = 100 × (viscera weight/body weight)

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

Intraperitoneal fat ratio (IPF, %) = 100 × (intraperitoneal fat weight/final body weight)

Table 4. Proximate composition, protein efficiency ratio (PER), lipid efficiency ratio (LER), protein retention efficiency (PRE) and lipid retention efficiency (LRE) of Asian seabass after 8 weeks feeding

Diets (Oil type, % of FO replacement)	Moisture (%)	Ash (%)	Protein (%)	Lipid (%)	PER	LER	PRE (%)	LRE (%)
Initial fish	75.62±074	3.68±11	15.79±0.08	3.79±0.17	-	-	-	-
Final fish								
1 (Control)	71.67±0.80	4.07±0.18	18.88±0.51^{ab}	6.22±0.61^{b}	1.57±0.03	7.36±0.15	30.12±1.06	47.85±5.43
2 (SO, 25%)	71.51±0.65	4.13±0.09	18.42±0.46^{b}	6.66±0.37^{ab}	1.58±0.12	7.20±0.57	29.53±1.47	50.62±5.22
3 (SO, 50%)	70.86±0.30	4.18±0.21	18.71±0.42^{ab}	7.27±0.43^{a}	1.55±0.09	6.80±0.37	29.58±1.78	53.09±5.66
4 (SO, 75%)	70.78±0.56	4.05±0.24	19.06±0.25^{a}	7.19±0.63^{a}	1.54±0.09	7.10±0.40	30.11±2.50	55.02±8.45
5 (PO, 25%)	71.57±0.53	3.98±0.21	19.00±0.32^{ab}	6.58±0.57^{ab}	1.57±0.03	6.92±0.15	30.72±0.69	48.86±5.15
6 (PO, 50%)	71.29±1.36	4.17±0.14	18.92±0.24^{ab}	6.62±0.36^{ab}	1.55±0.02	7.11±0.11	30.73±0.99	51.09±5.08
7 (PO, 75%)	71.73±0.53	4.19±0.23	18.70±0.59^{ab}	6.31±0.17^{b}	1.42±0.04	6.53±0.20	27.22±0.39	43.94±3.35
8 (SFO, 25%)	71.41±0.70	4.06±0.18	18.48±0.51^{ab}	6.04±0.57^{b}	1.57±0.09	7.05±0.41	29.46±1.58	44.58±4.69
9 (SFO, 50%)	71.60±0.81	4.18±0.21	18.47±0.39^{ab}	6.05±0.41^{b}	1.55±0.15	7.06±0.66	29.31±2.68	45.22±5.61
10 (SFO, 75%)	70.12±0.81	4.29±0.11	18.87±0.18^{ab}	7.06±0.40^{a}	1.52±0.04	7.08±0.19	29.01±1.15	52.75±4.06
Mean of main effects								
SO	71.20±0.67	4.11±0.18	18.76±0.47	6.84±0.66	1.56±0.08	7.11±0.40	29.83±1.55	51.64±6.09
PO	71.56±0.80	4.10±0.20	18.87±0.43	6.43±0.48	1.53±0.07	6.98±0.34	29.70±1.67	47.94±4.92
SFO	71.20±0.96	4.14±0.19	18.66±0.45	6.34±0.64	1.55±0.08	7.14±0.37	29.47±1.54	47.60±5.42
0% (Control)	71.67±0.72	4.07±0.17	18.88±0.51	6.22±0.58	1.57±0.03	7.36±0.13^{p}	30.12±0.92	47.85±4.71
25%	71.49±0.57	4.05±0.17	18.62±0.50	6.42±0.57	1.57±0.08	7.06±0.38^{pq}	29.90±1.29	48.02±5.12
50%	71.25±0.90	4.18±0.18	18.70±0.39	6.65±0.64	1.49±0.08	6.99±0.41^{pq}	29.87±1.80	49.80±5.91
75%	70.88±0.91	4.18±0.22	18.88±0.41	6.85±0.58	1.55±0.08	6.90±0.37 ^{q}	28.87±1.88	50.57±7.11
p-value								
Oil type	0.307	0.648	0.168	0.000	0.542	0.486	0.843	0.150
Level	0.079	0.085	0.096	0.000	0.115	0.048	0.259	0.654
Oil type × Level	0.221	0.477	0.044	0.000	0.764	0.422	0.280	0.264

