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# Dietary soybean lecithin affects growth performance, fillet biochemical composition and digestive enzyme activity in *Sparidentex hasta* juvenile

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**ABSTRACT**

An eight-week study was conducted on silvery-black porgy (*Sparidentex hasta*) juveniles to evaluate four isoproteic, isolipidic and isoenergetic different diets (50% crude protein, 20% crude lipids, 18.5 MJ $kg^{-1}$) containing graded levels of soybean lecithin (SBL) (0, 30, 60 and 90 g $kg^{-1}$ diet) at the expense of fish oil (FO). Fish fed the 60 g SBL $kg^{-1}$ diet had significantly higher weight gain (32.4%) and feed intake (8.8 g $fish^{-1}$) than the control group (SBL 0) ($P < 0.05$). The fillet fatty acid (FA) profiles were correlated with the FA profile of the experimental diets. Fish fed with SBL-supplemented diets had higher fillet phosphatidylcholine levels than the control group ($P < 0.05$). Plasma total immunoglobulin was higher in fish fed 60 and 90 g SBL $kg^{-1}$ diets than in the other groups ($P < 0.05$). Total protease activity was higher in fish fed the 90 g SLB $kg^{-1}$ diet than other treatments ($P < 0.05$). Results indicated that substitution of dietary FO with SBL diet up to 67% (60 g SLB $kg^{-1}$ diet) improved somatic growth performance and profoundly affected the fillet fatty acid profile in silvery-black porgy juveniles.

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## Introduction

Phospholipids (PLs) play a major role in maintaining the structure, integrity, fluidity and function of cellular membranes (Tocher et al. 2008). Dietary PLs have been reported to improve growth performance, survival rates, stress resistance and digestive functions in different fish species, both in larvae and early juveniles, and can decrease the incidence of skeletal deformities at larval stages (see reviews by Coutteau et al. 1997; Tocher et al. 2008; Cahu et al. 2009). In addition, PLs by stimulating lipoprotein synthesis in enterocytes can enhance lipid transport, improve the intestinal absorption of long-chain polyunsaturated fatty acids (LC-PUFA) and reduce intestinal steatosis (Fontagné et al. 2000; Gisbert et al. 2005; Tocher et al. 2008). In this regard, soybean lecithin (SBL) because of its high availability and reasonable price in comparison to marine PL sources has been commercially used as a ubiquitous source of PLs in aquafeeds (Tocher et al. 2008). From a nutritional point of view, SBL may also serve as a feed attractant, providing vitamins and EFAs that are vital for fish growth (see reviews by Coutteau et al. 1997; Tocher et al. 2008; Cahu et al. 2009). Several studies conducted in different fish species have reported positive effects of dietary SBL supplementation on growth performance (Kenari et al. 2011; Kumar et al. 2012; Taylor et al. 2015), digestive processes (Hamza et al. 2008; Kenari et al. 2011; Adel et al. 2017) and antioxidant enzyme activities (Gao et al. 2014; Kumar et al. 2014; Adel et al. 2017), as well as stress and disease resistance (Kumar et al. 2012, 2014; Adel et al. 2017).

Silvery-black porgy *S. hasta* is recognized as one of the most promising candidates for promoting mariculture activities in the south of Iran. Thus, considerable research has been focused on establishing the nutritional requirements of this species in order to optimize its diet formulation (Mozanzadeh et al. 2017). Thus, in order to continue improving the formulation of compound diets for this fish species, the current study was designed to evaluate the effects of dietary SBL inclusion on growth performance, humoral immune responses as well as digestive and antioxidant enzymes activities of *S. hasta* juveniles.

## Materials and methods

### Experimental design

For evaluating the effects of dietary SBL inclusion on *S. hasta* juveniles performance, an eight-week feeding trial was conducted using four isonitrogenous (*ca.* 500 g $kg^{-1}$ crude protein), isoenergetic (*ca.* 18.5 MJ $kg^{-1}$) and isolipidic (*ca.* 200 g $kg^{-1}$ crude lipids) diets containing graded levels of SBL (0, 30, 60 and 90 g $kg^{-1}$ diet) (Tables 1–3) at the expense of fish oil (FO) as the main lipid source. Experimental diets were prepared as described in Mozanzadeh et al. (2015). Diets were prepared by mixing all dry ingredients including fish meal, wheat meal, gluten meal, beef gelatin and premixes for 30 min. Then, FO, SBL and sufficient distilled water were added to form a soft dough and mechanically extruded to

