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Research Article

Combined effects of dietary soybean lecithin and different lipid sources on growth, fatty acid profile, immune response, and plasma biochemistry in juvenile Asian seabass (*Lates calcarifer*)

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Abstract

A six-week investigation was conducted to examine the interactive influences of dietary lipid sources and soybean lecithin (SBL) levels on some physiological responses of Asian seabass (*Lates calcarifer*) juveniles (50.2±2.3 g). A research design containing three SL levels, including 0, 2 and 4%, and four lipid sources, including fish oil (FO), vegetable oils (VO, canola and soybean oils; 1:1), rendered animal fats (RAF, lamb and poultry oil; 1:1), and their mixture (MIX) (FO, VO, and RAF; 1:1:1). Based on this twelve experimental groups were designed as follow, 1) FO (FO without SBL), 2) FO^{+2} (FO+2% SBL), 3) FO^{+4} (FO+4% SBL), 4) VO (VO without SBL), 5) VO^{+2} (VO+2% SBL), 6) VO^{+4} (VO+4% SBL), 7) RAF (RAF without SBL), 8) RAF^{+2} (RAF+2% SBL), 9) RAF^{+4} (RAF+4% SL), 10) MIX (MIX without SBL), 11) MIX^{+2} (MIX+2% SBL) and 12) MIX^{+4} (MIX+4% SBL). Asian seabass juveniles were distributed into thirty-six 300-L container (15 fish in each replicate) filled with 220 L of sea water (45.0±0.5 g/L). The experimental diets were offered to fish three times daily (27.1±1.2°C). The lowest final weight was in VO group and the highest growth performance were in FO^{+2}, RAF^{+2}, RAF^{+4}, MIX^{+2} and MIX^{+4} groups (p<0.05). In addition, feed intake (FI) and feed conversion ratio (FCR) ratio decreased in fish fed VO diet, but SL supplementation increased FI and FCR values (p<0.05). The amounts of n-3 / n-6 polyunsaturated fatty acids (PUFA) ratio, long chain-PUFA in fish fed diets containing FO increased. Plasma total protein and globulin in fish fed FO, VO^{+2} and RAF^{+4} were higher than those fed VO and RAF^{+2} diets. The highest and lowest plasma alternative complement activities were in FO^{+2} and VO groups, respectively (p<0.05). The fish fed with VO and RAF^{+2} diets had the highest and lowest plasma triglycerides levels, respectively. The highest and lowest plasma cholesterol were in RAF and FO^{+4}, respectively. The findings of this investigation showed that dietary FO replacement with mixture of various lipid sources and SBL supplementation at 2% can improve growth, fatty acid profile and humoral immune responses in *L. calcarifer* juveniles.

Introduction

Asian seabass (*Lates calcarifer*) is a euryhaline, voracious carnivorous and catadromous fish species. This, hardy nature protandrous hermaphrodite fish has suitable characteristics for developing cage culture industry particularly in tropical and subtropical regions such as high market preferences, high growth rate, high fecundity, suitable feed conversion ratio (Mozanzadeh *et al.,* 2021). Its annual global production surpassed 120,000 tons in 2020 with an expected 5% yearly increase in its production until 2035. It is expected that its market increase from US$ 960 million in 2023 to US$ 1492 million in 2033 (Future market insights, 2023). This species also considered as the best alternative for whiteleg shrimp (*Penaeus vannamei*) culture in the earthen ponds in the south of Iran, in which shrimp aquaculture industry extremely suffered due to infectious diseases. It has been confirmed dietary long-chain polyunsaturated fatty acids (LC-PUFA) requirement for *L. calcarifer* is about 1% (Glencross and Rutherford 2011; Salini *et al.* 2015a) and this species lack Δ5 desaturase activity to synthetize LC-PUFA from their precursors (Mohd-yusof *et al.,* 2010). Dietary LC-PUFA deficiency can reduce growth and the initiation of essential fatty acid (EFA) deficiency symptoms such as worsen FCR, fin erosion, abnormal reddening of the fins and fainting response (Glencross and Rutherford, 2011). However, Morton *et al.* (2014) reported that *L. calcarifer* juveniles may be fed diets containing as low as 0.1% docosahexaenoic acid (DHA) without compromising growth and health status. Furthermore, it has been proved that dietary total fish oil (FO) replacement with alternative lipid sources (ALS) is possible in *L. calcarifer* through a fish meal (FM) base diet to provide the optimum LC-PUFA for this species (Alhazzaa *et al.,* 2011a,b). However, the FO sparing in low FM diets could be complicated in this species and may compromise its growth and health condition (Glencross *et al.,* 2016). In this regard, it has been reported complete dietary FO substitution with vegetal oils decreased growth after 6 weeks in *L. calcarrifer* juveniles (19 g initial body weight, IBW) (Raso and Anderson, 2003). However, partial or complete dietary replacement of FO with rendered poultry fat (PF) did not compromise growth and survival over 12 weeks in *L. calcarrifer* (208 g, IBW) as long as FM containing residual FO, is used in the formulations (Salini *et al.,* 2015a). In a challenging scenario, Glencross *et al*. (2016) reported that dietary FO can be totally replaced with rice bran oil, while up to 70-90% of FM can be replaced with poultry and soybean meals without causing growth performance problems in *L. calcarrifer* (155 g IBW) after eight weeks. These research showed that *L. calcarrifer* can tolerate low FM and FO diets; however, the modulation of diet formulation by using feed additives such as emulsifiers could improve dietary FM and FO sparing in diet for this species. In this regard, it has been suggested that *L. calcarifer* at juvenile stages requires intact phospholipids (PL) to promote its growth and feed utilization (Salini et *al.,* 2016). Salini *et al.* (2016) reported that partial (88%) dietary FO substitution with soybean lecithin (SL) did not compromise growth

performance in *L. calcarifer* juveniles (47 g IBW) and avoided gross signs on EFA deficiency; however, dietary FO replacement with soybean oil compromised growth rate in this species. Due to their biological importance in the lipoproteins structure, PL assist the extracellular transportation of lipids and can improve feed efficiency, growth and health condition in fish. Moreover, PL are key precursors for eicosanoids, diacylglycerol and inositol phosphates, which are biological active mediators of many physiological processes. Soybean lecithin (SL) is a vegetal source of PL and has high market availability, economic price and relative stable biochemical composition (Tocher *et al.,* 2008). SL rich in linoleic (18:2n-6, LA) and linolenic (18:3n-3, LNA) acids, but devoid of LC-PUFA compared to marine PL sources such as krill oil (Tocher *et al.,* 2008; Wee *et al.,* 2023). It increases feed palatability due to the presence of the trimethyl and inositol groups on the choline base of phosphatidylcholine and phosphatidylinositol, respectively and it increases lipid digestibility in the gut because of its bio-surfactant property (Hertrampe and Piedad-Pascual, 2000). The use of ALS and SL in feed formulation can profoundly affect dietary fatty acid profile which can influence fish immune responses (Montero *et al.,* 2008, 2010, 2015). Dietary FA composition can profoundly affect immune responses in fish by modulating eicosanoids biosynthesis (Lin and Shiau, 2007; Geay *et al.,* 2010), antioxidant capacity (Castro *et al.,* 2016), and the gut microbiome (Torrecillas *et al.,* 2017, 2018). Therefore, in this investigation, it was aimed to examine the influences of complete replacement of dietary FO with ALS by SL (2 and 4%) supplementation on growth, immune responses and some health indices of *L. calcarifer* juveniles.

Materials and methods

Research design

A 3 × 4 factorial experiment was designed with two soy lecithin levels, including 2 and 4%, and four lipid sources, including FO, vegetable oils (canola and soybean oils; 1:1), rendered animal fats (lamb and poultry oil; 1:1) and their mixtures (MIX) (FO, VO, RAF; 1:1:1). According to this twelve experimental treatments were designed (Table 1) as follow, FO (FO without SBL), FO^{+2} (FO+2% SBL), FO^{+4} (FO+4% SBL), VO (VO without SBL), VO^{+2} (VO+2% SBL), VO^{+4} (VO+4% SBL), RAF (RAF without SBL), RAF^{+2} (RAF+2% SBL), RAF^{+4} (RAF+4% SBL), MIX (MIX without SBL), MIX^{+2} (MIX+2% SBL) and MIX^{+4} (MIX+4% SBL). In summary, dry feedstuffs were mixed for 20 min and then SL and lipid sources were included and blended for 10 min. Eventually, gelatin was dissolved in warm water and added to the mixture to make a soft dough. The dough was pelleted with a kitchen meat grinder (3 mm) and the pellets were dried (25ºC, 48 h) and kept in a freezer (-20°C). The biochemical profile of the diets were examined by standard procedures (AOAC, 2005).

Sampling methods

After six-week husbandry trial, the weight and length of all fish were individually measured. Fish were not fed for a day before sampling. The fish were anesthetized (2-phenoxyethanol, 300 mg/L, 9 fish per treatment) and blood was collected from the caudal vein with heparinized syringes.

Table 1: Formulation (g/kg) and proximate composition (%) of experimental diets.

