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Original Article

Effects of fish oil replacement by a combination of soybean and palm oil in Asian seabass (*Lates calcarifer*) diet on growth, fatty acid profile, digestive enzyme activity, immune parameters and salinity challenge♣

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Abstract

The experiment was in completely randomized design. Seven iso-nitrogenous and iso-lipidic diets were formulated having soybean and palm oil, an equal combination, to replace fish oil (FO) in diets at 0%, 25%, 37.5%, 50%, 62.5%, 75%, and 100%, respectively. Each diet was fed twice daily to Asian seabass juvenile (3.00±0.26g) in quintuplicate for eight weeks and performances were investigated. Finally, the fish were challenged with 30 ppt salinity to observe osmoregulatory responses. Zero mortality was observed in all feeding groups. Fish growth responses, diet consumption and feed conversion ratio were significantly affected ($p<0.05$). Crude lipid and viscerosomatic index of final fish were influenced ($p<0.05$). Fatty acid profiles of the whole-body reflected the dietary intake. Arachidonic acid (20:4n-6), eicosapentaenoic acid (20:5n-3), docosahexaenoic acid (22:6n-3) and n-3/n-6 ratio were better in fish fed 37.5% FO replaced diet. Specific digestive enzyme activities in stomach, pyloric caeca and intestine were unaffected ($p>0.05$). White blood cells, respiratory burst activity and serum protein were influenced by the diets ($p<0.05$). For salinity challenge, similar osmoregulatory responses were observed among the dietary groups. In short, the combination of soybean and palm oil substituted 75% FO in Asian seabass juvenile diet without affecting growth performance and health status.

Keywords: alternative lipid source, Asian seabass, growth, fatty acid profile, salinity challenge

1. Introduction

Lipids play important roles in many physiological functions in aquatic animals including cells constituents, source of energy, absorption and transportation of fat-soluble vitamins, essential fatty acids, precursors for certain hormones, vitamins and protein sparing (Turchini, Torstensen, & Ng, 2009). Fish are unable to synthesize long chain n-3 and highly unsaturated fatty acids, it is obligatory to supply them in formulated diet to attain normal growth performance as well as physiological functions. Currently, fish oil (FO) is used as main lipid source in aquatic animal diets to supply highly unsaturated fatty acid (HUFA), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) which are essential and showed beneficial effects on many cultured fish (Tocher, 2010). In 2016, approximately 75% of

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total FO production was used in aquafeed industry (Bachis, 2017). While the demand for aquafeed, animal feed and human consumption is expanding, FO production is inadequate due to static fish source, leading to increase prices. Therefore, suitable alternative lipid sources to replace FO is a prime need for sustainable development of aquaculture industry. Over the past years, plant origin sources have appeared as sustainable alternative to FO, as they are rich in C18-polyunsaturated fatty acids (C18-PUFA) such as linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3) but lack of HUFA (Turchini *et al*., 2009). However, marine fish species have limited capacity to synthesize HUFA from C18-PUFA but they are considered as essential fatty acid (Tocher, 2010). Thus, emphasis should be provided on feed formulation while replacing FO with vegetable oils (VO). Complete replacement of FO in large yellow croaker (Mu *et al*., 2020) negatively affected on growth performances but partial replacement is allowed. On the contrary, a blend of palm (PO) and linseed oil (LO) in greater amberjack (Monge-Ortiz *et al*., 2018) could replace without reducing growth and nutrient utilization at 100%. All fish species have not equally responded to different alternative lipid sources. However, manipulation of dietary fatty acid due to replacement of FO with VOs can be affected on digestive enzyme activity (Bowyer, Qin, Smullen, & Stone, 2012) and immune cells by changing fatty acid compositions of diets (Montero *et al*., 2015). Therefore, general health status should be considered when VOs using as an alternative lipid source.

Asian seabass (*Lates calcarifer*) is one of the most important food species in southeastern Asia and Australia. The production of this species has been increased over the past decades due to rapid expansion of global market of this species in different countries (Lim, Yusoff, Shariff, Kamarudin, & Nagao, 2019). Being a carnivorous euryhaline species, farming of Asian seabass predominantly depends on energy rich formulated diets which is expensive due to high price of ingredients such as fish meal and fish oil. Thus, this study aimed to investigate the effects of replacing FO by combination of soybean oil (SO) and PO on growth performance, fatty acid profile, nutrient utilization, health status and osmoregulatory responses in salinity challenge of Asian seabass.

2. Materials and Methods

2.1 Experimental design and diet preparation

The experiment was conducted on completely randomized design having 7 iso-nitrogenous and iso-lipidic formulated diets with five replications. An equal combination of food grade soybean oil (SO) (Thai Vegetable Oil Public Company Limited) and palm oil (PO) (P.K. Trading, Thailand, Company Limited) were sourced for fish oil (FO) replacement levels at 0%, 25%, 37.5%, 50%, 62.5%, 75%, and 100%. Diets were kept at -20 ºC until fed. Diet composition and fatty acid profile of the diets are presented in Tables 1 and 2, respectively.

2.2 Fish and feeding trial

The study was performed at Kidchakan Supamattaya Aquatic Animal Health Research Center, Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla, Thailand. Asian seabass fingerlings were nursed after obtaining from Coastal Aquaculture Technology and Innovation Research and Development Center, Songkhla, Thailand, in 1,000 L circular tank with nursery diet. The fish were gradually adapted to freshwater environment from 20 to 0 ppt (5 ppt/day). Feeding trial was carried out using fish fries having an average initial weight of 3.00±0.26 g (12 fish/aquarium), in 35 aquaria (100L, 60cm×40cm×50cm). An air blower was used to supply aeration. Eighty percent water was changed daily and water quality parameters were observed accordingly. Fish were fed twice daily at 8:30 and 17:00 to apparent satiation for eight weeks and daily feed consumption was recorded.