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**Table 1.** Ingredient and proximate composition of the experimental diets.

| | SBL (g kg$^{-1}$) | | | |
|---|---|---|---|---|
| Ingredients (g kg$^{-1}$) | 0 | 30 | 60 | 90 |
| Fish meal[a] | 560 | 560 | 560 | 560 |
| Beef gelatin[b] | 51 | 51 | 51 | 51 |
| Gluten meal | 120 | 120 | 120 | 120 |
| Wheat meal[c] | 101 | 101 | 101 | 101 |
| Fish oil[a] | 135 | 105 | 75 | 45 |
| Soybean lecithin[d] | 0 | 30 | 60 | 90 |
| Vitamin premix[e] | 15 | 15 | 15 | 15 |
| Mineral premix[f] | 15 | 15 | 15 | 15 |
| Antioxidant[g] | 3 | 3 | 3 | 3 |
| ***Proximate composition (g kg$^{-1}$)*** | | | | |
| Dry matter | 920.4 | 915.4 | 910.4 | 912.4 |
| Crude protein | 485.0 | 488.0 | 482.0 | 490.0 |
| Crude lipid | 260.0 | 253.0 | 245.0 | 249.0 |
| Ash | 85.0 | 84.0 | 89.0 | 89.0 |

[a]Fish meal (*Clupeonella* sp.); Parskilka Mazandaran, Iran (635 g kg$^{-1}$ crude protein, 177 g kg$^{-1}$ crude lipid).
[b]Gelatine; Beyza feed mill, Shiraz, Iran. (850 g kg$^{-1}$ crude protein, 42 g kg$^{-1}$ crude lipid).
[c]Wheat meal; Beyza feed mill, Shiraz, Iran. (120 g kg$^{-1}$ crude protein, 30 g kg$^{-1}$ crude lipid).
[d]Behpak Industrial Company, Behshahr, Mazandaran, Iran.
[e]Vitamin premix (mg kg$^{-1}$) of premix: vitamin A, 5,000,000 IU; vitamin D3, 500,000 IU; vitamin E, 3000 mg; vitamin K3, 1500; vitamin B1, 6000; vitamin B2, 24,000; vitamin B5, 52,000; vitamin B6, 18,000; vitamin B12, 60,000; folic acid, 3000; nicotinamide 180,000; antioxidant, 500, Damloran pharmaceutical company, Borujerd, Iran.
[f]Mineral premix (mg kg$^{-1}$) of premix: copper, 3000; zinc, 15,000; manganese, 20,000; Iron, 10,000; potassium iodate, 300. Microvit®, Razak laboratories, Tehran, Iran.
[g]Butylated hydroxyl toluene, GarmabShimi, Iran.

obtain pellets (3 mm). Pellets were dried in a convection oven at 25°C and stored in re-sealable plastic bags at −20°C until use.

**Table 2.** Fatty acid composition of experimental diets (mg g$^{-1}$ total fatty acids).

| | SLB (g kg$^{-1}$) | | | |
|---|---|---|---|---|
| Fatty acids | 0 | 30 | 60 | 90 |
| 14:0 | 25.2 | 30.1 | 25.5 | 20.2 |
| 16:0 | 210.5 | 234.4 | 213.4 | 220.4 |
| 18:0 | 52.5 | 52.9 | 48.6 | 50.1 |
| 20:0 | 13.5 | 13.1 | 13.3 | 7.2 |
| 22:0 | 1.6 | 2.1 | 2.9 | 2.6 |
| 24:0 | 4.3 | 6.1 | 5.0 | 10.2 |
| SFA[a] | 307.6 | 338.7 | 308.7 | 310.7 |
| 14:1n-5 | 1.2 | 2.3 | 1.6 | 0.6 |
| 16:1n-7 | 42.3 | 40.9 | 35.9 | 25.9 |
| 18:1n-7 | 27.2 | 23.2 | 21.0 | 18.1 |
| 18:1n-9 | 284.7 | 277.9 | 244.4 | 232.3 |
| 20:1n-9 | 2.7 | 2.7 | 1.7 | 0.9 |
| MUFA[b] | 358.1 | 347.0 | 304.6 | 277.8 |
| 18:2n-6 (LA)[c] | 94.8 | 105.6 | 168.4 | 248.9 |
| 20:2n-6 | 1.6 | 2.2 | 3.9 | 3.3 |
| 20:4n-6 (ARA)[d] | 2.2 | 5.2 | 3.5 | 0.9 |
| n-6 PUFA | 98.6 | 112.4 | 175.8 | 253.1 |
| 18:3n-3 (LNA)[e] | 17.6 | 18.9 | 27.4 | 39.1 |
| 20:3n-3 | 1.3 | 1.0 | 1.7 | 0.3 |
| 20:5n-3 (EPA)[f] | 40.6 | 36.8 | 37.4 | 21.8 |
| 22:6n-3 (DHA)[g] | 114.6 | 95.4 | 94.7 | 59.2 |
| n-3 PUFA | 174.1 | 152.1 | 161.2 | 120.4 |
| n-3/n-6 | 1.8 | 1.4 | 0.9 | 0.5 |
| ARA/EPA | 0.05 | 0.1 | 0.03 | 0.02 |
| DHA/EPA | 2.8 | 2.6 | 2.5 | 2.7 |