Ingredients[1]	Experimental diets[*]											
	FO	FO^{+2}	FO^{+4}	VO	VO^{+2}	VO^{+4}	RAF	RAF^{+2}	RAF^{+4}	MIX	MIX^{+2}	MIX^{+4}
Fishmeal[2]	355	355	355	355	355	355	355	355	355	355	355	355
Poultry meal[3]	145	145	145	145	145	145	145	145	145	145	145	145
Beef gelatin	10	10	10	10	10	10	10	10	10	10	10	10
Soy meal[4]	100	100	100	100	100	100	100	100	100	100	100	100
Corn gluten[4]	120	120	120	120	120	120	120	120	120	120	120	120
Wheat gluten[4]	120	120	120	120	120	120	120	120	120	120	120	120
Wheat middling	42	42	42	42	42	42	42	42	42	42	42	42
L- lysine	2	2	2	2	2	2	2	2	2	2	2	2
DL-methionine	1	1	1	1	1	1	1	1	1	1	1	1
Vitamin premix[5]	10	10	10	10	10	10	10	10	10	10	10	10
Mineral premix[6]	10	10	10	10	10	10	10	10	10	10	10	10
L-ascorbic acid (50%)[7]	5	5	5	5	5	5	5	5	5	5	5	5
Butyric acid[8]	5	5	5	5	5	5	5	5	5	5	5	5
Dicalcium phosphate	5	5	5	5	5	5	5	5	5	5	5	5
Fish oil[2]	70	50	30	-	-	-	-	-	-	14	10	5
Soybean oil[4]	-	-	-	35	25	15	-	-	-	14	10	5
Canola oil[4]	-	-	-	35	25	15	-	-	-	14	10	5
Lamb fat	-	-	-	-	-	-	35	25	15	14	10	5
Poultry oil	-	-	-	-	0	-	35	25	15	14	10	5
Soy lecithin[9]	-	20	40	-	20	40	-	20	40	-	20	40
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
	Proximate composition (%)											
Crude protein	45.9	46.1	46.3	45.8	45.6	46.3	46.2	45.8	45.8	46.1	46.2	45.8
Crude lipid	16.8	16.5	16.6	16.9	16.3	16.3	16.8	16.4	15.9	16.7	16.1	16.2
Ash	14.1	13.8	14.2	14.4	14.7	14.3	14.2	14.3	14.1	14.2	14.1	13.9
Moisture	6.1	6.2	6.9	6.2	7.4	7.3	7.3	7.1	6.6	7.1	6.9	7.2

[*]Abbreviations: FO: fish oil; FO^{+2}: fish oil + 2% soy lecithin; FO^{+4}: fish oil + 4% soy lecithin; VO: vegetable oil; VO^{+2}: vegetable oil + 2% soy lecithin; VO^{+4}: vegetable oil + 4% soy lecithin; RAF: rendered animal fat; RAF^{+2}: rendered animal fat + 2% soy lecithin; RAF^{+4}: rendered animal fat + 4% soy lecithin; MIX: mixture of all lipid sources; MIX^{+2}: mixture of all lipid sources + 2% soy lecithin; MIX^{+4}: mixture of all lipid sources + 4% soy lecithin.

[1] Composition of ingredients as % Dry-weight basis [fish meal (60.5% crude protein, 18.0% crude lipid); poultry meal (51.2% crude protein, 15.5% crude lipid), beef gelatin (85% crude protein, crude lipid, 4.2);soy meal (41% crude protein, 4.2% crude lipid); corn gluten (71.4% crude protein, 4.1% crude lipid); wheat gluten (53.3% crude protein, 2.8% crude lipid); wheat middling (12% crude protein, 3.0% crude lipid)].

[2] Parskilka Mazandaran, Iran (*Clupeonella* sp.).

[3] Nazdaneh Sepahan, Isfahan, Iran.

[4] Product of Kesht Va Sanat Shomal Vegetable Oil Factories Complex (Neca, Iran).

[5] Vitamin premix (IU/kg of premix): Ascorbic acid, 350000; Retinol, 1000000000; Cholecalciferol, 500000000; Tocopherols, 500000; Vitamin K3, 960000; thiamine, 980000; riboflavin, 800000; pyridoxine, 990,000; folic acid, 950000; cobalamin, 10000; biotin, 20000; Niacin, 995000; pantothenic acid, 980,000.

[6] Mineral premix mg/kg of premix: magnesium, 6400; copper, 2000; ferrous, 11,000; zinc, 7000; selenium, 100; iodine, 300; cobalt, 50; natrium, 5000. ATA

[7] Company, Tabriz, Iran. k Rooyan Darou, Semnan, Iran.

[8] Merck, Germany.

[9] Behpak indastrial company, Behshahr, Mazandaran, Iran.

The blood was centrifuged (5000 g; 10 min; 4°C), and plasma was separated. Plasma was aliquoted (2 × 200 µL aliquots for each sample) and transferred to a -20°C freezer. The same fish were sacrificed (using an overdose (1000 mg/L) of the anesthetic) after bleeding, and their whole body were kept in a -80°C freezer.

Fatty acid (FA) profile evaluation

Fatty acid composition of the diets and whole body was evaluated based on Christie (1993) method. A gas chromatograph (GC) (Agilent Technologies Inc., Santa Clara, CA, USA), equipped with an auto-sampler, a flame ionization detector and a cyanopropyl-phenyl capillary column (DB-225MS, 30 m × 0.250 mm inner diameter × 0.25 μm film thickness) was used for FA evaluation. An external standard FA methyl ester mixture (GLC-68d; Nu-Chek Prep., Waterville, MN, USA) was used to FA detection. The fatty acid profile of the experimental diets was provided in Table 2.

Table 2: Fatty acid profile (%) of the experimental diets.

Fatty acids[1]	Experimental diets*											
	FO	FO^{+2}	FO^{+4}	VO	VO^{+2}	VO^{+4}	RAF	RAF^{+2}	RAF^{+4}	MIX	MIX^{+2}	MIX^{+4}
14:0	2.5	2.0	0.6	1.5	1.3	1.2	3.0	2.3	1.9	2.5	1.7	1.3
16:0	21.0	18.8	17.5	22.6	22.1	21.7	32.4	32.8	31.4	26.9	24.0	22.3
18:0	11.7	9.6	8.8	8.5	7.5	7.5	18.0	15.1	13.6	11.2	10.6	9.4
20:0	0.1	0.5	0.8	0.8	0.7	0.7	0.2	0.3	0.5	0.6	0.6	0.8
22:0	0.5	0.5	0.8	0.6	0.6	0.5	0.2	0.4	0.3	0.4	0.5	0.8
SFA	35.8	31.4	28.5	34.0	32.2	31.6	53.8	50.9	47.7	41.6	37.4	34.6
14:1n-5	0.3	0.4	0.4	0.3	0.7	0.5	0.3	0.2	0.2	0.4	0.5	0.9
16:1n-7	3.5	2.5	1.8	1.9	1.8	1.8	3.5	3.2	2.3	1.7	1.8	1.4
18:1n-7	2.0	2.1	2.8	2.0	1.6	1.4	1.7	1.8	1.5	1.5	1.7	2.0
18:1n-9	26.8	19.4	17.8	42.0	32.1	29.3	26.2	24.6	23.3	28.1	24.3	21.1
20:1n-9	0.1	0.2	0.3	1.8	0.6	0.3	0.1	0.2	0.2	0.2	0.3	0.4
MUFA	32.7	24.6	23.1	48.0	36.8	33.3	31.8	30.0	23.5	28.3	28.6	25.8
18:2n-6 (LA)	8.1	20.3	23.3	8.4	21.2	26.2	7.7	14.7	19.0	13.8	17.9	23.6
20:2n-6	0.1	0.4	1.0	0.1	0.1	0.1	0.1	n.d.	0.2	0.2	0.2	0.1
20:4n-6 (AA)	1.8	1.5	1.2	0.2	0.3	0.2	0.2	0.2	0.3	0.8	0.6	0.5
18:3n-3 (LNA)	0.6	2.0	2.4	0.4	1.8	2.8	0.5	1.1	1.7	1.7	2.2	2.7
20:5n-3 (EPA)	5.9	5.7	5.4	0.3	0.4	0.4	0.2	0.4	0.6	3.0	2.7	2.4
22:6n-3 (DHA)	11.2	11.0	10.1	1.4	1.0	1.0	0.8	1.2	1.3	4.5	3.7	3.5
n-6 PUFA	10.0	22.2	25.5	8.7	21.6	26.5	8.0	14.9	19.5	14.8	18.7	24.2
n-3 PUFA	17.7	18.7	17.9	2.1	3.2	4.2	1.5	2.7	3.6	9.2	8.6	8.6
n-3 / n-6 PUFA	1.8	0.8	0.7	0.2	0.2	0.2	0.2	0.2	0.2	0.6	0.5	0.4

*Abbreviations: FO: fish oil; FO^{+2}: fish oil + 2% soy lecithin; FO^{+4}: fish oil + 4% soy lecithin; VO: vegetable oil; VO^{+2}: vegetable oil + 2% soy lecithin; VO^{+4}: vegetable oil + 4% soy lecithin; RAF: rendered animal fat; RAF^{+2}: rendered animal fat + 2% soy lecithin; RAF^{+4}: rendered animal fat + 4% soy lecithin; MIX: mixture of all lipid sources; MIX^{+2}: mixture of all lipid sources + 2% soy lecithin; MIX^{+4}: mixture of all lipid sources + 4% soy lecithin.

[1] SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; LA: linoleic acid; AA: arachidonic acid; LNA: linolenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; n.d: not detected.*Fish husbandry*

Asian seabass juveniles (50.2±2.3 g, initial body initial weight ± standard error) were individually weighted and stocked into thirty three polyethylene circular tanks (300 L, 15 fish in each tank) that filled with 220 L of disinfected sea water in a flow-through system. Each dietary treatment had three replicates. Water exchange rate was 100% daily. Fish were fed the experimental feeds for six weeks three times a day (08:00, 12:00, and 16:00) and unfed feeds were collected one h after each feeding. Temperature (31.2±1.5°C), pH (7.9±0.2), dissolved oxygen (6.5±0.3 mg/L), and ammonia (< 0.2 ppm) were evaluated once a week. The photoperiod was set at 12 h light: 12 h darkness.