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

Ingredients (% of diet)	Diets (% of fish oil replacement)						
	T1 (Control)	T2 (25%)	T3 (37.5%)	T4 (50%)	T5 (62.5%)	T6 (75%)	T7 (100%)
Poultry by product meal (63.18% protein)	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Soybean meal1 (49.09% protein)	13.00	13.00	13.00	13.00	13.00	13.00	13.00
Soy protein concentrate (57.82% protein)	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Wheat flour	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Ground rice husk	7.49	7.49	7.49	7.49	7.49	7.49	7.49
Fish oil	7.30	5.50	4.60	3.60	2.70	1.80	-
Soybean oil	-	0.90	1.35	1.85	2.30	2.75	3.65
Palm oil	-	0.90	1.35	1.85	2.30	2.75	3.65
Vitamin and mineral premix[1]	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
DL-Methionine	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Tuna viscera powder[2]	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Proximate composition[3] (%)							
Moisture	5.30	5.86	4.70	4.77	5.10	4.66	5.46
Ash	10.95	11.11	11.17	11.14	11.13	11.51	11.11
Crude protein	44.19	44.22	45.18	44.95	45.05	45.69	45.04
Crude lipid	11.31	11.39	11.27	11.53	11.36	11.24	11.39

[1]Obtained from Thai Union Group, Thailand. [2]Tuna viscera powder was made freshly at the laboratory. [3]By analysis (AOAC, 1995)

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

Fatty acids (% of total fatty acids)	Diets (% of fish oil replacement)						
	T1 (Control)	T2 (25%)	T3 (37.5%)	T4 (50%)	T5 (62.5%)	T6 (75%)	T7 (100%)
Butyric acid (4:0)	0.15	0.12	0.11	0.14	0.12	0.12	0.10
Lauric acid (12:0)	0.26	0.27	0.29	0.31	0.31	0.31	0.31
Myristic acid (14:0)	2.47	1.97	1.74	1.50	1.27	1.04	0.61
Pentadecylic acid (15:0)	0.33	0.26	0.22	0.20	0.15	0.12	-
Palmitic acid (16:0)	20.78	21.87	22.40	23.05	23.61	24.03	24.80
Margaric acid (17:0)	0.24	0.20	0.19	0.17	0.19	0.16	0.12
Stearic acid (18:0)	4.65	4.75	4.79	4.85	4.98	5.00	5.17
Nonadecylic acid (19:0)	1.82	1.97	2.25	1.99	2.16	2.17	2.08
Arachidic acid (20:0)	0.17	0.20	0.19	0.20	0.21	0.22	0.25
Behenic acid (22:0)	0.16	0.19	0.16	0.20	0.21	0.22	0.21
Total SFA	31.04	31.79	32.35	32.61	33.22	33.40	33.65
Myristoleic acid (14:1n-5)	0.20	0.18	-	0.18	-	-	-
Palmitoleic acid (16:1n-7)	4.86	3.97	3.62	3.16	2.83	2.41	1.65
Vaccenic acid (18:1n-7)	3.13	2.88	2.72	2.54	2.37	2.28	1.97
Oleic acid (18-1n-9)	28.90	29.65	30.05	30.39	30.57	31.09	31.61
Gondoic acid (20:1n-9)	5.81	3.78	3.00	2.30	1.76	1.16	0.20
Erucic acid (22:1n-9)	0.73	0.54	0.45	0.35	0.27	0.20	-
Nervonic acid (24:1n-9)	0.73	0.56	0.48	0.38	0.32	0.23	-
Total MUFA	44.36	41.57	40.31	39.29	38.12	37.37	35.43
Linoleic acid (18:2n-6)	12.40	16.51	18.60	20.80	22.42	24.27	27.91
(Eicosadienoic acid (20:2n-6)	0.32	0.24	0.26	0.21	0.19	0.18	-
Dihomo-gamma-linolenic acid (20:3n-6)	0.11	-	-	-	-	-	-
Arachidonic acid (20:4n-6)	0.76	0.66	0.60	0.54	0.52	0.48	0.42
Adrenic acid (22:4n-6)	0.16	0.14	-	-	-	-	-
Osbond acid (22:5n-6)	0.78	0.29	-	-	-	-	-
Total n-6 PUFA	14.54	17.84	19.46	21.55	23.12	24.93	28.33
Alpha-linolenic acid (18:3n-3)	-	1.75	1.92	2.02	2.15	2.27	2.37
Stearidonic acid (18:4n-3)	0.35	0.25	0.22	0.18	-	-	-
Eicosapentaenoic acid (20:5n-3)	2.38	1.70	1.42	1.10	0.84	0.55	-
Clupanodonic acid (22:5n-3)	0.55	0.60	0.50	0.38	0.30	0.19	-
Docosahexaenoic acid (22:6n-3)	5.44	3.79	3.23	2.41	1.86	1.29	0.22
Total n-3 PUFA	8.72	8.09	7.29	6.09	5.14	4.30	2.59
n-3/n-6	0.61	0.45	0.37	0.28	0.22	0.17	0.09

[1]SFA: (4:0), (12:0), (14::0), (15:0), (16:0), (17:0), (18:0), (19:0), (20:0) and (22:0). [2]MUFA: (14:1n-5), (16:1n-7), (18:1n-7), (18-1n-9), (20:1n-9), (22:1n-9) and (24:1n-9). [3]n-6 PUFA: (18:2n-6), (20:2n-6), (20:3n-6), (20:4n-6), (22:4n-6) and (22:5n-6). [4]n-3 PUFA: (18:3n-3), (18:4n-3), (20:5n-3), (22:5n-3) and (22:6n-3)

2.3 Growth performance and feed utilization measurement

At the end of the feeding trial, fish were starved for two meals and individually weighed on the next day. Survival rate (SR, %), weight gain (WG, g/fish), % weight gain (%, WG), diet consumption (g/fish), feed conversion ratio (FCR) and specific growth rate (SGR, %/day) were assessed. Two fish from three replications of each treatment were used to determine viscerosomatic index (VSI), hepatosomatic index (HSI) and intraperitoneal fat (IPF). Protein efficiency ratio (PER), protein retention efficiency (PRE, %), lipid efficiency ratio (LER) and lipid retention efficiency (LRE, %) were assessed following Martino, Cyrino, Portz, & Trugo (2005).

2.4 Proximate composition and fatty acid profile analysis

Proximate composition of the fish from three replications were determined using standard methods of AOAC (1995). For fatty acid profile, two fish per replication were collected, freeze-dried (LABCONCO, FreeZone 6), ground (Philips HR2115), pooled and vacuum packed according to treatments. Fatty acid profile of the diets and freeze-dried samples were analyzed at the laboratory of Fisheries and Oceans Canada, West Vancouver Laboratories, British Columbia, Canada as described by Arney, Liu, Forster, McKinley, & Pearce (2015).

2.5 Digestive enzyme activity determination

Stomach, intestine and pyloric caeca were collected from three replications (6 fish/treatment) and preserved in liquid nitrogen for analysis of specific trypsin, lipase and α-amylase activity as described by Srichanun, Tantikitti, Utarabhand, & Kortner (2013). Crude enzyme was extracted from the sample by homogenizing in 2.5 volumes (v/w) of ice-cold distilled water and centrifuged at 4 °C, 12,000*g* for 30 min. Each enzymatic activity was measured under optimum pH at room temperature. The enzymatic activities were expressed as specific enzyme activity (U/mg protein/min). A modified Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) was applied to determine protein content using bovine serum albumin as standard.