Notes: The table provide the detected fatty acids by gas chromatography. Abbreviation: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; LA: linoleic acid; ARA: arachidonic acid; LNA: linolenic acid; EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid.

**Table 3.** Lipid classes of experimental diets (%).

| | SBL (g kg$^{-1}$) | | | |
|---|---|---|---|---|
| Lipid classes (mg g extracted lipid$^{-1}$) | 0 | 30 | 60 | 90 |
| PC | 2.1 | 1.8 | 2.3 | 3.6 |
| PS + PI | – | 1.5 | 3.1 | 7.6 |
| PE | – | 1.2 | 1.8 | 3.5 |
| UK | – | – | – | 2.6 |
| PL | 2.1 | 4.5 | 7.2 | 17.3 |
| CHOL | 6.3 | 6.7 | 7.2 | 7.6 |
| FFA | 8.9 | 15.1 | 7.1 | 11.1 |
| TAG | 74.6 | 69.0 | 61.6 | 57.3 |
| SE + W | 8.1 | 4.7 | 16.9 | 6.7 |
| NL | 97.9 | 95.5 | 92.8 | 82.7 |

Notes: PC: phosphatidylcholine; PS: phosphatidylserine + PI: phosphatidylinositol; PE: phosphatidylethanolamine; UK: unknown; PL: phospholipids; CHOL: cholesterol; FFA: free fatty acids; TAG: triacylglycerol; SE: sterolsters; W: wax; NL: neutral lipids.

## Fish maintenance and feeding

This study was carried out at the Mariculture Research Station of the South Iranian Aquaculture Research Center (SIARC), Sarbandar, Iran. Fish were randomly distributed into 12 cylindrical polyethylene tanks (functional volume = 250 L), and each tank was stocked with 15 fish ($BW_i = 38.0 \pm 0.1$ g, mean ± standard error). Before beginning of the nutritional trial, fish were adapted to the experimental condition for two weeks. Tanks were supplied with running sea water (1 L min$^{-1}$) in a flow-through system and the mean values for salinity, tempreture, pH and dissolved oxygen were 48.2 ± 0.2 ppt, 25.1 ± 1.6°C, 7.7 ± 0.1 and 6.8 ± 0.4 mg L$^{-1}$, respectively. The photoperiod condition during experiment was 16L:8D (light:darkness). Each diet was tested by triplicate and fish were fed one of the above-mentioned diets by hand to visual satiation two times per day (0800 and 1500 h) for 56 days. Uneaten feed was removed from the bottom of the tank by siphoning 1 h after feeding, dried in an oven (60°C for 24 h) and weighed to determine feed intake values. All fish from each replicate were measured to the nearest 0.1 g for their body weight ($BW_f$) and their standard length (SL) was measured to the nearest 1 mm. Four specimens from each replicate were sacrificed with an overdose 2-phenoxyethanol for evaluateing the weight of the liver, intraperitoneal fat and viscera. Sample collection for blood ($n = 2$ fish per replicate) and plasma ($n = 2$ fish per replicate), digestive ($n = 2$ fish per replicate) and antioxidant enzymes ($n = 2$ fish per replicate) was done as previously reported by Pagheh et al. (2017).