Plasma immunological and biochemical parameters

Plasma immune parameters, including total protein (TP) and albumin (ALB) were evaluated by diagnostic kits (Biorex, Fars, Shiraz, Iran). Plasma globulin (GLOB) levels were calculated after subtracting albumin from total protein content. Plasma lysozyme activity was determined through turbidimetry method by measuring lysis degree of the *Micrococcus luteus* (Sigma, St Louis, USA) cell (Demers and Bayne, 1997). Fifty microlitre of the homogenate was added to 3 ml of the bacterial suspension in an ice bath. The absorbance was recorded at 570 nm immediately (A1). The mixture was incubated at 37°C for 30 min, transferred to ice bath to stop the reaction and immediately the second absorbance was recorded. To evaluate alternative complement (ACH50) activity, the volume of plasma complement that causes 50% hemolysis in rabbit red blood cells was determined (Sunyer and Tort, 1995). To evaluate respiratory burst activity (RBA) blood (50 µL) was placed in 2 mL microtube, and an equal amount of 0.2% nitroblue tetrazolium (NBT) solution containing 1 mL N, N-dimethyl formamide (Sigma, Germany) was added and incubated (30 min, 25ºC) then centrifuged (5 min at 3000 g). The optical density (OD) of supernatant was measured in a spectrophotometer (Biophotometer, Eppendorf, Germany) at 620 nm according to Secombes (1990). Plasma biochemical parameters, including triglycerides (TRIG), cholesterol (CHOL), high density lipoprotein (HDL), low density lipoprotein (LDL), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), phosphorus (P), calcium (Ca) and manganese (Mg), glucose (Glu), and creatinine (CRE) were spectrophotometrically measured by applying diagnostic kits (Biorex, Fars, Shiraz, Iran).

Statistical analysis

Statistics was done using SPSS software (version 23.0, Chicago, IL, USA). Lecithin level and lipid sources individual effects and their interactions were analyzed using a two-way ANOVA. Tukey's HSD test was used for pairwise comparison among treatments at $p<0.05$. Results are presented as mean ± standard error of the mean (n=3).

Results

Growth indices

There was not any mortalities among various groups (Table 3). The lowest FBW, daily growth rate, weight gain, feed intake (FI) and feed efficiency ratio (FER) were in fish fed VO (VO without SBL) that followed by those fed VO^{+2} group and the highest growth performance were in FO^{+2}, RAF^{+2}, RAF^{+4}, MIX^{+2} and MIX^{+4}, meanwhile the other groups showed intermediate values ($p<0.05$). Condition factor and HSI values were not affected by dietary treatments ($p>0.05$). Growth performance parameters were significantly affected by SBL levels and dietary lipid sources in which by including SBL in diet growth performance improved and among various lipid sources RAF and blends of all lipid sources had better effects on growth compare to FO. Supplementing VO group with SBL markedly improved growth performance in *L. calcarifer* juveniles.

Table 3: Growth performance of *Lates calcarifer* juveniles fed different experimental diets for 42 days. Data are presented as the mean ± pooled SE of three replicates.

Parameters	Experimental groups*												Two Way ANOVA			
	FO	FO^{+2}	FO^{+4}	VO	VO^{+2}	VO^{+4}	RAF	RAF^{+2}	RAF^{+4}	MIX	MIX^{+2}	MIX^{+4}	Pooled SE	LL level	LS	Interaction
IBW (g)	50.4	50.3	50.4	50.4	50.1	50.3	50.4	50.2	50.5	50.2	50.3	50.6	2.3	0.940	0.885	0.990
IBL (cm)	16.0	16.1	16.1	16.1	16.0	16.1	16.1	16.0	16.2	16.1	16.0	16.2	0.3	1.000	1.000	1.000
FBW (g)	86.2^{ab}	104.7^{a}	99.6^{ab}	65.6^{c}	76.0^{b}	94.2^{ab}	81.1^{ab}	106.0^{a}	103.6^{a}	89.2^{ab}	106.6^{a}	107.8^{a}	9.7	0.003	0.034	0.813
FBL (cm)	19.1^{ab}	20.4^{a}	19.9^{ab}	17.6^{c}	18.4^{b}	19.6^{ab}	18.7^{ab}	20.6^{a}	20.7^{a}	19.0^{ab}	20.7^{a}	20.9^{a}	0.6	0.013	0.047	0.788
SGR[1] (%/day)	1.3^{ab}	1.74^{a}	1.62^{ab}	0.62^{c}	1.0^{b}	1.50^{ab}	1.13^{ab}	1.78^{a}	1.71^{a}	1.37^{ab}	1.79^{a}	1.80^{a}	0.2	0.023	0.015	0.635
FCR[2]	1.5^{ab}	1.1^{a}	1.2^{a}	2.1^{c}	1.7^{b}	1.3^{a}	1.5^{ab}	1.2^{a}	1.1^{a}	1.4^{ab}	1.1^{a}	1.0^{a}	0.2	0.001	0.001	0.001
FI (g/fish)[3]	53.7^{ab}	59.8^{a}	59.0^{a}	31.9^{c}	44.0^{b}	57.1^{ab}	46.0^{bc}	67.0^{a}	58.4^{a}	54.6^{ab}	61.9^{a}	57.2^{ab}	0.1	0.056	0.005	0.001
SUR[4] (%)	100	100	100	100	100	100	100	100	100	100	100	100	0.0	1.000	1.000	1.000

Values within a column with a common superscript letter are not significantly different from the other dietary groups (p>0.05). The significance of the two main effects (fish meal replacement level and acidifier level) and their interaction were analyzed using two-way ANOVA.

Abbreviations: FO: fish oil; FO^{+2}: fish oil + 2% soy lecithin; FO^{+4}: fish oil + 4% soy lecithin; VO: vegetable oil; VO^{+2}: vegetable oil + 2% soy lecithin; VO^{+4}: vegetable oil + 4% soy lecithin; RAF: rendered animal fat; RAF^{+2}: rendered animal fat + 2% soy lecithin; RAF^{+4}: rendered animal fat + 4% soy lecithin; MIX: mixture of all lipid sources; MIX^{+2}: mixture of all lipid sources + 2% soy lecithin; MIX^{+4}: mixture of all lipid sources + 4% soy lecithin; IBW, initial body weight; IBL: initial body total length; FBW, final body weight; FBL: final body total length; SGR: specific growth rate; FCR, feed conversion ratio; SURV: survival; LL, lecithin level; LS, lipid source.

[1]Specific growth rate (SGR, %/day)=100 × [(ln FBW – ln IBW)/number of feeding days]

[2]Feed conversion ratio (FCR)=total feed intake (g) / weight gain (g)

[3]FI: feed intake=(total feed intake (g) / number of fish)

[4]Survival (%)=100 × (final number of Fish / initial number of Fish).

In addition, FI and FCR significantly reduced including VO in diet, but supplementing VO diet with SBL increased FI and FER (p<0.05).

Fatty acid profile

The amount of SFA markedly reduced by supplementing diet with SBL and the amount of this FA class in fish fed VO source, except for VO group, was relatively lower than those fed the other sources (Table 4). The amount of MUFA, especially OA in fish fed VO and VO^{+2} were increased; however, fish in FO^{+2} and RAF groups had the lowest whole body MUFA values (p<0.05). The amounts of n-6 PUFA, particularly LA increased in fish fed VO^{+2} and VO^{+4} and the lowest values were in F group. The highest and lowest ARA values were in MIX^{+4} and VO$^{+4,}$ respectively and it was affected by individual and interactive effects of SBL level and lipid source (p<0.05). n-3 PUFA, n-3/ n-6 ratio, LC-PUFA, EPA and DHA levels in fish fed diets containing FO increased compared to fish fed other treatments. The highest and lowest whole body DHA were in FO^{+2} and MIX groups, respectively (Table 5).

Plasma biochemistry

Fish fed VO and RAF^{+2} diets had the highest and lowest plasma TRIG levels, respectively and it was affected by LL, LS and their interactions (Table 6). The highest and lowest plasma CHOL were in RAF and FO^{+4}, respectively and it was affected by LL, LS and their interactions (p<0.05). Fish fed FO diet had the highest plasma HDL and the lowest values in FO^{+2} and FO^{+4}. The highest and lowest plasma LDL levels were in fish fed RAF and FO^{+4} diets,

respectively. The amount of plasma ALP in fish fed FO, VO^{+2}, RAF and RAF^{+4} were higher than other groups and those in FO^{+4} and VO had the lowest values. The plasma ALT values in fish fed RAF, RAF^{+2} and MIX^{+2} were higher than other groups and those in FO^{+2} and FO^{+4} had the lowest values. The highest levels of plasma AST was in RAF and the lowest values were in FO^{+2}, FO^{+4}, VO and VO^{+4}. All plasma enzymes were influenced by LL, LS and their interactions (p<0.05).

Fish fed FO and FO^{+2} had highest plasma P levels and those in MIX^{+4} had the lowest value. Fish fed FO diet had the highest plasma Ca level and the lowest levels were in FO^{+4} and RAF^{+2} (p<0.05). Plasma Mg did not affect by the experimental diets. The highest plasma GLU was in fish fed VO^{+4} and the lowest levels were in VO, RAF and MIX diets. Plasma CRE did not affect by the experimental diets.

Table 4: Fatty acid profile (%) of the fish whole body fed different experimental diets for 42 days. Data are presented as the mean ± pooled SE of three replicates.