The trypsin (EC 3.4.21.4) activity was assessed using N-benzoyl-DL-arginine-p-nitroanilide (BAPNA, Sigma B4875) as a substrate, as described by Erlanger, Kokowsky, & Cohen (1961). Briefly, BAPNA was dissolved in 43.5 mg: 1 mL dimethyl sulfoxide (DMSO) and diluted to 100 mL with 0.05 M Tris–HCl buffer containing 0.02 M $CaCl_2H_2O$. The assays were carried out at 8.5 pH. The absorbance was determined at 410 nm optical density in 96 well plate every 20 sec for 5 min. The unit of specific trypsin activity (U) was expressed as µmol of p-nitroaniline released per mg protein per min.

The activity of lipase (EC 3.1.1.3) was measured using para-nitrophenylpalmitate (p-NPP, Sigma N2752) as a substrate, as described by Winkler & Stuckman (1979). The substrate solution was prepared by swirling two solutions until completely dissolved, solution A (30 mg p-NPP in 10 mL of isopropanol) and solution B (0.1 g gum arabic and 1 mL Triton-X100 in 90 mL 50 mMTris–HCl buffer pH 8.5). The absorbance was read at 410 nm optical density every 20s for 5 min. The unit of specific lipase activity (U) was expressed as µmol of p-nitrophenol liberated per mg protein per min.

The activity of alpha-amylase activity was assessed using 1% starch solution in 50mM Tris-HCl buffer with 10mM NaCl (pH 8.0). Substrate solution (0.25 mL) and buffer (0.25 mL) were incubated with 0.05 mL crude enzyme for 10 min at room temperature. The produced reducing sugars were determined by dinitrosalicylic acid method (Somogyi, 1945). Finally, 0.5 mL 1% dinitrosalicylic acid (DNS) was added to the samples and incubated in boiling water bath for 5 min. The absorbance was recorded at 540 nm optical density. The maltose standard curve was used to determine the amount of maltose in this assay. One unit of specific alpha-amylase activity (U) was reported as µmol of maltose produced per min.

2.6 Determination of haemato-immunological parameters

Two fish from three replications (6 fish/treatment) were used to assess innate immune parameters. Blood was collected and processed to determine total blood cell (red; RBC and white blood cell; WBC), hemoglobin (Hb), hematocrit (Ht), respiratory burst activity, total serum protein and lysozyme activity. The counts of RBC and WBC were determined using the method of Supamattaya (1995). Briefly, 0.5 µL of blood was taken in diluting pipette and mixed upto 1 mL Yokoyama solution. Finally, haematocytometer were used to count RBC and WBC under microscope.

Hb level was measured colorimetrically by detecting the production of cyanamethaemoglobin (Blaxhall & Daisley, 1973). Briefly, 20 µL of blood sample was mixed with 5 mL Drabkin's solution and incubated at room temperature for 20 min. Then, the absorbance was read using microplate spectrophotometer (Multiskan GO Microplate Spectro photometer, Thermo Scientific) at 540 nm optical density. Ht was determined using the method of Larsen & Snieszko (1961). Blood was drawn to capillary tube and centrifuged at 10000*g* for 5 min. The percentage of hematocrit was calculated using the formula below.

Hematocrit (Ht, %) = (Packed cell volume / Total blood volume) × 100

Total plasma protein was assessed colorimetrically by the method of Lowry *et al*. (1951). Serum (5 µL) and double distilled water (995 µL) were mixed. Then 2 mL alkaline copper solution was applied and incubated at room temperature for 10 min. After that, 3 mL of Folin regent was employed and incubated at room temperature for another 10 min. Finally, the absorbance was read at 640 nm optical density in 96 well plate reader.

Serum lysozyme activity was determined by turbidimetric assay using microplate reader (Demers & Bayne, 1997). In brief, 25 µL of serum was mixed with 175 µL *Micrococcus lysodeikticus* solution and then the absorbance was read at 450 nm optical density. Hen egg lysozyme powder (Sigma) was employed as an external standard. Using the standard curve, the absorbance reduction rate of the samples was converted to lysozyme concentration (µg/ml). The reduction of nitroblue tetrazolium to formozan as a measure of superoxide anion (O_2^-) generation was used to evaluate the respiratory burst activity of leucocytes. The absorbance was measured at 640 nm with microplate reader where DMSO/KOH was used for blank (Stasiak & Baumann, 1996). For phagocytic activities, three fish per treatment were used and head kidney leucocytes were collected and prepared as described by Suwannasang, Dangwetngam, Issaro, Phromkunthong, and Suanyuk (2014).

2.7 Salinity challenge and blood sampling

After 8 weeks, two replications per feeding groups (12 fish/aquarium) were reared for another 1 week. Then, fish were fasted for 24 hours and a seven-days salinity exposure trial was conducted at 0 ppt and 30 ppt. Fish from each replication were randomly withdrawn and assigned into two aquaria (6 fish/aquarium) previously set with salinity 0 ppt and 30 ppt. Fish were sampled at 0 and 3 hours post salinity exposure. To maintain similar sampling time, fish of each aquarium was exposed at 10 minutes intervals. Two fish from each aquarium (4 fish/treatment) were anesthetized and blood was withdrawn for immediate preparation of RBC count and Ht determination. The remaining blood samples were used for serum collection. Serum electrolytes (Na^+, Cl^- and K^+), glucose and osmolality were determined at Hematology Laboratory, Songklanagarind Hospital, Hat Yai, Thailand. Mortality rate was observed with rest of the fish till seven days of post salinity exposure.

2.8 Statistical analysis

After checking normality and homogeneity of variance, one-way ANOVA was applied to analyze all the data by using the SPSS 22 for Windows. The difference between the treatment means was determined by Tukey's HSD test at 95% confidence level ($p<0.05$). A three-way ANOVA was performed for the parameters of salinity challenge.

3. Results

3.1 Acceptance of diets

The results showed an influence on diet consumption when replacing FO ($p<0.05$) with varying levels of SO and PO combination (Table 3). The highest

consumption was found in fish fed 100% FO replacement diet but was not significantly different from those of the control and treatment groups except the group fed with 25% and 50% FO replacement diets.

3.2 Survival and growth performance

The survival rate of fish in all treatments was 100%. In T4 (50% FO replacement), the fish of one replication died due to oxygen supply failure at week 5. Growth performance of Asian seabass fed with the FO replaced diets for eight weeks is shown in (Table 3). In comparison with the control group, dietary FO replacement by an equal combination of SO and PO significantly increased ($p<0.05$) WG, %WG, and SGR except for those fed 25% and 50% FO replacement diets. The fish fed 37.5% FO replacement diet showed the highest growth performance. Consistently, the fish fed 37.5% FO replacement diet had significantly the lowest FCR which was statistically like those of 62.5% and 75% replacement group and lower than the control group ($p<0.05$). VSI significantly increased in fish fed with 100% FO replaced diet ($p<0.05$) whereas HSI and IPF were unaffected ($p>0.05$).