Values are mean ± SD. n=4 for proximate composition. n=3 for PER, LER, PRE and LRE. Mean of main effects in the same row with different superscripts (Sources: a, b, c; Levels: p, q, r) are significantly different ($p < .05$)

Protein efficiency ratio (PER) = weight gain /total protein intake

Lipid efficiency ratio (LER) = weight gain /total lipid intake

Protein retention efficiency (PRE, %) = 100 × [{(final body weight ×final body protein) – (initial body weight × initial body protein)}/total protein intake]

Lipid retention efficiency (LRE, %) = 100 × [{(final body weight ×final body lipid) – (initial body weight × initial body lipid)}/total lipid intake]

0% and 25% was statistically similar (p>0.05) and higher than those at 25%, 50% and 75% replacement.

3.3 Proximate composition of fish carcass

Proximate composition of whole-body carcass is shown in Table 4. Moisture and ash contents of final fish showed similar results (p>0.05) as compared with those of the control group. Crude protein content was not affected by single effect of either vegetable oil types or replacement levels, but an interaction of both effects was observed among the feeding groups. Crude lipid content of final fish was significantly affected (p<0.05) by both VO types and levels of FO replacement. Besides, there were interactions between the two factors. In case of VO types, highest carcass protein content was found in PO followed by SO and SFO, respectively. Lipid content was the highest in fish fed SO diet followed by PO and SFO, respectively. For replacement levels, protein and lipid contents of fish carcass showed an inverse relationship, as the level of FO replacement increased, protein contents decreased. On the contrary, lipid contents in the carcass increased as VOs replacement levels decreased. The lowest lipid content was in the control fish group which was significantly lower than those of 25%, 50% and 75 % replacement level groups (p<0.05).

3.4 Fatty acid composition of fish carcass

The fatty acid profile of the final fish carcass reflected dietary intake (Table 5). Increasing levels of VOs replacing for FO significantly affected (p<0.05) carcass FA profile. The increased VO levels resulted in decreased levels of SFA (9:0, 14:0, 16:0, 18:0 and 19:0), and increased levels of MUFA (18:1n-9 and 20:1n-9) and C18-PUFA (18:2n-6 and 18:3n-3). Ratios of 18:3n-3 and 18:2n-6 of fish carcass were significantly altered (p<0.05) by oil types and levels of replacement. The highest ratio was observed in SO diet fed group followed by PO and SFO diet groups, respectively and decreased with increasing replacement levels in the diets.

3.5 Digestive enzyme activity

The digestive enzyme activity in pyloric caeca and intestine of fish fed diets with different types of VOs replacing for FO is reported in Table 6. The specific trypsin and α-amylase activity were comparatively higher in pyloric caeca

Table 5. Fatty acid profile of Asian seabass carcass after 8 weeks of feeding with diets containing different levels of vegetable oils replacing for fish oil (% of total fatty acids)