	Experimental groups*												Two Way ANOVA			
Fatty acids[1]	FO	FO^{+2}	FO^{+4}	VO	VO^{+2}	VO^{+4}	RAF	RAF^{+2}	RAF^{+4}	MIX	MIX^{+2}	MIX^{+4}	Pooled SE	LL level	LS	Interaction
14:0	1.5^{ab}	1.4^{ab}	1.4^{ab}	1.6^{ab}	1.6^{ab}	1.2^{b}	1.6^{ab}	1.9^{a}	1.8^{a}	2.1^{a}	2.0^{a}	2.1^{a}	0.1	0.027	0.001	0.001
16:0	20.9^{ab}	20.2^{ab}	20.8^{ab}	18.3^{b}	18.7^{b}	19.0^{b}	22.5^{a}	22.6^{a}	20.6^{ab}	21.1^{ab}	19.1^{b}	20.5^{ab}	0.4	0.001	0.153	0.266
18:0	9.4^{a}	8.6^{ab}	8.0^{ab}	7.1^{b}	6.6^{c}	7.5^{b}	9.9^{a}	8.8	7.5^{b}	10.1^{a}	8.7^{ab}	7.5^{b}	0.2	0.001	0.236	0.696
SFA	32.3^{ab}	30.6^{ab}	31.2^{ab}	30.2^{ab}	27.5^{b}	28.4^{b}	34.7^{a}	33.5^{a}	30.3^{ab}	35.0^{a}	30.0^{ab}	30.1^{ab}	0.7	0.001	0.122	0.404
14:1n-5	1.4	1.7	1.7	1.1	1.1	1.1	1.2	0.8	0.9	2.4	1.7	1.8	0.2	0.428	0.238	0.782
16:1n-7	2.1^{b}	2.5^{ab}	2.5^{ab}	2.8^{a}	2.4^{ab}	2.0^{b}	1.8^{b}	2.9^{a}	3.3^{a}	3.2^{a}	2.0^{b}	2.9^{a}	0.1	0.133	0.101	0.009
18:1n-7	2.0^{ab}	2.3^{ab}	1.6^{b}	2.0^{ab}	1.9^{ab}	1.7^{b}	1.4^{b}	1.7^{b}	1.6^{b}	2.8^{a}	2.9^{a}	2.6^{a}	0.1	0.115	0.012	0.001
18:1n-9	21.8^{b}	19.7^{b}	20.4^{b}	28.7^{a}	28.0^{a}	24.1^{ab}	21.3^{b}	24.9^{ab}	25.8^{ab}	25.1^{ab}	24.8^{ab}	21.5^{b}	0.5	0.198	0.002	0.015
MUFA	28.0^{b}	26.3^{c}	26.9^{c}	34.6^{a}	33.3^{a}	28.9^{b}	26.4^{c}	31.0^{ab}	31.4^{ab}	31.6^{ab}	31.4^{ab}	28.9^{b}	0.5	0.233	0.002	0.004
18:2n-6 (LA)	17.7^{d}	21.0^{c}	22.1^{c}	24.4^{b}	28.5^{a}	30.1^{a}	21.6^{c}	22.4^{c}	24.3^{b}	24.3^{b}	23.1^{b}	21.9^{c}	0.6	0.001	0.001	0.267
20:2n-6	0.6^{a}	0.2^{c}	0.3^{bc}	0.2^{c}	0.2^{c}	0.3^{bc}	0.3^{bc}	0.3^{bc}	0.3^{bc}	0.2^{c}	0.4^{b}	0.2^{c}	0.0	0.847	0.033	0.001
20:4n-6 (AA)	1.0^{bc}	1.3^{b}	0.9^{bc}	0.8^{c}	0.7^{cd}	0.6^{d}	1.1^{bc}	0.9^{c}	0.8^{c}	0.6^{d}	1.2^{b}	3.8^{a}	0.2	0.001	0.001	0.001
18:3n-3 (LNA)	4.3^{a}	4.0^{ab}	4.5^{a}	4.8^{a}	3.7^{b}	3.5^{b}	4.8^{a}	4.5^{a}	4.9^{a}	3.3^{b}	3.8^{ab}	2.1^{c}	0.1	0.263	0.001	0.405
20:5n-3 (EPA)	1.3^{a}	1.3^{a}	1.0^{ab}	0.8^{ab}	0.8^{ab}	0.6^{b}	0.8^{ab}	0.9^{ab}	0.8^{ab}	0.7^{b}	0.8^{ab}	0.7^{b}	0.0	0.095	0.001	0.001
22:6n-3 (DHA)	6.4^{ab}	7.5^{a}	5.8^{b}	4.2^{bc}	3.4^{c}	3.9^{bc}	4.6^{bc}	3.8^{c}	3.6^{c}	2.4^{d}	5.3^{b}	4.5^{bc}	0.2	0.027	0.001	0.749
n-6 PUFA	19.3^{d}	22.5^{c}	23.2^{c}	25.4^{b}	29.4^{a}	31.1^{a}	23.9^{c}	22.6^{c}	25.4^{b}	25.1^{b}	24.6^{b}	25.9^{b}	0.6	0.001	0.001	0.001
n-3 PUFA	12.0^{a}	12.8^{a}	11.3^{a}	9.9^{ab}	7.9^{b}	8.0^{b}	10.2^{ab}	9.2^{ab}	9.3^{ab}	6.5^{c}	9.9^{ab}	7.3^{b}	0.3	0.104	0.001	0.001
LCPUFA	9.3^{a}	10.4^{a}	8.0^{b}	6.1^{c}	5.1^{c}	5.5^{c}	7.7^{b}	5.8^{c}	5.5^{c}	4.0^{d}	7.6^{b}	9.2^{a}	0.3	0.508	0.001	0.004
n-3 / n-6 PUFA	0.5^{a}	0.6^{a}	0.5^{a}	0.4^{ab}	0.3^{b}	0.3^{b}	0.4^{ab}	0.4^{ab}	0.4^{ab}	0.3^{b}	0.4^{ab}	0.3^{b}	0.0	0.001	0.001	0.001

*Abbreviations: FO: fish oil; FO^{+2}: fish oil + 2% soy lecithin; FO^{+4}: fish oil + 4% soy lecithin; VO: vegetable oil; VO^{+2}: vegetable oil + 2% soy lecithin; VO^{+4}: vegetable oil + 4% soy lecithin; RAF: rendered animal fat; RAF^{+2}: rendered animal fat + 2% soy lecithin; RAF^{+4}: rendered animal fat + 4% soy lecithin; MIX: mixture of all lipid sources; MIX^{+2}: mixture of all lipid sources + 2% soy lecithin; MIX^{+4}: mixture of all lipid sources + 4% soy lecithin.

[1] SFA: saturated fatty acids including 20:0 and 22:0; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; LA: linoleic acid; AA: arachidonic acid; LNA: linolenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; LCPUFA: long chain polyunsaturated fatty acids, including 20:2n-6, AA, EPA and DHA; n.d: not detected.

Table 5: Serum immune parameters of *Lates calcarifer* juveniles fed different experimental diets for 42 days. Data are presented as the mean ± pooled SE of three replicates.

Parameters	Experimental groups*												Two Way ANOVA			
	FO	FO^{+2}	FO^{+4}	VO	VO^{+2}	VO^{+4}	RAF	RAF^{+2}	RAF^{+4}	MIX	MIX^{+2}	MIX^{+4}	Pooled SE	LL level	LS	Interaction
TP (g/dL)	5.3^{a}	4.6^{ab}	4.3^{ab}	3.4^{c}	4.9^{a}	4.4^{ab}	4.6^{ab}	3.6^{c}	4.9^{a}	4.5^{ab}	4.5^{ab}	3.9^{bc}	0.3	0.198	0.936	0.001
ALB (g/dL)	1.2^{a}	0.9^{ab}	0.5^{b}	0.6^{b}	1.0^{ab}	1.3^{a}	1.0^{ab}	0.7^{b}	1.1^{ab}	1.2^{a}	1.1^{ab}	0.9^{ab}	0.2	0.476	0.579	0.001
GLOB (g/dL)	4.1^{a}	3.7^{a}	3.8^{a}	2.8^{b}	3.9^{a}	3.1^{b}	3.6^{ab}	2.9^{b}	3.8^{a}	3.3^{ab}	3.4^{ab}	3.0^{b}	0.2	0.363	0.597	0.006
LYZ (U/ mL)	60.7	66.0	66.3	65.7	62.8	62.0	61.3	62.0	63.8	61.3	60.7	61.0	2.1	0.775	0.282	0.504
ACH50 (U/ mL)	51.0^{ab}	50.6^{ab}	50.0^{ab}	46.3^{b}	51.7^{ab}	55.3^{a}	53.7^{ab}	49.7^{ab}	53.7^{ab}	51.3^{ab}	52.6^{ab}	52.3^{ab}	1.6	0.039	0.068	0.375
BA(CFU)	313.7	319.7	311.7	318.3	326.0	316.3	315.3	314.7	312.0	311.3	311.0	312.0	7.1	0.626	0.686	0.993
RBA (OD 540)	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.13	0.14	0.13	0.0	0.736	0.089	0.909

*Abbreviations: FO: fish oil; FO^{+2}: fish oil + 2% soy lecithin; FO^{+4}: fish oil + 4% soy lecithin; VO: vegetable oil; VO^{+2}: vegetable oil + 2% soy lecithin; VO^{+4}: vegetable oil + 4% soy lecithin; RAF: rendered animal fat; RAF^{+2}: rendered animal fat + 2% soy lecithin; RAF^{+4}: rendered animal fat + 4% soy lecithin; MIX: mixture of all lipid sources; MIX^{+2}: mixture of all lipid sources + 2% soy lecithin; MIX^{+4}: mixture of all lipid sources + 4% soy lecithin; TP, total protein; ALB, albumin; GLOB, globulin; 111111lyz, lysozyme; ACH50, alternative complement pathway activity; BA, bactericidal activity; RBA, respiratory burst activity; AA, antibacterial activity.

Values within a column with a common superscript letter are not significantly different from the other dietary groups (p>0.05). The significance of the two main effects (fish meal replacement level and acidifier level) and their interaction were analyzed using two-way ANOVA.

Table 6: Plasma biochemical parameters of *Lates calcarifer* juveniles fed different experimental diets for 42 days. Data are presented as the mean ± pooled SE of three replicates.