3.3 Proximate composition of fish carcass and nutrient efficiency

Dietary fish oil replacement with combination of SO and PO had no significant effect ($p>0.05$) on moisture, ash and crude protein in the final fish carcass (Table 4). On the contrary, fish fed with 100% FO replacement diet presented higher carcass lipid than those fed the control, 25%, 37.5% and 50% FO replacement diets, while fish fed 62.5% and 75% FO replacement diets did not change lipid content compared to the other feeding groups. For nutrient utilization, no significant difference was found in PER, LER, PRE and LRE when FO was replaced at different levels by SO and PO equal combination.

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

Growth parameters	Diets (% of fish oil replacement)							p-value
	T1 (Control)	T2 (25%)	T3 (37.5%)	T4 (50%)	T5 (62.5%)	T6 (75%)	T7 (100%)	
Initial weight (g/fish)	3.0±0.27	3.0±0.26	3.0±0.25	3.0±0.27	3.0±0.25	3.0±0.28	3.0±0.28	
Weight gain (g/fish)	25.78±1.78^{a}	25.10±1.60^{a}	29.81±0.65^{b}	25.14±1.99^{a}	26.49±1.66^{ab}	27.24±2.36^{ab}	27.00±0.32^{ab}	0.004
% Weight gain	859.80±58.28^{a}	836.05±53.20^{a}	992.54±22.77^{b}	838.04±68.47^{a}	883.68±55.98^{ab}	908.30±78.06^{ab}	899.79±10.77^{ab}	0.005
Survival rate (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	
Consumed feed (g/fish)	32.07±1.24^{abc}	31.45±1.37^{a}	33.75±0.56^{bc}	31.83±0.81^{ab}	32.76±1.54^{abc}	33.12±0.67^{abc}	34.04±0.37^{c}	0.005
FCR	1.25±0.05^{b}	1.25±0.04^{b}	1.13±0.03^{a}	1.27±0.08^{b}	1.24±0.05^{ab}	1.22±0.08^{ab}	1.26±0.02^{b}	0.016
SGR (%/Day)	4.03±0.11^{a}	3.99±0.11^{a}	4.27±0.04^{b}	4.00±0.13^{a}	4.08±0.10^{ab}	4.12±0.14^{ab}	4.11±0.02^{ab}	0.008
VSI (%)	5.97±0.63^{a}	6.76±0.50^{ab}	6.70±0.88^{ab}	6.43±0.20^{ab}	6.25±1.09^{ab}	6.79±1.34^{ab}	7.94±0.84^{b}	0.022
HSI (%)	1.63±0.18	1.91±0.37	1.63±0.20	1.47±0.19	1.55±0.42	1.65±0.15	1.84±0.46	0.197
IPF (%)	1.98±0.49	2.35±0.35	2.84±0.86	2.54±0.93	2.75±1.36	3.41±1.47	3.71±0.43	0.061

Values are mean ± SD of five replicates except for T4 where n=4 and VSI, HSI and IPF where n=6. Means of main effects in the same row with different superscripts (a, b and c) 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 (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 with fish oil replacement diets

	Initial fish	Diets (% of fish oil replacement)							p-value
		T1 (Control)	T2 (25%)	T3 (37.5%)	T4 (50%)	T5 (62.5%)	T6 (75%)	T7 (100%)	
Moisture (%)	76.54	71.64±0.84	71.58±0.93	71.27±0.23	71.39±0.70	70.49±1.26	71.04±0.34	70.02±0.44	0.153
Ash (%)	3.54	4.58±0.15	4.50±0.15	4.55±0.16	4.58±0.24	4.63±0.29	4.21±0.15	4.43±0.31	0.112
Protein (%)	15.51	17.93±0.33	17.71±0.64	17.83±0.25	17.92±0.59	17.68±0.85	17.50±0.30	17.40±0.29	0.477
Lipid (%)	3.64	5.83±0.43^{a}	5.74±0.08^{a}	5.57±0.23^{a}	5.61±0.15^{a}	6.21±0.99^{ab}	6.53±0.20^{ab}	6.94±0.44^{b}	0.001
PER	-	1.79±0.06	1.84±0.05	1.94±0.03	1.70±0.17	1.82±0.04	1.77±0.15	1.75±0.04	0.146
LER	-	7.01±0.24	7.14±0.18	7.76±0.12	6.61±0.67	7.22±0.15	7.19±0.61	6.93±0.17	0.063
PRE (%)	-	32.63±0.92	32.96±1.01	34.97±0.79	30.89±3.03	32.57±1.37	31.36±3.07	30.85±0.87	0.156
LRE (%)	-	42.65±1.25	42.69±1.88	44.45±1.76	38.91±2.08	46.94±10.20	49.33±4.70	50.64±4.04	0.093

Values are mean ± SD. n=3. Mean of main effects in the same row with different superscripts (a, b, c) are significantly different ($p<0.05$). 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]

3.4 Fatty acid profile of diets and fish carcass

The fatty acid profile of the diets reflected FO replacement with SO and PO at different levels (Table 2). The control diet had higher levels of n-3 and n-6 PUFA such as EPA, 20:5n-3, DHA, 22:6n-3 and ARA, 20:4n-6 as well as n-3/n-6 ratio. The contents of n-3 and as well as n-3/n-6 ratio gradually decreased with increasing replacement levels whereas, n-6 PUFA gradually increased.

The fatty acid composition of the whole-body carcass was influenced by the dietary intake (Table 5). Generally saturated fatty acids were comparatively higher in fish carcass than the supplied diets. Total monounsaturated fatty acids decreased in fish fed diets with increased levels of fish oil replacement except in fish fed with 62.5% FO replacement diet. Oleic acid (OA, 18-1n-9) was the richest fatty acid in the fish carcass across the treatments. As expected, LA and ALA contents were the highest in those fish fed with 100% vegetable oil which decreased with increasing levels of FO in the diets. The fish fed 37.5% FO replacement showed comparatively higher ARA, EPA and DHA than other treatment groups.

3.5 Digestive enzyme activities

Compared to the control group, dietary fish oil replacement by an equal combination of SO and PO had no significant effect ($p>0.05$) on specific trypsin, lipase and amylase activity in stomach, pyloric caeca and intestine (Table 6).