## Lipid classes and fatty acid (FA) analyses

Total lipids from diets and fish fillets were extracted by sample homogenization in chloroform/methanol (2:1, v/v) (Folch et al. 1957). Lipid class separation was performed by high-performance thin-layer chromatography (HPTLC) (Olsen and Henderson 1989). The HPTLC plates (10 × 10, Nano-sil 20, 0.2 mm of Nano-silica gel 60, Fiers, Kuurne, Belgium) were used for the separation of lipid classes. In this regard, plates were placed in chloroform: methanol (2:1) for 24 h, then they were transferred in the oven at 110°C for 30 min and let them cool in a desicator. A volume of 10 µl of samples was transferred to plates and developed using a mixture of methylacetate: isopropanol: chloroform:

methanol: KCl (2:2:2:1:1). Then, plates were dried in a desicator for 15 min, and placed in a second solvent (29.75 ml of hexan + 5.25 ml of diethylether + 0.35 ml glacial acetic acid) for 15 min. Fewster mix (3% copper acetate in 8% orthophosphoric acid) was pulverized on the plates. Finally, plates were placed in the oven at 160°C for 20 min, and after cooling the lipid classes were quantified by densitometry (BioRad, GS-900, USA).

For determining the diet and fillet FA's profiles, FA methyl esters were prepared by acidic methanolysis of lipid extracts using sulphuric acid in methanol (Christie 1993). In this regard, the lipid sample (up to 50 mg) is dissolved in 2.5% sulphuric acid in methanol (2 mL) in a test tube. The mixture was left for 1 h at 80°C, then samples were cooled down at room temperature. After that, water (1.5 mL) containing sodium chloride (0.9%) was added and the required esters extracted with hexane (2 × 1 mL) using Pasteur pipettes to separate the layers. The solution centrifuged (4000 *g*, 50 min, 4°C) and the upper layer, which contained FA methyl esters was separated and evaporated under a stream of nitrogen. Finally, the remained dry FA methyl esters were dissolved in isooctane (1 mL) and determined by gas chromatography. The FA composition of diet ($n = 1$) and fish fillet ($n = 3$) were determined by an auto sampler gas chromatography (GC, Agilent technologies 7890 N, USA), equipped with aflame ionization detector (FID) and a cyanopropyl–phenyl capillary column (DB-225MS, 30 m × 0.250 mm ID × 0.25 µm Film thickness, USA). Carrier gas was ultra-high purity nitrogen at a flow rate of 1 mL $min^{-1}$. The column temperature was programmed as follows: holding at 100°C for 2 min, raising to 182°C at a rate of 30°C $min^{-1}$, and again raising to 220°C at a rate of 2°C $min^{-1}$, holding for 5 min, and finally column heating at a rate of 3°C $min^{-1}$–230°C, then holding at this temperature for 3 min. The injector and detector temperatures were set at 230°C and 300°C, respectively. The split ratio was 30:1 and the sample volume injected for each analysis was 1 µL (total run time = 40 min per sample). The identification of fatty acids was performed by comparing their retention time with those of an external commercial standard mixture (GLC-68d, NuChek Prep., MN, USA) (Agh et al. 2014).

### Hematological and antioxidant status

Complete blood count was assessed according to Blaxhall and Daisley (1973). Haemolytic and lysozyme activities, as well as total immunoglobulin (Ig) levels in the plasma were determined according to Andani et al. (2012), Ellis (1990) and Siwicki et al. (1994), respectively. Superoxide dismutase (SOD) and catalase (CAT) activities, as well as total antioxidant capacity (TAC) in liver samples were assayed according to Kono (1978), Koroluk et al. (1988) and Benzie and Strain (1996), respectively.

### Activity of pancreatic digestive enzymes

Samples were processed and handled following the indications of Solovyev and Gisbert (2016) regarding the time of sample storage and process. Dissected digestive tracts from the same tank were pooled and homogenized (1–2 min at 0–4°C; 3 volumes v/w of 50 mM 2 mM Tris–HCl buffer, pH 7.0) (Chong et al. 2002). Total alkaline proteases were assayed according to method described by García-Carreño and Haard (1993). Bile salt-activated lipase activity was assayed according to the method described by Iijima et al. (1998). The soluble protein of crude enzyme extracts was quantified by means of the Bradford's method (1976). All the assays were made in triplicate (methodological replicates). All oxidative stress condition parameters and digestive enzymes activities were measured in triplicate with a microplate scanning spectrophotometer (PowerWave HT, BioTek®, USA).