Fatty acids[1]	Experimental groups*												Two Way ANOVA			
	FO	FO^{+2}	FO^{+4}	VO	VO^{+2}	VO^{+4}	RAF	RAF^{+2}	RAF^{+4}	MIX	MIX^{+2}	MIX^{+4}	Pooled SE	LL level	LS	Interaction
Lipid components																
TRIG (mg/dL)	83.0^{c}	66.0^{c}	68.0^{c}	155.3^{a}	75.0^{c}	70.0^{c}	71.3^{c}	49.0^{d}	71.0^{c}	101.7^{b}	62.7^{c}	79.8^{c}	4.0	0.001	0.001	0.001
CHOL (mg/dL)	118.0^{b}	72.7^{d}	69.3^{d}	92.7^{c}	100.3^{c}	121.7^{b}	159.7^{a}	111.8^{b}	89.3^{c}	126.7^{b}	114.3^{b}	99.7^{c}	8.4	0.002	0.028	0.001
HDL (mg/dL)	78.7^{a}	34.3^{d}	36.7^{d}	52.7^{c}	49.8^{c}	57.3^{b}	68.3^{b}	48.3^{c}	63.7^{b}	66.0^{b}	60.0^{b}	57.0^{b}	4.5	0.004	0.001	0.003
LDL (mg/dL)	50.3^{b}	33.3^{d}	26.3^{e}	34.3^{d}	38.3^{d}	32.7^{d}	65.0^{a}	49.7^{b}	46.0^{c}	55.7^{b}	51.3^{b}	44.0^{c}	5.3	0.002	0.196	0.005
Liver enzymes																
ALP (U/ L)	102.3^{a}	93.7^{b}	74.3^{c}	74.0^{c}	104.7^{a}	96.0^{b}	106.3^{a}	96.3^{b}	107.7^{a}	98.3^{b}	98.0^{b}	88.0^{bc}	4.2	0.001	0.001	0.001
ALT (U/ L)	5.3^{b}	3.3^{c}	2.7^{c}	4.3^{b}	4.3^{b}	4.0^{b}	6.0^{a}	6.3^{a}	5.0^{b}	4.0^{b}	6.7^{a}	4.3^{b}	0.4	0.001	0.001	0.001
AST (U/ L)	33.7^{c}	22.3^{d}	24.7^{d}	22.0^{d}	34.0^{c}	26.0^{d}	56.3^{a}	40.0^{b}	43.7^{b}	34.7^{c}	47.3^{b}	46.0^{b}	1.6	0.001	0.001	0.001
Electrolytes																
P (mg/dL)	12.8^{a}	12.3^{a}	8.5^{bc}	9.6^{b}	10.2^{b}	9.6^{b}	10.8^{b}	9.9^{b}	9.7^{b}	8.7^{bc}	9.0^{b}	7.2^{c}	0.4	0.001	0.001	0.001
Ca (mg/dL)	11.9^{a}	10.6^{b}	9.4^{c}	9.9^{b}	11.1^{ab}	10.5^{b}	10.9^{ab}	9.5^{c}	11.2^{ab}	10.6^{b}	10.9^{ab}	10.3^{b}	0.4	0.136	0.904	0.002
Mg (mg/dL)	3.9	3.3	3.2	3.2	3.0	3.0	2.9	3.6	2.8	3.0	3.2	3.0	0.1	0.307	0.166	0.089
Miscellaneous																
GLU (mg/dL)	75.0^{b}	47.0^{d}	55.6^{c}	34.7^{e}	48.3^{d}	92.3^{a}	26.7^{e}	57.0^{c}	54.3^{c}	31.3^{e}	56.0^{c}	70.7^{b}	3.9	0.001	0.001	0.001
CRE (mg/dL)	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.0	0.053	0.104	0.128

Abbreviations: FO: fish oil; FO^{+2}: fish oil + 2% soy lecithin; FO^{+4}: fish oil + 4% soy lecithin; VO: vegetable oil; VO^{+2}: vegetable oil + 2% soy lecithin; VO^{+4}: vegetable oil + 4% soy lecithin; RAF: rendered animal fat; RAF^{+2}: rendered animal fat + 2% soy lecithin; RAF^{+4}: rendered animal fat + 4% soy lecithin; MIX: mixture of all lipid sources; MIX^{+2}: mixture of all lipid sources + 2% soy lecithin; MIX^{+4}: mixture of all lipid sources + 4% soy lecithin; CHOL, cholesterol;

Values within a column with a common superscript letter are not significantly different from the other dietary groups (p>0.05). The significance of the two main effects (fish meal replacement level and acidifier level) and their interaction were analyzed using two-way ANOVA.

Discussion

As aquaculture of *L. calcarifer* is developing, sustainable ALS to FO are required to support this fast-growing industry (Alhazzaa *et al.,* 2012). In our investigation, complete dietary FO replacement with VO significantly decreased growth, FI and FCR in *L. calcarifer*. It seems that high amounts of C18 PUFA in VO sources reduced their digestibility and adversely affected growth and FCR in this species. Also, Alhazzaa *et al*. (2011a,b) reported that complete dietary FO replacement with echium oil markedly suppressed growth in *L. calcalifer* fingerlings that was in concomitant with reduced FE in this species. In addition, Alhazzaa *et al.* (2012) reported that growth in *L. calcarifer* was decreased by complete dietary FO replacement with linseed oil. In another study, partial (88%) or total dietary FO replacement with a blend of palm oil, palm flake and olive oil retarded growth in *L. calcarifer* juveniles and signs of EFA deficiencies also were noticed in fish fed FO free diet (Salini *et al.,* 2015b). Also, Rahman *et al*. (2022) demonstrated growth and FI reduction in *L. calcarifer* fingerlings, when over 25% of dietary FO replaced with VO sources. In contrast, complete dietary FO substitution with RAF or mixture of various lipid sources did not compromise growth and feed utilization in this species. It seems that SFA and MUFA rich sources such as RAF can be utilized efficiently by marine tropical carnivorous fish species to produce energy compared to C18 PUFA rich sources (Alhazza *et al.,* 2019). In addition, Glencross *et al.* (2016) reported that total FO replacement with rice bran oil did not compromise growth in *L. calcarifer* if 10% FM was included in the diet. However, in FM-free diet total FO replacement decreased growth and feed utilization in this species (Glencross *et al.,* 2016). In addition, in the current research the mixture of various lipid sources increased growth in *L. calcarifer* may due to supplying more digestible energy and a better balance among the SFA, MUFA and C18 PUFA as previously reported in other studies (Raso and Anderson, 2003; Salini *et al.,* 2015b; Glencross *et al.,* 2016).

Freshwater fish require about 2% dietary PL, whereas marine fish need more PL requirement up to 7% (Tocher *et al*., 2008). In the present study 2 or 4% SL supplementation in the experimental diets increased growth, FE and FI in *L. calcarifer*, particularly in those fed VO containing dietsas previously confirmed by Salini *et al.* (2016). Also, supplementing diet with SL improved growth and feed utilization in Sobaity seabream (*Sparidentex hasta*, Pagheh *et al.,* 2019). The presence of trimethyl group on the choline or inositol groups in phospholipid spources can trigger the gustatory response of fish and increase FI in fish fed SL supplemented diets (Izquierdo and Koven, 2010; La *et al.,* 2018). Furthermore, dietary SL by increasing lipid digestibility and lipoproteins synthesis can provide more digestible energy for fish (Tocher *et al.,* 2008).

In this investigation, the combination of 4% SL and mixed lipid sources increased VSI values in *L. calcarifer* as a consequence of greater lipid deposition. However, the combination of SL at 2% and FO or RAF significantly decreased VSI value suggesting hypolipidemic effects

with this lipid combination. In addition, the interactive effects of 2% SL and RAF or mixture sources increased FI in *L. calcarifer* suggesting synergistic effects of SL level and lipid sources in this species.

The capacity of juvenile *L. calcarifer* to synthesize endogenously LC-PUFA from LA and LNA is relatively restricted (Alhazzaa *et al.,* 2011a; 2013b). In the present investigation, the whole body FA profile in *L. calcarifer* significantly influenced by various lipid sources, the amount of SL and their interaction. The inclusion of VO or increasing SL level in diet drastically reduced SFA due to the high levels of C18 PUFA, especially LA which could be displaced SFA in the whole body. In addition, SFA-rich sources, including FO and RAF increased SFA retention in groups fed FO, RFA and mixed feed in comparison with groups fed VO diets. In this regard, dietary FO with lamb tallow enhanced SFA in the fillet and liver of sobaity sea bream; however, FO replacement with canola or sunflower oils decreased SFA in these tissues (Mozanzadeh *et al.,* 2016). Also, similar to the findings of the current study it has been demonstrated that SFA level in the fillet of sobaity sea bream decreased by increasing SL level in diet because LA displaced SFA (Pagheh *et al.,* 2019). Furthermore, in our study the amounts of SFA in RAF and mixed diets was greater than their levels in the whole body, suggesting these FA class may be catabolized to produce energy (Mozanzadeh *et al.,* 2016). In addition, fish fed MUFA-rich lipid sources, including VO and RAF had higher whole body MUFA levels as previously reported by Salini *et al.* (2015a) in *L. calcarifer* fed diets containing poultry fat. In addition, in our research the inclusion of 4% SL in diet decreased MUFA level in the whole body in *L. calcarifer* as a consequence of MUFA displacement by ALA. Salini *et al.* (2016) demonstrated the replacement of dietary FO with SL markedly decreased MUFA in the neutral lipids but it did not affect MUFA level in polar lipids in whole body of *L. calcarifer*.