3.6 Haemato-immunological parameters

RBC, Ht, Hb and serum lysozyme activity were not affected by FO replacement with SO and PO as compared with the control group ($p>0.05$) whereas, WBC, total serum protein and serum respiratory burst activity showed a significant difference ($p<0.05$) among the feeding groups (Table 7). A decreasing trend of WBC was observed in the fish fed with FO replaced diets at different levels as compared

Table 5. Fatty acid profile of Asian seabass carcass after 8 weeks of feeding with fish oil replacement diets

Fatty acids (% of total fatty acids)	Initial fish	Diets (% of fish oil replacement)						
		T1 (Control)	T2 (25%)	T3 (37.5%)	T4 (50%)	T5 (62.5%)	T6 (75%)	T7 (100%)
Caproic acid (6:0)	0.25	0.16	0.10	-	-	0.22	0.15	0.07
Lauric acid (12:0)	-	0.14	0.14	0.13	0.14	0.16	0.15	0.15
Tridecylic acid (13:0)	0.13	-	-	-	-	-	-	-
Myristic acid (14:0)	5.58	3.53	2.74	2.21	2.09	2.06	1.58	1.13
Pentadecylic acid (15:0)	1.77	0.50	0.38	0.29	0.27	0.26	0.21	0.13
Palmitic acid (16:0)	32.80	26.99	26.28	24.07	24.48	26.61	24.90	23.69
Margaric acid (17:0)	1.52	0.34	0.28	0.22	0.21	0.23	0.20	0.15
Stearic acid (18:0)	12.40	6.23	6.02	5.60	5.81	6.43	6.16	5.65
Nonadecylic acid (19:0)	3.76	1.18	1.32	1.43	1.58	0.99	1.25	1.32
Arachidic acid (20:0)	0.49	0.17	0.18	0.17	0.17	0.21	0.21	0.19
Behenic acid (22:0)	0.35	-	0.09	0.09	-	0.10	0.10	0.11
SFA[1]	59.05	39.23	37.53	34.23	34.74	37.27	34.91	32.59
Myristoleic acid (14:1n-5)	-	0.13	0.10	0.09	0.09	0.10	0.08	-
Palmitoleic acid (16:1n-7)	7.61	6.85	5.57	4.66	4.40	4.18	3.38	2.54
Vaccenic acid (18:1n-7)	4.54	3.92	3.44	3.15	2.92	2.98	2.73	2.24
Oleic acid (18-1n-9)	17.65	34.59	34.82	34.34	34.04	38.48	36.91	35.74
Gondoic acid (20:1n-9)	0.80	4.68	3.89	2.71	2.13	2.17	1.37	0.35
Erucic acid (22:1n-9)	-	0.50	0.43	0.37	0.27	0.25	0.18	-
Nervonic acid (24:1n-9)	0.60	0.24	0.30	0.21	0.14	0.47	0.37	0.18
MUFA[2]	31.20	50.91	48.55	45.52	43.99	48.62	45.01	41.05
Linoleic acid (18:2n-6)	1.79	5.85	10.19	13.91	15.74	11.05	16.14	21.86
Gamma-linolenic acid (18:3n-6)	-	0.18	0.37	0.71	0.76	0.28	0.54	1.25
Eicosadienoic acid (20:2n-6)	-	0.34	0.27	0.31	0.28	0.29	0.23	0.28
Dihomo-gamma-linolenic acid (20:3n-6)	-	-	0.20	0.28	0.34	0.15	0.23	0.33
Arachidonic acid (20:4n-6)	0.74	0.34	0.36	0.47	0.35	0.17	0.24	0.27
Adrenic acid (22:4n-6)	-	-	-	0.09	-	-	-	-
Osbond acid (22:5n-6)	0.34	-	-	-	-	0.16	-	-
n-6 PUFA[3]	2.87	6.71	11.40	15.77	17.48	12.09	17.37	23.99
Alpha-linolenic acid (18:3n-3)	0.23	0.22	0.12	0.72	1.17	0.38	0.91	1.40
Stearidonic acid (18:4n-3)	0.82	0.68	0.10	0.18	0.16	0.65	0.34	0.11
Eicosapentaenoic acid (20:5n-3)	0.62	0.34	0.41	0.62	0.42	0.18	0.21	0.13
Clupanodonic acid (22:5n-3)	0.29	0.17	0.23	0.44	0.31	0.08	0.14	-
Docosahexaenoic acid (22:6n-3)	3.53	0.93	1.14	1.95	1.27	0.58	0.77	0.42
n-3 PUFA[4]	5.49	2.34	2.00	3.91	3.34	1.88	2.36	2.05
n-3/n-6	1.91	0.35	0.18	0.25	0.19	0.16	0.14	0.09

1SFA: (6:0), (12:0), (13:0), (14::0), (15:0), (16:0), (17:0), (18:0), (19:0), (20:0) and (22:0). 2MUFA: (14:1n-5), (16:1n-7), (18:1n-7), (18-1n-9), (20:1n-9), (22:1n-9) and (24:1n-9). 3n-6 PUFA: (18:2n-6), (18:3n-6), (20:2n-6), (20:3n-6), (20:4n-6), (22:4n-6) and (22:5n-6). 4n-3 PUFA: (18:3n-3), (18:4n-3), (20:5n-3), (22:5n-3) and (22:6n-3)

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

Parameters	Diets (% of fish oil replacement)							*p*-value
	T1 (Control)	T2 (25%)	T3 (37.5%)	T4 (50%)	T5 (62.5%)	T6 (75%)	T7 (100%)	
RBC ($\times 10^9$ cell/mL)	3.67±0.30	3.60±0.17	3.66±0.45	3.65±0.26	3.63±0.13	3.72±0.15	3.61±0.48	0.995
WBC ($\times 10^7$ cell/mL)	4.45±0.86^{c}	3.87±0.51^{bc}	3.30±0.10^{abc}	3.65±0.84^{abc}	3.16±0.89^{ab}	2.95±0.28^{ab}	2.44±0.62^{a}	0.000
Ht (%)	34.92±2.78	32.20±3.51	32.73±6.73	28.78±3.03	31.20±2.17	30.16±3.59	28.61±2.43	0.096
Hb (g/dL)	5.16±0.70	5.31±0.16	5.06±0.40	5.51±0.49	5.66±0.77	5.94±0.66	5.83±0.27	0.093
Serum protein (mg/mL)	100.36±7.77^{b}	106.41±5.88^{b}	102.84±4.25^{b}	102.75±5.90^{b}	104.64±7.11^{b}	100.35±7.15^{b}	55.18±6.59^{a}	0.000
Serum respiratory burst activity (OD 640)	0.33±0.04^{ab}	0.38±0.09^{ab}	0.44±0.06^{b}	0.42±0.03^{ab}	0.34±0.08^{ab}	0.34±0.03^{ab}	0.31±0.08^{a}	0.012
Serum lysozyme activity (µg/mL)	3.85±0.10	4.06±0.18	4.08±0.42	4.25±0.29	4.18±0.44	4.19±0.20	4.12±0.24	0.594
Phagocytosis (%)	16.00±2.29	18.75±1.06	17.75±0.35	16.33±3.21	16.33±2.52	16.00±2.29	18.50±2.83	0.738
Phagocytosis index	8.16±0.79	5.82±0.46	5.77±0.11	5.12±2.16	5.92±1.26	4.57±0.55	4.96±1.99	0.101

Values are mean ± SD. n=6. Means of main effects in the same row without superscripts are not significantly different ($p > 0.05$).