Diets (Oil type, % of FO replacement)	Nonanoic acid (9:0)	Lauric acid (12:0)	Myristic acid (14:0)	Pentadecylic acid (15:0)	Palmitic acid (16:0)	Margaric acid (17:0)	Stearic acid (18:0)	Palmitoleic acid (16:1n-7)	Oleic acid (18:1n-9)	Gondoic acid (20:1n-9)	Linoleic acid (18:2n-6)	α-Linolenic acid (18:3n-3)	SFA [1]	UFA [2]	MUFA [3]	PUFA [4]	18:3n-3 / 18:2n-6
Initial fish	1.41±0.17	0.79±0.02	8.87±0.64	3.98±0.38	1.01±0.22	2.90±0.30	32.13±0.74	4.47±0.29	9.57±0.14	7.63±0.21	20.36±1.35	6.87±0.63	51.09±1.09	48.91±1.09	21.68±0.36	27.23±0.73	0.34±0.05
1 (Control)	1.17±0.56	0.67±0.27	7.91±0.66	1.44±0.34	2.11±1.20	0.65±0.23	16.48±1.84	8.32±1.12	7.29±1.03	3.78±1.27	44.67±2.34^{d}	5.51±3.45^{e}	30.43±3.69	69.57±3.69	19.40±1.66	50.18±4.56	0.12±0.07^{d}
2 (SO, 25%)	0.66±0.13	0.63±0.51	5.68±0.83	1.08±0.14	2.20±1.81	0.48±0.25	15.64±2.10	6.56±1.40	6.71±0.73	4.11±2.45	47.32±4.16^{cd}	8.43±4.92^{cde}	26.21±3.99	73.13±5.20	17.38±1.84	55.75±7.02	0.18±0.10^{cd}
3 (SO, 50%)	0.86±0.40	0.52±0.24	4.24±0.12	1.00±0.31	0.99±0.61	0.32±0.10	13.93±1.52	5.20±0.48	5.36±0.57	2.87±0.90	46.36±0.64^{cd}	18.37±4.06^{ab}	21.86±3.04	78.14±3.04	13.42±1.01	64.73±3.86	0.40±0.09^{ab}
4 (SO, 75%)	0.71±0.07	0.37±0.03	2.85±0.11	0.74±0.08	0.39±0.08	0.16±0.06	12.85±0.13	3.51±0.06	4.99±0.06	2.02±0.31	45.90±0.26^{cd}	25.51±0.44^{a}	18.07±0.15	81.93±0.15	10.52±0.30	71.41±0.25	0.56±0.01^{a}
5 (PO, 25%)	1.22±0.13	0.62±0.12	5.81±0.52	1.19±0.20	1.75±0.54	0.41±0.12	15.31±0.38	7.00±0.10	6.61±1.14	3.86±0.58	49.14±0.89^{bcd}	7.08±3.02^{de}	26.31±0.72	73.69±0.72	17.48±1.76	56.22±2.46	0.14±0.06^{d}
6 (PO, 50%)	0.89±0.30	0.57±0.09	4.39±0.22	0.84±0.07	1.37±0.68	0.25±0.07	14.60±1.00	5.54±0.46	5.52±0.38	2.82±0.85	53.37±1.67^{c}	9.85±1.73^{cde}	22.91±1.95	77.09±1.95	13.87±0.91	63.22±2.72	0.18±0.03^{cd}