### Statistical analyses

Data were analysed using SPSS ver.19.0 (Chicago, Illinois, USA). All data are presented as mean ± standard error of the mean calculated from three replicates (tanks). Arcsine transformations were conducted on data expressed as percentage. One-way ANOVA was performed at a significance level of 0.05 following confirmation of normality and homogeneity of the variance. Duncan's procedure was used for multiple comparisons when statistical differences were found among groups by the one-way ANOVA.

## Results

### Fatty profile and lipid classes of experimental diets

As presented in Table 2, the levels of dietary polyunsaturated FA, mainly (linoleic and linolenic acids), increased; wheareas the content of monounsaturated fatty acids (oleic acid, 18:1n-9), as well as that of n-3 LC-PUFA (especially EPA and DHA) decreased with the progressive replacement of dietary FO with SBL. As expected, PL levels, including phosphatidyl choline, ethnolamine, serin and inositol in diets increased, and the level of tryacyglycerols decreased with the progressive replacement of dietary FO with SBL (Table 3).

### Growth performance

In the present study, no mortality occurred throughout the experiment (Table 4). Growth performance of fish fed SBL-supplemented diets was improved in comparison with the control group. In this context, fish fed with the control (SBL 0) and 60 g SBL $kg^{-1}$ diets had the lowest and highest WG (96.0 *vs.* 128.0%) and SGR (1.2 *vs.* 1.5% $day^{-1}$) values, respectively, whereas the other groups showed intermediate values (Table 4). Values of the HSI were higher in fish fed the control diet than those fed SBL-supplemented diets, whereas there were no differences in other somatic indices including VSI, PFI and K among experimental groups ($P > 0.05$).

### Fillet lipid classes and FA profiles

Lipid classes and FA composition of fillets significantly changed depending on lipid classes and FA composition of experimental diets (Tables 5 and 6). Fillets of fish fed the control diet had the highest content in saturated fatty acids [mainly palmitic (16:0) and stearic (18:0) acids] ($P < 0.05$). The levels of monounsaturated fatty acids (MUFA), especially oleic acid (18:1n-9, OA), significantly decreased in the fillet of fish fed the 90 g SLB $kg^{-1}$ diet ($P < 0.05$). The amount of polyunsaturated fatty acids (PUFA), especially

**Table 4.** Growth response and survival of *S. hasta* juvenile fed experimental diets differing in their content in soybean lecithin (SBL) (mean ± SEM, $n = 3$).

| | SLB (g kg$^{-1}$) | | | | |
|---|---|---|---|---|---|
| Parameter | 0 | 30 | 60 | 90 | *P*-value |
| $BW_i$ (g) | 37.8 ± 0.1 | 38.1 ± 0.1 | 37.8 ± 0.1 | 37.9 ± 0.2 | 0.099 |
| $BW_f$ (g) | 74.1 ± 0.9$^{b}$ | 77.2 ± 2.7$^{ab}$ | 86.2 ± 1.7$^{a}$ | 82.6 ± 4.0$^{ab}$ | 0.043 |
| SGR (% day$^{-1}$)[1] | 1.2 ± 0.0$^{b}$ | 1.3 ± 0.1$^{ab}$ | 1.5 ± 0.0$^{a}$ | 1.4 ± 0.1$^{ab}$ | 0.043 |
| WG (%)[2] | 95.8 ± 2.1$^{b}$ | 102.7 ± 7.5$^{b}$ | 128.2 ± 4.8$^{a}$ | 117.7 ± 10.6$^{ab}$ | 0.043 |
| SUR (%)[3] | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 1.000 |
| FI (g fish$^{-1}$) | 55.7 ± 0.8$^{c}$ | 60.4 ± 0.8$^{b}$ | 64.5 ± 1.1$^{a}$ | 62.7 ± 2.0$^{ab}$ | 0.001 |
| FCR[4] | 1.5 ± 0.0 | 1.6 ± 0.1 | 1.3 ± 0.1 | 1.4 ± 0.1 | 0.224 |
| PER[5] | 1.3 ± 0.0 | 1.3 ± 0.1 | 1.5 ± 0.1 | 1.4 ± 0.1 | 0.237 |
| K (%)[6] | 3.0 ± 0.0 | 3.0 ± 0.1 | 3.1 ± 0.0 | 2.9 ± 0.1 | 0.347 |
| VSI (%)[7] | 8.0 ± 0.3 | 7.8 ± 0.7 | 8.3 ± 0.1 | 8.3 ± 0.3 | 0.929 |
| HSI (%)[8] | 1.8 ± 0.1$^{a}$ | 1.4 ± 0.1$^{b}$ | 1.5 ± 0.0$^{b}$ | 1.6 ± 0.0$^{b}$ | 0.030 |
| PFI (%)[9] | 3.1 ± 0.3 | 3.7 ± 0.6 | 3.3 ± 0.2 | 3.9 ± 0.2 | 0.761 |