In the current research, the inclusion of VO or SL in diet increased levels of n-6 PUFA, especially LA as also reported in gilthead seabream (*Sparus aurata*, Saleh *et al.,* 2015), *L. calcarifer* (Salini *et al.,* 2016) and sobaity seabream (Pagheh *et al.,* 2019). In addition, dietary FO replacement with ALS decreased LC-PUFA level and n-3 / n-6 ratio in *L. calcarifer* whole body as previously reported in the same species (Alhazzaa *et al.,* 2013b; Salini *et al.,* 2015a, b; Salini *et al.,* 2016). However, in the present research using mixture of various lipids and SL inclusion enhanced LC-PUFA retention, especially ARA and DHA in the whole body. It seems that using blends of various lipid sources and SL increase the selective retention of LC-PUFA in *L. calcarifer.* In this regard, supplementing diet with SL (Jafari *et al.,* 2021) or lyso-lecithin (Jafari *et al.,* 2024) increased LC-PUFA levels in the fillet of stellate (*Acipenser stellatus*). Further studies required at molecular level to elucidate the mode of action of ALS on LC-PUFA biosynthesis in marine fish.

Dietary lipid source can modulate immune response by changing the fluidity of leucocytes membranes, their membrane-associated enzymes and receptor sites. It can also affect immunocompetance by

modifying the biosynthesis of pro-inflammatory and anti-inflammatory eicosanoids and cytokines (Montero *et al.,* 2008; 2010). In this sense, Alhazzaa *et al.* (2013a) reported that during the infection period with *Streptococcus iniae*, complete dietary FO replacement with echium oil decreased eicosanoids levels, including thromboxane B2 and 6-keto-prostaglandin F1, in *L. calcarifer* plasma compared to those fed rapeseed oil. In addition, the authors stated that fish fed on FO and echium oil had an enduring response in their eicosanoid levels, after a week bacterial infection. Also, the modulations of immune responses depends on the FO replacement level, the FA profile of the ALS, and the cultured species (Montero *et al.*, 2010). In this sense, dietary FO replacement with ALS in marine fish with restricted LC-PUFA biosynthesis ability, can largely affect and alternate their immune system (Mourente *et al.,* 2007) in comparison with freshwater or salmonid species with high LC-PUFA biosynthesis capacity (Seierstad *et al.,* 2009; Kiron *et al.,* 2012). The results of our study showed, fish fed VO diet had lower plasma TP, ALB, GLOB and ACH50 activity that was in concomitant with lowest growth performance in *L. calcarifer*, indicating suppressed immune responses in this species. In addition, supplementing diet with SL, increased ACH50 activity in fish fed VO diet suggesting immunostimulatory effects of SL on *L. calcarifer*. In this regard, Montero *et al.* (2003) reported that dietary FO substitution with soybean oil at 60% after 203 days pronouncedly reduced serum ACH50 in gilthead seabream (*Sparus aurata*). In addition, they showed decreased phagocytic activity of macrophages in fish fed diets in which 60% or 80% of FO replaced with rapeseed or soybean oils, respectively. In addition, humoral immune responses were suppressed in large yellow croaker (*Larimichthys crocea*, Tan *et al.,* 2016) or Japanese Seabass (*Lateolabrax japonicus*, Tan *et al.,* 2017) fed VO diets. On the other hand, several studies reported that supplementing diet with SL improved humoral immune responses in stellate sturgeon (*Acipenser stellatus*, Jafari *et al.,* 2018), Caspian brown trout (*Salmo trutta caspius*, Haghparast *et al.,* 2019) and largemouth bass (*Micropterus salmoides*, Wu *et al.,* 2023). It seems that better availability of EFA from PL in comparison to neutral lipids might result in modified cell membrane FA profile of the experimental fish via stimulating eicosanoids production (Jafari *et al.,* 2018). Thus, in our investigation fish fed MIX diets had improved immune status because of more balanced FA profile in these diets.

In our research, VO group had higher plasma TRIG suggesting increased liver lipogenesis in *L. calcarifer*. However, dietary SL supplementing reduced plasma TRIG in fish suggesting hypolipidemic effects of SL in fish. In this regard, Song *et al*. (2024) showed increased serum TRIG in largemouth bass fed soy and rapeseed oils containing diets. It is suggested that, LA or oleic acid rich lipid sources such as VO could stimulate liver TRIG synthesis and secretion by provoking phosphatidylcholine synthesis through glycerol-3-phosphate pathway can induce liver lipid and VLDL secretion (Vegusdal *et al.,* 2005; Kjær *et al.,* 2008). On the other hand, Lin *et al.* (2017) reported that, the

serum TRIG level decreased by dietary phospholipids supplementation in juvenile hybrid snakehead (*Channa argus* × *Channa maculata*).

In our study, plasma cholesterol and LDL in RAF groups increased due to high levels of total SFA and cholesterol in RAF as previously reported in largemouth bass (Song *et al.,* 2024). In addition, supplementing diet with SL reduced plasma CHOL and LDL levels in fish indicating hypocholesterolemic effects of SL in *L. calcarifer.* In contrast, Salini *et al*. (2016) reported that supplementing diet with marine derive PL (krill phospholipid) increased plasma CHOL compared to SL due to the high levels of CHOL in krill PL. Moreover, Glencross *et al.* (2016) reported that serum CHOL levels declined significantly in response to FO replacement with rice bran oil in *L. calcarifer*. In juvenile hybrid snakehead, PL supplemented diets resulted in higher levels of HDL and lower levels of LDL than those fed with the PL deficient controls (Lin *et al.,* 2017).

In our study, fish fed FO diet had the highest plasma HDL levels that could be due to high levels of n–3 LC-PUFA in this diet as previously reported by Mozanzadeh *et al.* (2020) in sobaity seabream. Elevated plasma enzymes, including ALP, ALT and AST are considered a reliable marker of health status and nutrients metabolism in fish (Oliveira *et al.,* 2024). In the present study, the amount of plasma ALP in fish fed FO, VO^{+2}, RAF and RAF^{+4} were higher than other groups that could be related to increasing liver membrane injuries in these groups. In addition, in this study plasma ALT increased in fish fed RAF, RAF^{+2} and MIX^{+2} compared to the other treatments indicating a failure in amino acid metabolism or hepatocyte necrosis in this species. Moreover, the highest levels of plasma AST was in RAF that was associated with increasing ALP and ALT in this group suggesting high levels of SFA in this diet may resulted in liver steatosis and liver damages in this group. In contrast, Salini *et al.* (2015a) in which dietary FO replacement with ALS did not affect serum enzyme markers, including ALT and lactate dehydrogenase (LDH) in *L. calcarifer*. Song *et al.* (2024) reported that serum ALP increased but ALT decreased in largemouth bass fed diets in which FO replaced with rapeseed oil. In addition, these authors reported that serum AST increased in largemouth bass fed diets in which FO replaced with soybean oil. Regarding dietary SL supplementation, El-Sayed *et al.* (2021) reported that liver enzymes in serum reduced in Nile tilapia (*Oreochromis niloticus*) fed SL supplemented diets suggesting health promoting effects of this supplement. In the present study, the amounts of plasma P and Ca increased in fish fed FO diet that may due to high levels of vitamin D in this lipid source that enhance P and Ca absorption.

In the current study, fish fed VO^{+4} diet had higher plasma GLU level compared to the other treatments. In this sense, it was revealed that VO could impair glucose-stimulated insulin secretion of islets (Nunes *et al.,* 2007) and induce glucose intolerance and insulin resistance (Deol *et al.,* 2015), which may be the causes hyperglycemia in fish as also reported in blunt snout bream (*Megalobrama amblycephala,* Wang *et al.*

(2017), hybrid sturgeon (Yu *et al.,* 2020) and largemouth bass (Song *et al.,* 2024).

Conclusions

The findings of the present study showed that supplementing diet containing various lipid sources with 2-4% SL improved growth performance in *L. calcarifer* juveniles that attributed to increased FI and FCR in this species. In addition, whole body fatty acid profile pronouncedly affected by both dietary lipid sources and SL level and feeding fish with blends of various lipid sources in combination with SL supplementation improved FA profile of fish in regards to LC-PUFA level compared to those fed VO or RAF diets. In addition, using MIX oils by supplementing SL provided better health condition in regard to humoral immune responses and plasma biochemical parameters compared to those fed VO and RAF diets. Further studies are required to evaluate the interactive effects of dietary various ALS with SL levels on antioxidant status, immune responses, disease resistance and fatty acid metabolism evaluation at molecular level in *L. calcarifer*.

Conflicts of interest

The authors declare no conflicts of interest.

References

Alhazzaa, R., Bridle, A.R., Nichols, P.D., and Carter, C.G., 2011a. Replacing dietary fish oil with Echium oil enriched barramundi with C18 PUFA rather than long-chain PUFA. *Aquaculture,* 312(1-4), 162–171. DOI:10.1016/j.aquaculture.2010.12.023

Alhazzaa, R.,, Bridle, A.R., Nichols, P.D., and Carter, C.G., 2011b. Up-regulated desaturase and elongase gene expression promoted accumulation of polyunsaturated fatty acid (PUFA) but not long-chain PUFA in *Lates calcarifer*, a tropical euryhaline fish fed a stearidonic- and γ-linoleic acid enriched diet. *Journal of Agriculture and Food Chemistry,* 59, 840: 8423–8434. DOI:10.1021/jf201871

Alhazzaa, R., Bridle, A.R., Carter, C.G. and Nichols, P.D., 2012. Sesamin modulation of lipid class and fatty acid profile in early juvenile teleost, *Lates calcarifer*, fed different dietary oils. Food *Chemistry,* 134, 2057–2065. DIO: 10.1016/j.foodchem.2012.04.004

Alhazzaa, R., Bridle, A.R., Mori, T.A., Barden, A.E., Nichols, P.D. and Carter, C.G., 2013a. Echium oil is better than rapeseed oil in improving the response of barramundi to a disease challenge. *Food Chemistry,* 141, 1424–1432. DOI:10.1016/j.foodchem.2013.04.052

Alhazzaa, R., Sinclair, A.J. and Turchini, G.M., 2013b. Bioconversion of α-linolenic acid into n-3 long chain polyunsaturated fatty acid in hepatocytes and ad hoc cell culture optimisation. *PLoS One*, 8, e73719. DOI:10.1371/journal.pone.0073719

Alhazzaa, R,, Nichols, P.D. and Carter, C.G., 2019. Sustainable alternatives to dietary fish oil in tropical fish aquaculture. *Reviews in Aquaculture,* 11, 1195-1218. DOI:10.1111/raq.12287.