7 (PO, 75%)	0.58±0.10	0.52±0.05	4.03±0.22	0.56±0.06	0.43±0.17	0.11±0.01	12.50±0.31	4.60±0.15	4.62±0.22	2.05±0.19	60.09±0.65^{a}	9.89±0.64^{cde}	18.74±0.44	81.26±0.44	11.27±0.49	69.99±0.23	0.16±0.01^{cd}
8 (SFO, 25%)	0.67±0.25	0.36±0.05	6.11±0.42	1.03±0.17	1.16±0.18	0.39±0.14	14.83±0.89	7.74±0.33	6.49±0.61	2.94±0.54	49.26±2.39^{bcd}	9.02±4.52^{cde}	24.55±1.59	75.45±1.59	17.17±0.81	58.28±2.40	0.19±0.10^{cd}
9 (SFO, 50%)	0.61±0.16	0.39±0.09	4.26±0.19	0.72±0.09	0.53±0.17	0.34±0.13	13.27±0.70	5.26±0.19	5.59±0.99	3.11±0.56	49.44±2.21^{bc}	16.47±5.20^{cd}	20.12±1.48	79.88±1.48	13.96±1.58	65.92±3.03	0.34±0.12^{bc}
10 (SFO, 75%)	0.66±0.12	0.50±0.06	3.00±0.07	0.78±0.12	0.52±0.11	0.35±0.06	13.53±0.61	3.63±0.35	5.22±0.23	4.10±0.41	53.63±0.95^{c}	14.08±1.69^{bc}	19.34±0.93	80.66±0.93	12.95±0.67	67.71±1.41	0.26±0.03^{bc}
Mean of main effects																	
SO	-	-	-	-	-	-	-	-	6.09±1.16	-	46.06±2.37	14.45±8.85	24.14±5.54	75.69±5.80	15.18±3.75	60.52±9.38	0.31±0.19
PO	-	-	-	-	-	-	-	-	6.01±1.27	-	51.82±0.65	8.08±2.94	24.60±4.83	75.40±4.83	15.50±3.46	59.90±8.11	0.15±0.05
SFO	-	-	-	-	-	-	-	-	6.15±1.09	-	49.25±3.76	11.27±5.66	23.61±4.96	76.39±4.96	15.87±2.88	60.52±7.68	0.23±0.11
0% (Control)	-	-	-	-	-	-	-	-	7.30±0.93^{p}	-	44.67±2.11	5.51±3.12	30.43±3.34^{p}	69.58±3.34^{r}	19.40±1.50^{p}	50.18±4.13^{s}	0.12±0.07
25%	-	-	-	-	-	-	-	-	6.60±0.78^{p}	-	48.58±2.71	8.17±3.92	25.69±2.43^{q}	74.09±3.04^{q}	17.34±1.40^{q}	56.75±4.24^{r}	0.17±0.08
50%	-	-	-	-	-	-	-	-	5.49±0.64^{q}	-	49.72±3.35	14.90±5.22	21.63±2.37^{r}	78.37±2.37^{p}	13.75±1.12^{r}	64.62±3.15^{q}	0.31±0.12
75%	-	-	-	-	-	-	-	-	4.94±0.31^{q}	-	53.21±6.09	16.49±6.96	18.72±0.77^{s}	81.28±0.77^{p}	11.58±1.16^{s}	69.70±1.77^{p}	0.33±0.17
p- value																	
Oil type	-	-	-	-	-	-	-	-	0.877	-	0.000	0.000	0.547	0.573	0.341	0.856	0.000
Level	-	-	-	-	-	-	-	-	0.000	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Oil type × Level	-	-	-	-	-	-	-	-	0.963	-	0.000	0.001	0.804	0.791	0.488	0.669	0.000