Note: A different superscript in the same row denotes statistically significant differences ($P < .05$).
[1]Specific growth rate (SGR, % day$^{-1}$) = [(ln $BW_f$ − ln $BW_i$)/$t$] × 100, where $t$ is experimental period (56 days).
[2]Weight gain (WG, %) = [($BW_f$ − $BW_i$)/$BW_i$] × 100.
[3]Survival (S, %) = (number of fish in each group remaining on day 56/initial number of fish) × 100.
[4]Feed conversion ratio (FCR) = feed intake (g)/weight gain (g).
[5]Protein efficiency ratio (PER) = weight gain (g)/protein intake (g).
[6]Fulton's condition factor (K) = ($BW_f$/$SL^3$) × 100.
[7]Viscerosomatic index (VSI, %) = (visceral weight (g)/$BW_f$).
[8]Hepatosomatic index (HSI, %) = (liver weight (g)/$BW_f$) × 100.
[9]Intraperitoneal fat index (IPF, %) = (intraperitoneal fat weight (g)/$BW_f$) × 100.

linoleic acid (18:2n-6, LA) and α-linolenic acid (18:3n-3, α-LNA), significantly increased with increasing dietary SBL levels; however, the concentrations of LC-PUFA including ARA, EPA and DHA as well as the n−3/n−6 ratio in the fillet significantly decreased with increasing SBL in diets ($P < 0.05$). Fish fed with SBL-supplemented diets had higher fillet phosphatidylcholine than the control group, whereas the fillet of fish fed the 90 g SBL kg$^{-1}$ diet had the highest phosphatidylethanolamine levels ($P < 0.05$). However, levels of triacylglycerides were almost similar each other among different dietary groups ($P > 0.05$).

### *Hematological and antioxidant parameters*

In the present study, hematological parameters, as well as hematological indices, were not affected by the inclusion of SBL in the basal diet (Table 7, $P > 0.05$). Regarding, non-specific serological parameters, fish fed the control diet had the highest plasma lysozyme activity than other experimental groups (Figure 1(a); $P < 0.05$). However, plasma haemolytic activity (Figure 1(b)) was not affected in different experimental groups ($P > 0.05$). Plasma total Ig (Figure 1(c)) level was higher in fish fed diets supplemented with 60 and 90 g SBL kg$^{-1}$

**Table 5.** Fatty acid (mg g$^{-1}$ total fatty acids) composition of fillet of *S. hasta* juvenile fed experimental diets differing in their content in soybean lecithin (SBL) (mean ± SEM, $n = 3$).