AOAC., 2005. Official Methods of Analysis (18th ed.). Gaithersburg, MD:

Association of Official Analytical Chemists International.

Castro, C., Diógenes, A.F., Coutinho, F., Panserat, S., Corraze, G., Pérez-Jiménez, A., Peres, H. and OlivaTeles, A., 2016. Liver and intestine oxidative status of gilthead sea bream fed vegetable oil and carbohydrate rich diets. *Aquaculture,* 464,665–672. DOI:10.1016/j.aquaculture.2016.08.005

Christie, W.W., 1993. Preparation of ester derivatives of fatty acids for chromatographic analysis. In: Christie, W.W. (ed.) Advances in lipid methodology-two. Oily Press, Dundee. pp 69–111.

Demers, N.E. and Bayne, C.J., 1997. The immediate effects of stress on hormones and plasma lysozyme in rainbow trout. *Developmental & Comparative Immunology*, 21,363–373. DOI:10.1016/S0145-305X(97)00009-8

Deol, P., Evans, J.R., Dhahbi, J., Chellappa, K., Han, D.S., Spindler, S. and Sladek, F.M., 2015. Soybean oil is more obesogenic and diabetogenic than coconut oil and fructose in mouse: potential role for the liver. *PloS One,* 10(7), e0132672. DOI:10.1371/journal.pone.0132672

El-Naggar, K., Mohamed, R., El-katcha, M.I., Abdo, S.E. and Soltan, M.A., 2021. Plant ingredient diet supplemented with lecithin as fish meal and fish oil alternative affects growth performance, serum biochemical, lipid metabolism and growth-related gene expression in Nile tilapia. *Aquaculture Research*, 52(12), 6308-6321. DOI: 10.1111/are.15494

El-Sayed, A.F., Tammam, M.S. and Makled, S.O., 2021. Lecithin-containing bioemulsifier boosts growth performance, feed digestion and absorption and immune response of adult Nile tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition,* 27: 757–770. DOI:10.1111/anu.13221

Future Market Insight, 2023. Asian Sea Bass Market-Analysis, Outlook, Growth, Trends, Forecast. Future Market Insight. Available at: https:// www.futuremarketinsights.com/reports/ sea-bass-market (accessed on January 10, 2023).

Geay, F., Santigosa, I., Culi, E., Corporeau, C., Boudry, P., Dreano Y, Corcos L, Bodin, N., Vandeputte, M., Zambonino-Infante, J.L., Mazurais D. and Cahu, C.L, 2010. Regulation of FADS2 expression and activity in European sea bass (*Dicentrarchus labrax* L.) fed a vegetable diet. *Comparative Biochemestiry Physiollogy Part B: Biochemistry of Molecular and Biology,* 156,237–243. DOI:10.1016/j.cbpb.2010.03.008

Glencross, B.D. and Rutherford, N.R., 2011. A determination of the quantitative requirements for docosahexaenoic acid for juvenile barramundi (*Lates calcarifer*). *Aquaculture Nutrition*, 17: e536-13 e548. DOI:10.1111/j.1365-2095.2010.00795.x.

Glencross, B., Blyth, D., Irvin, S., Bourne, N., Campet, M., Boisot, P. and Wade, N.M., 2016. An evaluation of the complete replacement of both fishmeal and fish oil in diets for juvenile Asian seabass, *Lates calcarifer*.

Aquaculture, 451, 298-309. DOI:10.1016/j.aquaculture.2015.09.012

Haghparast, R.J., Moghanlou, K.S., Mohseni, M. and Imani, A., 2019. Effect of dietary soybean lecithin on fish performance, hemato-immunological parameters, lipid biochemistry, antioxidant status, digestive enzymes activity and intestinal histomorphometry of pre-spawning Caspian brown trout (*Salmo trutta caspius*). *Fish and Shellfish Immunology*, 91,50–57. DOI:10.1016/j.fsi.2019.05.022

Hertrampe, J.W. and Piedad-Pascual, F., 2000. Handbook on ıngredients for aquaculture feeds. Kluwer Academic Publishers, Dordrencht, The Netherlands. 573 P.

Izquierdo, M.S. and Koven, W.M., 2010. Lipids. In: Holt J, editor. Larval fish nutrition. Wiley and Sons Publisher, Oxford. pp 47–82.

Jafari, F., Agh, N., Noori, F., Tokmachi, A. and Gisbert, E., 2018. Effects of dietary soybean lecithin on growth performance, blood chemistry and immunity in juvenile stellate sturgeon (*Acipenser stellatus*). *Fish and Shellfish Immunology,* 80, 487-496. DOI:10.1016/j.fsi.2018.06.023

Jafari, F., Noori, F., Agh, N., Estevez, A., Ghasemi, A., Alcaraz, C. and Gisbert, E., 2021. Phospholipids improve the performance, physiological, antioxidative responses and, lpl and igf1 gene expressions in juvenile stellate sturgeon (*Acipenser stellatus*). *Aquaculture,* 541, 736809. DOI:10.1016/j.aquaculture.2021.736809

Jafari, F., Agh, N., Noori, F., Gisbert, E. and Mozanzadeh, M.T., 2024. Supplementing lysolecithin in corn-oil based diet enhanced growth and improved body biochemical composition in juvenile stellate sturgeon (*Acipenser stellatus*). *Animal Feed Science and Technology,* 310,115945. DOI:10.1016/j.anifeedsci.2024.115945

Kiron, V. 2012. Fish immune system and its nutritional modulation for preventive health care. *Animal Feed Science and Technology*, 173, 111–133. DOI: 10.1016/j.anifeedsci.2011.12.015

Kjær, M., Todorčević, M., Torstensen, B., Vegusdal, A. and Ruyter, B., 2008. Dietary n-3 HUFA affects mitochondrial fatty acid β-oxidation capacity and susceptibility to oxidative stress in Atlantic salmon. *Lipids*, 43,813-827. DOI:10.1007/s11745-008-3208-z

La, T.X., Ishikawa, M., Tola, S., Fukada, H. and Masumoto, T., 2018. Effects of dietary phospholipid level and fraction on the feed intake of non-fish meal diet in yellowtail, *Seriola quinqueradiata* Temminck & Schlegel, 1845. *Aquaculture Research,* 49, 569–575. DOI:10.1111/are.13488

Lin, S.M, Li, F.J., Yuangsoi, B. and Doolgindachbaporn, S., 2017. Effect of dietary phospholipid levels on growth, lipid metabolism, and antioxidative status of juvenile hybrid snakehead (*Channa argus*×*Channa maculata*). *Fish Physiology and Biochemistry,* 44,401–410. DOI:10.1007/s10695-017-0443-3

Lin, Y.H. and Shiau, S.Y., 2007. Effects of dietary blend of fish oil with corn oil on growth and non-specific immune

responses of grouper, *Epinephelus malabaricus*. *Aquaculture Nutrition,* 13, 137–144. DOI:10.1111/j.1365-2095.2007.00458.x

Mohd-Yusof, N.Y., Monroig, O., Mohd-Adnan, A., Wan, K.L. and Tocher, D.R., 2010. Investigation of highly unsaturated fatty acid metabolism in the Asian sea bass, *Lates calcarifer*. *Fish Physiology and Biochemistry,* 36,827-843. DOI:10.1007/s10695-010-9409-4

Montero, D., Benitez-Dorta, V., Caballero, M.J., Ponce, M., Torrecillas, S., Izquierdo, M., Zamorano, M.J. and Manchado, M., 2015. Dietary vegetable oils: effects on the expression of immune-related genes in Senegalese sole (*Solea senegalensis*) intestine. *Fish and Shellfish Immunology,* 44, 100–108.

Montero, D., Kalinowski, T., Obach, A., Tort, R.L.L., Caballero, M.J. and Izquierdo, M.S., 2003. Vegetable lipid sources for gilthead seabream (*Sparus aurata*), Effects on fish health. *Aquaculture,* 225, 353–370. DOI:10.1016/S0044-8486(03)00301-6

Montero, D., Grasso, V., Izquierdo, M.S., Ganga, R., Real, F., Tort, L., Caballero, M.J. and Acosta, F., 2008. Total substitution of fish oil by vegetable oils in gilthead seabream (*Sparus aurata*) diets: effects on hepatic Mx expression and some immune parameters. *Fish and Shellfish Immunology,* 24, 147–155.