[1]SFA: Saturated fatty acids: 9:0, 14:0, 16:0, 18:0 and 19:0; [2]UFA: Unsaturated fatty acids: 18:1n-9; 20:1n-9, 18:2n-6 and 18:3n-3; [3]MUFA: Monounsaturated fatty acid: 18:1n-9 and 20:1n-9; [4]PUFA: Polyunsaturated fatty acid: 18:2n-6 and 18:3n-3

Table 6. Digestive enzyme activity in pyloric caeca and intestine of Asian seabass after 8-week feeding with the experimental diets containing different levels of vegetable oils replacing for fish oil

Diets (Oil type, % of FO replacement)	Specific trypsin activity (U/mg protein/min)	Specific lipase activity (U/mg protein/min)	Specific α-amylase activity (U/mg protein/min)
Pyloric caeca			
1 (Control)	2.85±0.42	2.25±0.25	1.10±0.27
2 (SO, 25%)	2.68±0.44	1.75±0.27	1.04±0.23
3 (SO, 50%)	2.15±0.39	1.38±0.08	0.98±0.26
4 (SO, 75%)	2.14±0.24	1.38±0.42	0.86±0.09
5 (PO, 25%)	2.02±0.53	1.40±0.23	1.09±0.24
6 (PO, 50%)	3.01±0.12	1.43±0.24	0.74±0.23
7 (PO, 75%)	2.41±0.67	1.62±0.44	0.70±0.24
8 (SFO, 25%)	2.83±0.63	1.73±0.07	0.88±0.13
9 (SFO, 50%)	3.02±0.62	1.80±0.35	0.62±0.15
10 (SFO, 75%)	2.84±0.03	1.64±0.31	0.77±0.06
Mean of main effects			
SO	2.50±0.48	1.73±0.45	0.99±0.21
PO	2.67±0.53	1.69±0.45	0.90±0.29
SFO	2.88±0.45	1.88±0.35	0.83±0.22
0% (Control)	2.85±0.38	$2.25±0.23^{p}$	$1.10±0.23^{p}$
25%	2.61±0.58	$1.64±0.25^{q}$	$1.00±0.20^{pq}$
50%	2.70±0.56	$1.55±0.31^{q}$	$0.78±0.25^{q}$
75%	2.51±0.48	$1.55±0.37^{q}$	$0.78±0.15^{q}$
p-value			
Oil type	0.053	0.204	0.154
Level	0.213	0.000	0.002
Oil type × Level	0.107	0.420	0.552
Intestine			
1 (Control)	0.84±0.19	2.02±0.40	0.16±0.005
2 (SO, 25%)	0.91±0.01	1.75±0.66	0.16±0.03
3 (SO, 50%)	0.77±0.11	1.47±0.19	0.15±0.03
4 (SO, 75%)	0.82±0.12	1.76±0.65	0.16±0.03
5 (PO, 25%)	0.71±0.14	1.73±0.92	0.21±0.02
6 (PO, 50%)	0.67±0.03	1.76±0.77	0.20±0.01
7 (PO, 75%)	0.66±0.02	1.97±0.53	0.21±0.02
8 (SFO, 25%)	0.65±0.12	1.44±0.45	0.19±0.03
9 (SFO, 50%)	0.67±0.12	1.58±0.62	0.16±0.06
10 (SFO, 75%)	0.59±0.06	1.46±0.27	0.17±0.04
Mean of main effects			
SO	$0.82±0.12^{a}$	1.75±0.50	0.16±0.03
PO	$0.71±0.12^{ab}$	1.87±0.63	0.19±0.04
SFO	$0.68±0.15^{b}$	1.64±0.47	0.17±0.04
0% (Control)	0.84±0.17	2.02±0.36	0.16±0.04
25%	0.72±0.15	1.66±0.67	0.19±0.03
50%	0.70±0.10	1.60±0.54	0.16±0.04
75%	0.67±0.10	1.73±0.51	0.18±0.03
p-value			
Oil type	0.023	0.494	0.072
Level	0.059	0.266	0.450
Oil type × Level	0.636	0.961	0.863

Values are mean ± SD. n=4. Mean of main effects in the same row with different superscripts (Sources: a, b, c; Levels: p, q, r) are significantly different ($p < .05$)

than those in intestine but specific lipase activity was almost similar between pyloric caeca and intestine. VO types showed an effect only on the trypsin activity in the intestine. Fish fed SO containing diets showed the highest trypsin activity ($p<0.05$) whereas those of SFO diet groups were the lowest. Replacement levels showed an effect on specific lipase and α-amylase activity in pyloric caeca in that the control group and 25% replacement level exhibited the similarly highest activities ($p<0.05$) whereas those fed VOs containing diets at all levels had lower activities.

3.6 Haemato-immunological parameters

Innate immune parameters are shown in Table 7. RBC, Ht, Hb, respiratory burst activity and lysozyme activity in fish fed diets containing VOs at different replacement levels were similar with those of fish fed the control diet ($p>0.05$). WBC and total serum protein in fish fed diets with

Table 7. Red blood cell (RBC), white blood cell (WBC), hematocrit (Ht), hemoglobin (Hb), serum protein, respiratory burst activity and lysozyme activity in Asian Seabass after 8 week feeding with diets