Table 7. Red blood cell **(**RBC**)**, white blood cell **(**WBC**)**, hematocrit **(**Ht**)**, hemoglobin **(**Hb**)**, serum protein, respiratory burst activity, lysozyme activity and phagocytic activity in Asian seabass after 8 weeks feeding with fish oil replacement diets

Specific digestive enzyme (Unit/mg protein/min)	Diets (% of fish oil replacement)							p-value
	T1 (Control)	T2 (25%)	T3 (37.5%)	T4 (50%)	T5 (62.5%)	T6 (75%)	T7 (100%)	
Stomach								
Specific trypsin activity	0.34±0.09	0.37±0.12	0.36±0.12	0.33±0.09	0.36±0.11	0.32±0.16	0.35±0.09	0.988
Specific lipase activity	0.51±0.07	0.55±0.12	0.46±0.10	0.49±0.08	0.47±0.10	0.43±0.21	0.52±0.17	0.893
Specific amylase activity	1.30±0.14	1.28±0.51	1.40±0.35	1.30±0.37	1.44±0.19	1.31±0.34	1.44±0.64	0.982
Pyloric caeca								
Specific trypsin activity	0.97±0.14	1.10±0.28	1.12±0.33	1.04±0.19	1.00±0.14	1.27±0.40	1.23±0.16	0.365
Specific lipase activity	3.58±0.48	3.87±0.73	3.41±0.71	3.19±0.85	3.10±0.73	3.14±0.67	3.34±0.74	0.572
Specific amylase activity	1.32±0.56	1.42±0.32	1.25±0.20	1.20±0.30	1.30±0.20	1.13±0.27	1.29±0.32	0.860
Intestine								
Specific trypsin activity	0.70±0.13	0.62±0.07	0.83±0.13	0.84±0.20	0.73±0.28	0.86±0.10	0.88±0.13	0.136
Specific lipase activity	1.87±0.41	1.71±0.47	2.47±0.30	1.53±0.51	1.87±0.54	1.76±0.62	1.91±0.49	0.085
Specific amylase activity	1.14±0.19	1.00±0.15	1.03±0.22	1.22±0.27	0.96±0.12	1.09±0.35	1.31±0.35	0.230

Values are mean ± SD. n=6, except phagocytosis parameters where n=3. Means of main effects in the same row with different superscripts (a, b and c) are significantly different ($p < 0.05$).

with the control group. Phagocytosis and phagocytosis index in fish were not affected by the diets (p>0.05).

3.7. Salinity challenge

Salinity challenge results are presented in Figures 1 to 2 and the three-way ANOVA analysis is shown in Table 8. Survival rate of the fish in all feeding groups was 100% when challenged with 0 ppt and 30 ppt for seven days. All treatment groups showed higher serum sodium and chloride ion concentrations as well as osmolality when the fish were exposed to 30 ppt for 3 hours as compared to 0 ppt baseline level. SO and PO combination for FO replacement did not show any influence on Na^+, Cl^-, K^+ levels, serum osmolality, serum glucose, RBC and Ht levels (p>0.05). The salinity exposure time showed an effect on serum glucose concentration and RBC (p<0.05). Additionally, serum Na^+, Cl^- and osmolality were significantly affected by both salinity levels and exposure time as interaction of the factors was detected (p<0.05). Neither diets×exposure time nor diets×salinity levels interactions showed any statistical significance on serum electrolytes, RBC and Ht (p>0.05). Diets×exposure time interaction showed an effect on serum osmolality and glucose levels (p<0.05) whereas diets×salinity levels had no influence on them (p>0.05).

4. Discussion

In this study, the vegetable oils consisting of an equal mixture of SO and PO used to substitute FO at different levels did not have an effect on the survival rate of Asian seabass as 100% survival was obtained. Similar or higher levels of feed consumption of the fish in different treatment groups than those of the control diet fed group showed that the combination of SO and PO as alternative lipid sources did not impair diet palatability. Suitable intake of well balance diets generally leads to good growth. Growth performance and feed efficiency of the fish fed diets having SO and PO combination either as a partial or complete replacement for FO was good as compared to that of the control group. Moreover, the fish fed with 37.5% FO replaced diet showed better weight gain, FCR and SGR than other feeding groups (Table 3). In our previous

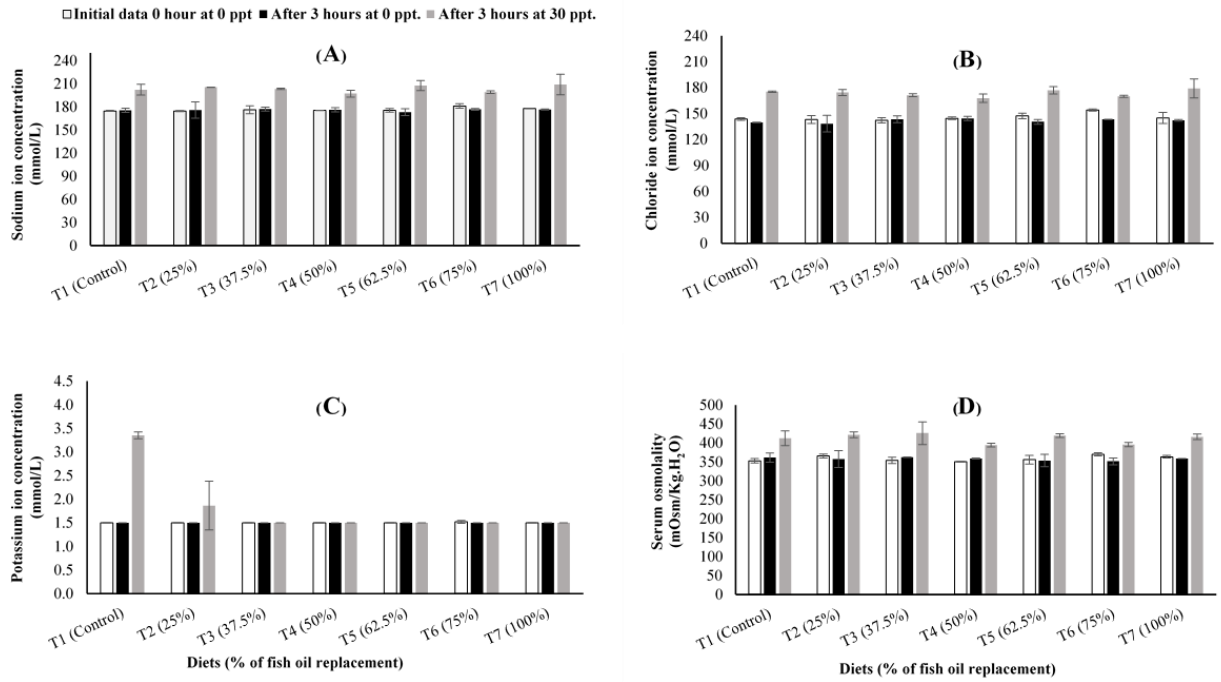

Figure 1. (A) Serum sodium ion, (B) chloride ion, (C) potassium ion concentrations and (D) serum osmolality in Asian seabass at 0 and 3 hours of salinity challenge at 0 and 30 ppt (mean±SD)