| | SLB (g kg$^{-1}$) | | | | |
|---|---|---|---|---|---|
| Fatty acids | 0 | 30 | 60 | 90 | *P*-value |
| 14:0 | 12.4 ± 0.1$^{c}$ | 18.7 ± 0.3$^{a}$ | 15.9 ± 1.9$^{ab}$ | 14.5 ± 0.8$^{bc}$ | 0.043 |
| 16:0 | 231.6 ± 1.5$^{a}$ | 215.6 ± 0.2$^{c}$ | 224.8 ± 1.7$^{b}$ | 214.9 ± 0.4$^{c}$ | 0.030 |
| 18:0 | 94.6 ± 1.3$^{a}$ | 67.9 ± 0.9$^{c}$ | 81.8 ± 5.6$^{b}$ | 77.9 ± 0.2$^{b}$ | 0.012 |
| 20:0 | 2.4 ± 0.7$^{b}$ | 2.5 ± 0.3$^{b}$ | 6.7 ± 1.8$^{a}$ | 7.4 ± 0.1$^{a}$ | 0.001 |
| 22:0 | 5.5 ± 0.1$^{a}$ | 1.1 ± 0.1$^{c}$ | 1.0 ± 0.1$^{c}$ | 2.6 ± 0.6$^{b}$ | 0.001 |
| 24:0 | 5.6 ± 1.3 | 4.5 ± 1.1 | 4.9 ± 1.1 | 3.5 ± 0.6 | 0.756 |
| SFA | 352.0 ± 2.0$^{a}$ | 310.4 ± 0.8$^{d}$ | 335.2 ± 2.6$^{b}$ | 320.7 ± 1.5$^{c}$ | 0.022 |
| 14:1n-5 | 2.7 ± 0.2 | 1.8 ± 0.4 | 2.0 ± 0.5 | 2.5 ± 0.2 | 0.351 |
| 16:1n-7 | 33.5 ± 0.5$^{a}$ | 34.5 ± 1.5$^{a}$ | 33.4 ± 2.0$^{a}$ | 26.4 ± 1.8$^{b}$ | 0.018 |
| 18:1n-7 | 36.4 ± 1.0$^{a}$ | 25.2 ± 0.8$^{bc}$ | 28.2 ± 1.2$^{b}$ | 22.5 ± 0.7$^{c}$ | 0.027 |
| 18:1n-9 | 228.5 ± 3.7$^{bc}$ | 247.3 ± 1.5$^{a}$ | 234.6 ± 6.5$^{ab}$ | 213.3 ± 7.2$^{c}$ | 0.035 |
| 20:1n-9 | 2.1 ± 0.5 | 2.0 ± 0.0 | 2.5 ± 0.3 | 1.8 ± 0.1 | 0.958 |
| 22:1n-9 | 4.8 ± 0.7$^{a}$ | 4.8 ± 0.0$^{a}$ | 6.0 ± 0.5$^{a}$ | 2.3 ± 0.2$^{b}$ | 0.040 |
| MUFA | 307.8 ± 4.7$^{a}$ | 315.7 ± 1.9$^{a}$ | 307.7 ± 5.6$^{a}$ | 268.8 ± 6.5$^{b}$ | 0.001 |
| 18:2n-6 | 87.4 ± 8.1$^{c}$ | 103.5 ± 5.0$^{b}$ | 117.2 ± 0.4$^{b}$ | 175.3 ± 1.4$^{a}$ | 0.001 |
| 20:2n-6 | 3.0 ± 0.4 | 1.6 ± 0.5 | 2.8 ± 0.9 | 3.3 ± 0.1 | 0.855 |
| 20:4n-6 | 11.0 ± 1.0$^{a}$ | 11.3 ± 0.3$^{a}$ | 9.8 ± 0.6$^{ab}$ | 8.4 ± 0.1$^{b}$ | 0.039 |
| n-6 PUFA | 101.4 ± 8.6$^{c}$ | 116.4 ± 4.8$^{bc}$ | 129.7 ± 0.7$^{b}$ | 187.0 ± 1.4$^{a}$ | 0.001 |
| 18:3n-3 | 9.8 ± 0.7$^{c}$ | 13.8 ± 0.7$^{b}$ | 13.8 ± 1.1$^{b}$ | 18.5 ± 8.5$^{a}$ | 0.001 |
| 20:3n-3 | 2.6 ± 0.1$^{a}$ | 1.9 ± 0.2$^{b}$ | 1.8 ± 0.1$^{b}$ | 1.3 ± 0.1$^{c}$ | 0.045 |
| 20:5n-3 | 41.6 ± 0.6$^{a}$ | 40.5 ± 1.7$^{a}$ | 40.3 ± 0.7$^{a}$ | 34.9 ± 0.9$^{b}$ | 0.020 |
| 22:6n-3 | 165.1 ± 3.6$^{a}$ | 150.4 ± 0.3$^{ab}$ | 142.9 ± 8.2$^{b}$ | 138.0 ± 8.2$^{b}$ | 0.001 |
| n-3 PUFA | 177.5 ± 4.4$^{a}$ | 166.1 ± 1.2$^{ab}$ | 158.4 ± 9.3$^{b}$ | 157.9 ± 8.8$^{b}$ | 0.001 |
| n-3/n-6 | 1.8 ± 0.2$^{a}$ | 1.4 ± 0.1$^{ab}$ | 1.2 ± 0.1$^{b}$ | 0.8 ± 0.1$^{c}$ | 0.001 |
| ARA/EPA | 0.3 ± 0.0 | 0.3 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.900 |
| DHA/EPA | 4.0 ± 0.1 | 3.7 ± 0.2 | 3.6 ± 0.3 | 4.0 ± 0.1 | 1.000 |