Diets (Oil type, % of FO replacement)	RBC cells/mL ($\times 10^9$ cells/mL)	WBC cells/mL ($\times 10^7$ cells/mL)	Ht (%)	Hb (g/dL)	Serum protein (mg/mL)	Respiratory burst activity (OD 640)	Lysozyme activity (μg/ml)
1 (Control)	4.12±0.08	3.58±0.46	41.57±3.32	9.18±0.52	82.62±3.86	0.027±0.016	4.12±0.17
2 (SO, 25%)	4.16±0.17	3.60±0.21	41.74±3.31	8.96±0.31	81.14±2.84	0.028±0.013	4.12±0.17
3 (SO, 50%)	4.17±0.15	3.82±0.58	41.44±3.71	8.95±0.38	81.30±2.63	0.025±0.013	4.05±0.31
4 (SO, 75%)	4.15±0.24	3.83±0.51	41.36±3.44	9.02±0.34	76.12±4.67	0.025±0.015	4.03±0.20
5 (PO, 25%)	4.09±0.23	3.48±0.39	41.65±2.46	9.16±0.23	81.90±5.03	0.022±0.005	4.02±0.20
6 (PO, 50%)	4.05±0.19	4.23±0.36	41.69±1.18	9.17±0.54	80.74±3.94	0.024±0.008	4.05±0.17
7 (PO, 75%)	4.09±0.20	4.40±0.35	41.24±2.86	9.16±0.83	77.41±3.73	0.023±0.010	4.03±0.23
8 (SFO, 25%)	3.96±0.18	4.09±0.40	41.95±1.13	9.19±0.15	81.77±2.09	0.019±0.006	3.95±0.25
9 (SFO, 50%)	4.05±0.28	4.05±0.21	40.90±1.30	9.16±0.44	81.57±6.09	0.018±0.005	3.97±0.20
10 (SFO, 75%)	4.05±0.21	4.09±0.13	39.18±1.24	9.11±0.41	76.69±3.38	0.020±0.007	4.13±0.20
Mean of main effects							
SO	4.15±0.16	3.72±0.47	41.53±3.14	9.02±0.39	80.48±3.93	0.026±0.013	4.08±0.21
PO	4.09±0.18	3.86±0.54	41.54±2.42	9.17±0.53	80.26±4.39	0.024±0.009	4.06±0.19
SFO	4.04±0.20	3.89±0.41	40.83±2.88	9.16±0.38	80.29±4.78	0.021±0.009	4.04±0.22
0% (Control)	4.12±0.08	3.58 ± 0.44^{q}	41.57±3.15	9.18±0.49	82.62 ± 3.57^{p}	0.027±0.014	4.12±0.17
25%	4.07±0.20	3.70 ± 0.42^{p}	41.76±2.36	9.08±0.26	81.51 ± 3.29^{p}	0.023±0.009	4.03±0.21
50%	4.08±0.21	3.98 ± 0.49^{p}	41.39±2.12	9.06±0.42	81.21 ± 4.24^{p}	0.022±0.009	4.02±0.23
75%	4.09±0.21	4.06 ± 0.44^{p}	40.55±2.66	9.11±0.56	76.84 ± 3.69^{q}	0.022±0.010	4.06±0.20
p-value							
Oil type	0.224	0.173	0.683	0.588	0.935	0.386	0.753
Level	0.893	0.006	0.631	0.948	0.000	0.662	0.319
Oil type × Level	0.924	0.290	0.943	0.997	0.998	0.972	0.680

Values are mean ± SD. n=8. Mean of main effects in the same row with different superscripts (Sources: a, b, c; Levels: p, q, r) are significantly different ($p < .05$)

different VOs levels were significantly affected, as the level of VOs increased, WBC increased while serum protein decreased. The lowest WBC was in fish fed 100% FO diet group which was significantly lower ($p<0.05$) than those fed with 25, 50 and 75% VOs diet groups. Total serum protein was reduced in the fish fed 75% VOs containing diets in comparison with 0%, 25 and 50% VOs fed groups.