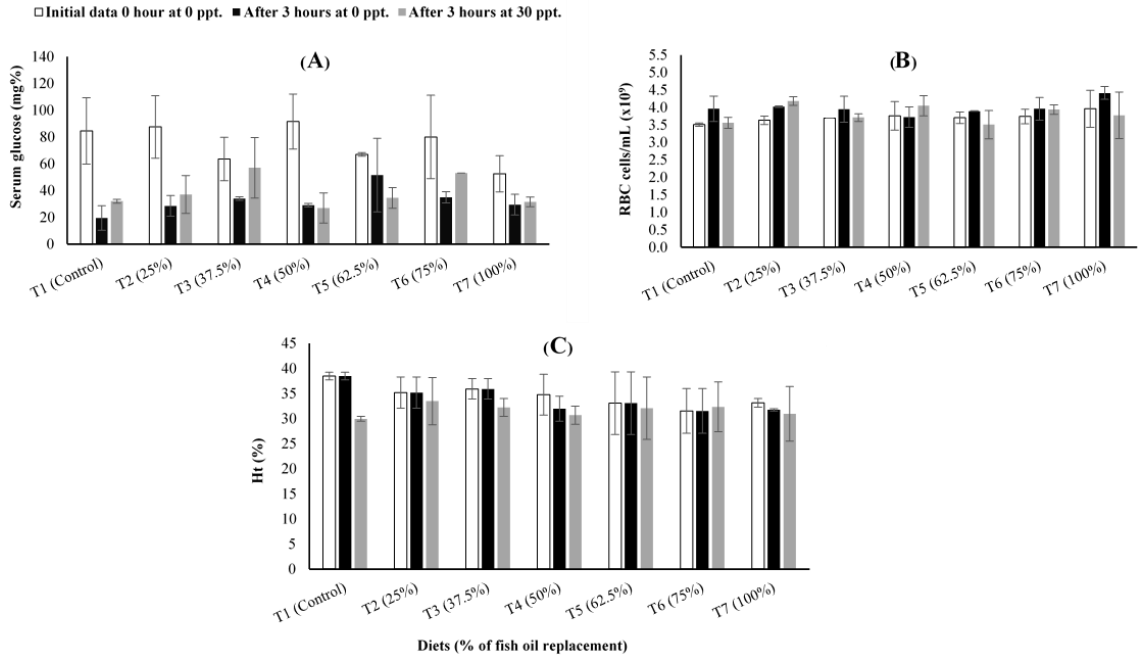

Figure 2. (A) Serum glucose concentrations (mg%), (B) red blood cell (RBC cells/mL) and (C) hematocrit (Ht, %) in Asian seabass at 0 and 3 hours of salinity challenge at 0 and 30 ppt (mean±SD)

study in Asian seabass, we observed reduced growth performance when the fish were fed with diets having either SO or PO replacing FO beyond 25% (Rahman *et al*., 2021). The lower level of SO or PO replacing for FO than this study may be due to imbalanced FA in the single source of oil to support maximum growth. The results of this study are similar with the study in greater amberjack which demonstrated feasibility of partial or complete replacement with a blend of PO and linseed oil (LO) (Monge-Ortiz *et al*., 2018). On the contrary, large yellow croaker showed negative growth responses on complete replacement of FO by rapeseed oil (Mu *et al*., 2020). However, species specific responses were observed when substituting FO by vegetable oils (Turchini *et al*., 2009). Generally, vegetable oils such as PO contain high

Table 8. Three-way ANOVA analysis (p-value) on the effects of diets (D), times (T), salinity levels (S) and their interaction for Asian Seabass under salinity challenge after transferring to 0 or 30 ppt and sampled at 0 and 3 h of post exposure

Factors	Parameters						
	Na^+	Cl^-	K^+	Serum osmolality	Serum glucose	RBC	Ht
D	0.495	0.080	0.531	0.208	0.281	0.196	0.148
T	0.000	0.000	0.218	0.000	0.000	0.016	0.104
S	0.000	0.000	0.204	0.000	0.480	0.247	0.201
D×T	0.422	0.060	0.507	0.048	0.039	0.728	0.750
D×S	0.694	0.478	0.520	0.719	0.920	0.673	0.959
T×S	0.000	0.000	0.204	0.000	0.480	0.247	0.278
D×T×S	0.694	0.478	0.520	0.719	0.920	0.673	0.934

level of SFA (16:0) and MUFA (18:1n-9) which are less digestible and lacking n-3 PUFA. SO contains high level of n-6 PUFA and essential n-3 PUFA necessary for some fish species which are more digestible than PO. Therefore, a high digestible oil with sufficient essential fatty acids availability would be a good lipid source to support good growth in fish. In this study, increased growth performance might be a result of suitable mixture of SO and PO which provides proper combination of essential fatty acids in the diets.

Optimal dietary lipid, in terms of both FA profile and levels, provides essential energy for maintaining proper physiological activities and prevents protein utilization for energy so that protein can be used for effective tissue formation. Lipid composition of fish carcass samples reflected dietary fatty acid. The carcass lipid increased with increasing FO replacement levels that might be related to the dietary fatty acid composition particularly, MUFA. Gradual lipid deposition in fish fed diets with increasing VO level may be due to an increased amount of OA and LA in the fish body that may be associated with lower levels of n-3 PUFA availability in diets. Previous study showed that dietary n-3 PUFA plays important role for very low-density lipoprotein (VLDL) production in liver cells of Atlantic salmon, the lack of VLDL hampers esterification of free fatty acids into triacylglycerol and phospholipid which leads to an increase of lipid deposition (Ruyter, Moya-Falcón, Rosenlund, & Vegusdal, 2006). In this study, the deposition of lipids in viscera increased when fish fed with 100% FO replaced diet by combining SO and PO. The accumulation of fat in visceral organs may be due to lack of some essential fatty acids in SO and PO lipids.