Montero, D., Mathlouthi, F., Tort, L., Afonso JM, Torrecillas S, Fernández-Vaquero A, Negrin D. and Izquierdo, M.S., 2010. Replacement of dietary fish oil by vegetable oils affects humoral immunity and expression of pro-inflammatory cytokines genes in gilthead sea bream *Sparus aurata*. *Fish and Shellfish Immunology, 29,* 1073–1081. DOI:10.1016/j.fsi.2010.08.024

Morton, K.M., Blyth, D., Bourne, N., Irvin, S. and Glencross, B.D., 2014. Effect of ration level and dietary docosahexaenoic acid content on the requirements for long-chain polyunsaturated fatty acids by juvenile barramundi (*Lates calcarifer*). *Aquaculture,* 433,164–172. DOI:10.1016/j.aquaculture.2014.05.047

Mourente, G., Good, J.E., Thompson, K.D. and Bell, J.G., 2007. Effects of partial substitution of dietary fish oil with blends of vegetable oils, on blood leucocyte fatty acid compositions, immune function and histology in European sea bass (*Dicentrarchus labrax* L.). *British Journal of Nutrition,* 98,770–779. DOI:10.1017/S000711450773461X

Mozanzadeh, M.T., Agh, N., Yavari, V., Marammazi, J.G., Mohammadian, T. and Gisbert, E., 2016. Partial or total replacement of dietary fish oil with alternative lipid sources in silvery-black porgy (*Sparidentex hasta*). *Aquaculture,* 451, 232–240. DOI:10.1016/j.aquaculture.2015.09.022

Mozanzadeh, M.T., Agh, N., Yaghoubi, M. and Marammazi, J.G., 2020. Effects of dietary different lipid sources on plasma metabolites in silvery -black porgy (*Sparidentex hasta*) juveniles. *Iranian Journal of Fisheries Sciences,* 19, 45-58. DOI:10.22092/ijfs.2018.116907

Mozanzadeh, M.T., Safari O, Oosooli, R., Mehrjooyan, S., Najafabadi, M.Z., Hoseini, S.J., Saghavi, H, Monem, J., 2021. The effect of salinity on growth performance, digestive and antioxidant enzymes, humoral immunity and stress indices in two euryhaline fish species: Yellowfin seabream (*Acanthopagrus latus*) and Asian seabass (*Lates calcarifer*). *Aquaculture*, 534, 736329. DOI:10.1016/j.aquaculture.2020.736329

Nunes, E., Peixoto, F., Louro, T., Sena, C., Santos, M., Matafome, P. and Seica, R., 2007. Soybean oil treatment impairs glucose-stimulated insulin secretion and changes fatty acid composition of normal and diabetic islets. *Acta Diabetologica*, 44(3), 121–130. DOI:10.1007/s00592-007-0252-8

Oliveira, J., Oliva-Teles, A. and Couto, A., 2024. Tracking biomarkers for the health and welfare of aquaculture fish. *Fishes,* 9, 289. DOI: 10.3390/fishes9070289

Pagheh, E., Agh, N., Marammazi, J.G., Nouri, F., Sepahdari, A., Gisbert, E. and Mozanzadeh, M.T., 2019. Dietary soybean lecithin affects growth performance, fillet biochemical composition and digestive enzyme activity in *Sparidentex hasta* juvenile. *Journal of Applied Animal Research,* 47(1), 24-33, DOI:10.1080/09712119.2018.1557663

Rahman, A., Tantikitti, C., Suanyuk, N., Forster, I., Hlongahle, B. and Tunpairoj, W., 2022. 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. *Songklanakarin Journal of Science and Technology,* 44, 79-90. DOI:10.14456/sjst-psu.2022.13

Raso, S. and Anderson, T.A., 2003. Effects of dietary fish oil replacement on growth and carcass proximate composition of juvenile barramundi (*Lates calcarifer*). *Aquaculturte Research*, 34, 813-819.

Saleh, M. Betancor, J. Roo, V. Benítez-Dorta, M.J. Zamorano, J.G. Bell, and Izquierdo, M., 2015. Effect of krill phospholipids versus soybean lecithin in microdiets for gilthead seabream (*Sparus aurata*) larvae on molecular markers of antioxidative metabolism and bone development *Aquaculture Nutrition*, 21, 474-488. DOI: 10.1111/anu.12177

Salini, M., Irvin, S., Bourne, N., Blyth, D., Cheers, S., Habilay, N. and Glencross, B., 2015a. Marginal efficiencies of long chain-polyunsaturated fatty acid use by barramundi (*Lates calcarifer*) when fed diets with varying blends of fish oil and poultry fat. *Aquaculture,* 449, 48-57. DOI:10.1016/j.aquaculture.2015.02.027

Salini, M.J., Turchini, G.M., Wade, N.M. and Glencross, B.D., 2015b. Rapid effects of essential fatty acid deficiency on growth and development parameters and transcription of key fatty acid metabolism genes in juvenile barramundi (*Lates calcarifer*). *British Journal of Nutrition,* 114, 1784–1796. DOI:10.1017/S0007114515003529.

Salini, M.J., Wade, N., Bourne, N., Turchini, G.M. and Glencross, B.D.,

2016. The effect of marine and non-marine phospholipid rich oils when fed to juvenile barramundi (*Lates calcarifer*). *Aquaculture,* 455, 125-135. DOI:10.1016/j.aquaculture.2016.01.013

Secombes, C.J. 1990. Isolation of salmonid macrophages and analysis of their killing activity. Tech. *Fish Immunology,* 1, 137–154.

Seierstad, S.L., Haugland, Ø., Larsen, S., Waagbø, R. and Evensen, Ø. 2009. Pro-inflammatory cytokine expression and respiratory burst activity following replacement of fish oil with rapeseed oil in the feed for Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 289, 212–218. DOI: 10.1016/j.aquaculture.2008.12.004

Song, R., Yao, X., Jing, F., Yang, W., Wu, J., Zhang, H., Zhang, P., Xie, Y., Pan, X., Zhao, L. and Wu, C., 2024. Effects of five lipid sources on growth, hematological parameters, immunity and muscle quality in juvenile largemouth bass (*Micropterus salmoides*). *Animals,* 14, 781. DOI:10.3390/ani140507814

Sunyer, J.O. and Tort, L., 1995. Natural hemolytic and bactericidal activities of sea bream *Sparus aurata* serum are effected by the alternative complement pathway. *Veterinary Immunology and Immunopathology*, 45(3), 333–345. DOI:10.1016/0165-2427(94)05430-Z

Tan, P., Dong, X., Mai, K., Xu, W. and Ai, Q., 2016. Vegetable oil induced inflammatory response by altering TLR-NF-kB signalling, macrophages infiltration and polarization in adipose tissue of large yellow croaker (*Larimichthys crocea*). *Fish and Shellfish Immunology,* 59, 398–405. DOI:10.1016/j.fsi.2016.11.009

Tan, P., Dong, X., Xu, H., Mai, K. and Ai, Q., 2017. Dietary vegetable oil suppressed non-specific immunity and liver antioxidant capacity but induced inflammatory response in Japanese sea bass (*Lateolabrax japonicus*). *Fish and Shellfish Immunology*, 63, 139–146. DOI:10.1016/j.fsi.2017.02.006

Tocher, D.R., Bendiksen, E.A., Campbell, P.J. and Bell, J.G., 2008. The role of phospholipids in nutrition and metabolism of teleost fish. *Aquaculture,* 280:21–34. DOI:10.1016/j.aquaculture.2008.04.034

Torrecillas, S., Caballero, M.J., Mompel, D., Montero, D., Zamorano, M.J., Robaina, L., Ramirez, F.R., Karalazos, V., Kaushik, S. and Izquierdo, M., 2017. Disease resistance and response against *Vibrio anguillarum* intestinal infection in European sea bass (*Dicentrarchus labrax*) fed low fish meal and fish oil diets. *Fish and Shellfish Immunology*, 67, 302–311. DOI:10.1016/j.fsi.2017.06.022

Torrecillas, S., Rivero-Ramirez, F., Izquierdo, M.S., Caballero, M.J., Makol, A., Suarez-Bregue, P., FernandezMontero, A., Rotllant, J. and Montero, D., 2018. Feeding European sea bass (*Dicentrarchus labrax*) juveniles with a functional synbiotic additive (mannan oilgosaccharides and *Pediococcus acidilactici*), An effective tool to reduce low fish meal and fish oli gut health effects? *Fish and Shellfish Immunology,* 81, 10–20. DOI:10.1016/j.fsi.2018.07.007

Vegusdal, A., Gjøen, T., Berge, R., Thomassen, M. and Ruyter, B., 2005. Effect of 18:1n− 9, 20: 5n− 3, and 22: 6n− 3 on lipid accumulation and secretion by Atlantic salmon hepatocytes. *Lipids,* 40, 477-486. DOI:10.1007/s11745-005-1407-z

Wang, B.K., Liu, W.B., Xu, C., Cao, X.F., Zhong, X.Q., Shi, H.J. and Li, X.F., 2017. Dietary carbohydrate levels and lipid sources modulate the growth performance, fatty acid profiles and intermediary metabolism of blunt snout bream *Megalobrama amblycephala* in an interactive pattern. *Aquaculture,* 481, 140–153. DOI:10.1016/j.aquaculture.2017.08.034

Wee, W., Téllez-Isaías, G., Abdul Kari, Z., Cheadoloh, R., Kabir, M.A., Mat, K., Mohamad Sukri S.A., Rahman, M.M., Rusli, N.D. and Wei, L.S., 2023. The roles of soybean lecithin in aquafeed: a crucial need and update. *Frontiers in Veternity Science* 10:1188659. DOI:10.3389/fvets.2023.1188659

Wu, J., Yang, W., Song, R., Li, Z., Jia, X., Zhang, H., Zhang, P., Xue, X., Li, S., Xie, Y., Zhang, R., Y,e J., Zhou, Z. and Wu, C., 2023. Dietary soybean lecithin improves growth, immunity, antioxidant capability and intestinal barrier functions in Largemouth Bass *Micropterus salmoides* juveniles. *Metabolites,* 13, 512. DOI:10.3390/metabo13040512

Yu, H., Xing, W., Li, T., Xu, G., Ma, Z., Jiang, N. and Luo, L., 2020. Effects of alternative dietary lipid sources on growth performance, health status and fillet fatty acid composition of hybrid sturgeon (*Acipenser baeri* Brandt ♀ × *Acipenser schrenckii* Brandt ♂). *Aquaculture NutrItion,* 00:1–12. DOI:10.1111/anu.1308