4. Discussion

In the present study, Asian seabass responded well to all diets without affecting survival rate which indicated that alternative lipid sources did not have adverse effects on fish health. The results on diet consumption indicated that Asian seabass accepted the experimental diets differently depending upon the oil types and the levels of replacement for FO that related to nutrients available for fish growth. Growth performances of fish fed diets having SO, PO and SFO replacing FO at 25% were similar. However, increasing levels of FO replacement in Asian seabass diets beyond 25% reduced FW, WG and SGR regardless of vegetable oil sources. The reduced growth performance of the fish might be as results of the reduced amount of consumed feed which reduced when VOs were included to replace FO particularly, in the groups fed soybean oil. Similarly, a reduction in fish growth when VOs replaced FO in diets was observed in other fish species. In black seabream and large yellow croaker, SO and rapeseed oil (RO) could replace 60-80% (Peng *et al*., 2008) and 50% of FO (Mu *et al.*, 2020), respectively without compromising growth while complete FO replacement in both studies reduced growth. In European seabass, 60% partial replacement of FO was successful with a blend of RO, linseed oil (LO) and PO (Mourente, Dick, Bell, & Tocher, 2005). Total replacement of FO by SO in diets for sharp-snout seabream (Piedecausa, Mazón, García García, & Hernández, 2007), by SFO for Atlantic salmon (Bransden, Carter, & Nichols, 2003) and a blend of PO and LO for greater amberjack (Monge-Ortiz *et al*., 2018) had no adverse effects on growth and feed utilization. However, these studies used fish meal as protein source that also supplied FO in the basal diet and could contribute in good growth and nutrient utilization. In our study, the reduced growth responses of fish fed high levels of VOs in comparison with those of the control group were due to lower diet consumption as well as alteration of dietary FA profile that might affect lipid utilization. The reduced LER with increasing levels of VO indicated lower lipid utilization. The FA composition of lipid sources could have an effect on feed intake by pre- or post-absorptive mechanisms through affecting palatability and digestibility (Morais *et al*., 2006). Abimorad & Carneiro (2007) reported that dietary nutrient digestibility could negatively be affected through high inclusion level of VOs which results in low feed efficiency and, consequently, fish weight gain.

Dietary lipid supports required energy to maintain physiological activities and prevent dietary protein utilization to produce energy so that protein will be used for tissue formation effectively. In this study, fish protein and lipid composition were affected by VO replacement for FO though, the difference was very minor. Decreased carcass lipid in low FO replacing groups might be related to FA profile of the diets. High VO levels in diets may lead to decreased lipid digestion and absorption; therefore lower VO containing diet

fed groups may utilize lipids more efficiently for energy than those of high VO diet fed groups. The results were in line with previous studies in Atlantic salmon (Ruyter, Moya-Falcón, Rosenlund, & Vegusdal, 2006; Torstensen, Frøyland, Ørnsrud, & Lie, 2004). However, such effects were not observed in European seabass (Richard, Mourente, Kaushik, & Corraze, 2006) and humpback grouper (Shapawi, Mustafa, & Ng, 2008).

The obtained FA profile using the analysis method in the present study did not give the profile of HUFA (C≥20) of neither experimental diets nor fish carcass which should be rich HUFA. Thus, the reported FA profile in the final fish carcass and the diets lack the HUFA families. Taking this limitation into consideration, our findings indicated that increased levels of C-18 PUFA and decreased SFA levels with increasing dietary VO levels corresponded to types of oils incorporated in the diets. Furthermore, replacing FO by VOs at levels higher than 25% decreased utilization of fatty acids that resulted in deposition of lipids in body where protein might be used as energy source. Increased carcass levels of α-linoleic acid and α-linolenic acid in fish fed diets having high levels of VOs might indicate low capability of FA utilization for important metabolism. Lipid deposition was higher in SO fed groups that might be a result of inefficient utilization of C-18 PUFA (α-linoleic acid and α-linolenic acid) for longer chain unsaturated FA and consequently being deposited in body tissue. It is assumed that dietary FO replacement by high levels of SO reduced the HUFA (C≥20) in diets which may hamper lipid metabolism through reducing the activity of enzymes associated in the esterification of free FA into triacylglycerol and phospholipid to produce very low-density lipoprotein (VLDL) (Ruyter, Moya-Falcón, Rosenlund, & Vegusdal, 2006). Vegusdal, Gjøen, Berge, Thomassen, & Ruyter (2005) demonstrated that n-3 HUFA is crucial for producing VLDL in Atlantic salmon liver cell where lower level of n-3 HUFA in diet reduced production of VLDL that resulted in deposition of linoleic acid and α-linolenic acid in cell lipid. The results were similar to the findings in other species such as silvery-black porgy (Mozanzadeh *et al*., 2016), and turbot (Peng *et al*., 2014). The concentration of SFA in whole body carcass of all treatments was low indicating that SFA were mainly catabolized for energy production which was also reported in other species (Nasopoulou & Zabetakis, 2012; Sales & Glencross, 2011).