The storage of lipid in fish liver is commonly used as indicator of unsuitable lipid ingestion, may cause a fatty liver syndrome which is a well-established issue while replacing FO by VOs (Piedecausa, Mazón, García García, & Hernández, 2007). This may be related to increased lipid peroxidation as well as inefficient nutrient utilization (Craig, Washburn, & Gatlin, 1999). In our study, HSI was similar in all the feeding groups suggesting no hepatic problem with increasing levels of combined SO and PO inclusion.

The effects of using alternative lipid sources to replace FO in fish diets on growth, feed performance, fatty acid composition and deposition is still in contradiction (Turchini *et al*., 2009). Previous findings recommend that SFA and MUFA may influence long chain PUFA utilization from diet to tissue (Bowzer, Jackson, & Trushenski, 2016). In our study, despite ARA, EPA, DHA and n-3/n-6 ratio in the 37.5% FO replaced diet were less than that of the control diet, the levels of these FAs were higher than those of other treatment groups. Dietary replacement by SO-PO combination at 37.5% for FO may provide a balanced proportion of SFA: MUFA: PUFA and n-3/n-6 ratio for maintaining and enhancing LC-PUFAs in body tissues.

The overall responses of specific digestive enzyme activities (trypsin, lipase and ∞-amylase) differed among the organs, activities in pyloric caeca were generally higher than those in the stomach and intestine. Principally, the digestive enzyme activity is directly associated with dietary intake (Eusebio & Coloso, 2002). However, the results demonstrated that substitution of FO in Asian seabass diets by SO-PO mixture had no effect on trypsin, lipase and ∞-amylase activities in stomach, pyloric caeca and intestine.

Hematological assessment is a useful tool to monitor metabolic disorders, deficiencies, adaptation processes to various environmental influences and chronic stress status (Peres, Santos, & Oliva-Teles, 2013). In this study, FO substitution by SO and PO combination showed an effect on WBC, serum protein and respiratory burst activity among the feeding groups. The FO replacement in diets by VOs may lead to change of whole-body fatty acid profile, especially n-3/n-6 ratio, which may modulate the response of immune system by altering fatty acid composition and membrane fluidity of the immune cells (Montero *et al*., 2008). It may also modify non-specific responses both cellular and humoral (Lin & Shiau, 2007), eicosanoid production (Bell, Tocher, MacDonald, & Sargent, 1994) and gene expression (Montero *et al*., 2015). In this study, fish fed with control diet had the highest WBC, which decreased in fish fed with diets having SO and PO higher than 50% replacement. WBC plays crucial role in inflammation which may be affected by alteration of fatty acids in diets, especially ARA which enhance proinflammatory eicosanoids (Bell *et al*., 1994). This result is contradictory with our previous report on Asian seabass where we have observed increased number of WBC in fish fed with increasing levels of FO replaced diet (Rahman *et al*., 2021). In case of total serum protein, we found similar findings with our previous study that dramatically decreased in fish fed with 100% FO replaced diets. Total serum protein supply may be affected by several factors such as sex, age, spawning, food, light, temperature, osmotic pressure, hibernation hormones, oxygen depletion and season (Booke, 1964). Thus, further studies are required to rule out possible reasons of lowering WBC and serum protein in fish fed with higher level of FO replaced diet by combining SO and PO. The lysozyme activity was not affected but serum respiratory burst activity was altered by the diets. Previously, Subhadra,

Lochmann, Rawles, & Chen (2006) reported similar findings in largemouth bass where dietary FO was substituted by poultry fat. The lower serum respiratory burst activity in fish fed with 100% FO replacement diet compared to the other feeding groups may be attributed to the lack of an optimal n-3/n-6 ratio in this diet. Therefore, the results obtained in this study indicated that total replacement of dietary FO in juvenile Asian seabass diet with equal mixture of SO and PO had negative effects on immune parameters which may have an impact on health when feeding with alternative lipid sources for long time.

Seabass is a euryhaline fish which can survive in a wide range of salinity through functional changes in osmoregulatory organs such as gills and intestine (Rasmussen, 1991). However, 7 days of salinity challenge, we observed an outstanding capacity to adapt with the acute changes in salinity from 0 ppt to 30 ppt in which 100% survival rate observed in all feeding groups. In this study, the metabolic response of Asian seabass to the salinity challenge was not affected by diets but the salinity levels and time of exposure influenced the metabolic responses. The results of serum ions (Na^+, Cl^- and K^+) and osmolality displayed an ionic and osmotic balance in Asian seabass when subjected to changes in salinity from 0 to 30ppt. Similar findings was observed in European seabass, another euryhaline fish when exposed to 35ppt from 15ppt (Silva-Brito *et al*., 2019). The fish challenged in hyperosmotic environment need to drink more seawater to maintain physiological homeostasis by balancing the instant fluxes of water and electrolytes, specially Na^+, Cl^- and K^+ (Whittamore, 2012). Na^+ and Cl^- are extracellular and K^+ is intracellular dominant osmotic components. The acclimation with altered salinity requires more energy. In our study, hyperglycemic response was observed after 3 hours of exposure at 30ppt comparing to the 0ppt. The elevation of serum glucose at 3 hours of salinity exposure at 30ppt was higher than 0ppt but lesser than initial data due to higher energy demands in hyperosmotic condition than hypoosmotic condition. This is further supported by the total number of RBC in fish, which play important role in oxygen transportation in cell that was influenced by the time of exposure. Additionally, an interaction was observed between diets and time of exposure on serum osmolarity and glucose level. This specified that diets may influence in the acclimatization at the beginning of salinity stress, but zero mortality after 7 days of exposure demonstrated that the fish adapted well to the abrupt salinity changes. However, the responses to salinity challenge on serum ions and osmolality indicated that Asian seabass fed with FO replaced diets by SO and PO at different levels are able to recover from salinity stress ranging from 0 ppt to 30 ppt.

5. Conclusions

The results suggested that combination of soybean and palm oil can substitute up to 75% of fish oil in young Asian seabass diet without any apparent detrimental effect on growth and health. It is worth noting that soybean and palm oil combination at 75% and 25% fish oil in the present study provide required essential fatty acids for seabass to maintain proper health status. Inclusion of the oil mixture at higher level than 75% replacement for fish oil might result in increased carcass lipid and reduced white blood cell and serum protein. The study also showed that Asian seabass fed with FO replaced diets were able to regulate osmoregulation in salinity stress conditions. However, the study was carried out for a standard eight-week growth trial in young seabass, the validation for the alternative oil sources over a longer culture period to reach marketable size fish may be required for commercial application.

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