Specific lipase activity is known to change with degree of saturation and chain length of the FA of dietary lipid (Turchini, Torstensen, & Ng, 2009). Though we have some limitation on fatty acid profile analysis, the fatty acid profile results showed correlation between lipase activity and the lipid composition in the fish body. In our study, fish fed diet with 100% FO exhibited better specific lipase activity in pyloric caeca than those of diets replaced with VOs. Similarly, lower lipase activity was observed in yellowtail kingfish when fed diet incorporated with canola oil comparing with FO containing diet (Bowyer, Qin, Adams, Thomson, & Stone, 2012). Contrarily, increased lipase activity was found in European seabass when fed with coconut oil containing diet (Morais *et al*., 2004).

Hematological assessment is important for monitoring fish health status (Hrubec, Cardinale & Smith, 2000). In this study, replacement for FO by VOs at increasing levels showed a changes in WBC and total serum protein. The WBC increased with increasing VOs levels in the diets. This phenomenon was also observed in other species such as Vundu (*Heterobranchus longifilis*) and African Catfish (*Clarias gariepinus*), (Babalola, Adebayo, Apata, & Omotosho, 2009; Ochang, Fagbenro, & Adebayo, 2007). WBC plays a crucial role in immune responses, mainly in inflammation, which influenced by dietary eicosanoid profile (Tocher *et al*., 2006). A diet containing high n-6 PUFAs produces high levels of pro-inflammatory 2-series prostaglandins and 4-series leucotrienes (LTs) and lipoxins (LXs) resulting from ARA. Contrarily, high n-3 PUFAs containing diets produce anti-inflammatory 3-series PGs and 5-series LTs and LXs resulting from EPA. Diets rich in n-6 PUFA enhance immune response due to pro-inflammatory eicosanoids (Bell, Tocher, MacDonald, & Sargent, 1994). On the other hand, blood serum protein is fairly impatient biochemical system that reflects the condition of the organisms which may change under external and internal factors (Babalola, Adebayo, Apata, & Omotosho, 2009). The factors that may alter demand of total serum protein supply are sex, age, spawning, food, light, temperature, osmotic pressure, hibernation hormones, oxygen depletion and season (Booke, 1964). Hille (1982) validated that serum protein alteration is an indication of osmoregulatory dysfunction, damage of tissues surrounding blood vessels and hemodilution alteration in rainbow trout. The lower serum protein in the present study in Asian seabass may be a response to an unknown stress factor. The relationship between the changes of serum protein and high vegetable oil level replacing for fish oil should be further studied.

5. Conclusions

The results of this study suggest that substitution of FO in Asian seabass diet can be possible using vegetable oils such as SO and PO at 25% level of replacement without compromising growth performance, nutrient utilization, digestive enzyme activity and immunity. As young carnivorous fish including Asian seabass may not be able to fully utilize vegetable oils for important metabolic functions, the present study was conducted on this important stage. However, to fully understand the ability of this species on vegetable oil utilization for practical fish production, further studies using larger fish and culturing to marketable size should be investigated.

Acknowledgements

Md. Arefin Rahman was supported by Thailand's Education Hub Scholarship for ASEAN Countries (TEH-AC), Prince of Songkla University, Hat Yai, Thailand.

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