Notes: A different superscript in the same row denotes statistically significant differences ($P < .05$). The table provide values of the detected fatty acids by gas chromatography. Abbreviation: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; LA: linoleic acid; ARA: arachidonic acid; LNA: linolenic acid; EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid.

**Table 6.** Lipid classes (mg $g^{-1}$ extracted lipid) of fillet of *S. hasta* juvenile fed experimental diets differing in their content in soybean lecithin (SBL) (mean ± SEM, $n = 3$).

| | SBL (g $kg^{-1}$) | | | | |
|---|---|---|---|---|---|
| Lipid classes (mg g extracted $lipid^{-1}$) | 0 | 30 | 60 | 90 | *P*-value |
| PC | 1.2 ± 0.1[b] | 1.6 ± 0.1[a] | 1.7 ± 0.1[a] | 1.6 ± 0.1[a] | 0.035 |
| PE | 0.3 ± 0.0[c] | 0.4 ± 0.0[c] | 0.8 ± 0.0[b] | 1.1 ± 0.0[a] | 0.026 |
| PL | 1.5 ± 0.1[b] | 2.0 ± 0.5[ab] | 2.5 ± 0.5[a] | 2.7 ± 0.4[a] | 0.032 |
| MAG | 6.2 ± 0.2 | 5.9 ± 0.1 | 6.1 ± 0.3 | 5.7 ± 0.0 | 0.135 |
| CHOL | 1.5 ± 0.1 | 1.4 ± 0.2 | 1.6 ± 0.0 | 1.6 ± 0.1 | 0.914 |
| FFA | 26.3 ± 0.8 | 32.6 ± 3.5 | 32.0 ± 0.3 | 28.4 ± 0.1 | 0.424 |
| TAG | 59.4 ± 0.6 | 53.1 ± 4.6 | 51.1 ± 0.7 | 58.0 ± 0.3 | 0.478 |
| SE + W | 5.0 ± 0.4 | 5.0 ± 1.1 | 6.7 ± 0.9 | 3.6 ± 0.4 | 0.491 |
| NL | 98.5 ± 0.1 | 98.0 ± 0.2 | 97.5 ± 0.5 | 97.3 ± 0.9 | 0.933 |

Notes: PC: phosphatidylcholine; PE: phosphatidylethanolamine; PL: phospholipids; MAG: monoacylglycerol; CHOL: cholesterol; FFA: free fatty acids; TAG: triacylglycerol; SE: sterolsters; W: wax; NL: neutral lipids.

diets than in the other groups ($P < 0.05$). There were not significant differences in liver antioxidant parameters including SOD (Figure 2(a)), CAT (Figure 2(b)) and TAC (Figure 2(c)) among experimental groups ($P > 0.05$).

### *Digestive enzyme activity*

In the present study, fish fed the 90 g SBL $kg^{-1}$ diet had the highest total protease activity in comparison to the other groups (Figure 3(a); $P < 0.05$); however, bile salt-activated lipase activity was not affected by dietary SBL supplementation (Figure 3(b); $P > 0.05$).

## Discussion

Supplementing diets with functional feed additives (e.g. acidifiers, phospholipids, pro-, pre- and synbiotics) not only increases nutrient digestibility but also improves growth performance and general health in farmed aquatic animals (Hussain et al. 2017; Rabia et al. 2017; Wang et al. 2017). Regarding the importance of dietary PLs, several studies have revealed that juvenile fish may also need dietary PLs supplementation for optimal somatic growth performance (Uyan et al. 2007, 2009; Salini et al. 2016) as they have a limited synthesis capacity (Tocher et al. 2008). In the present study, the improvement in somatic growth of fish fed SBL-supplemented diets could be explained by different reasons. For instance, increasing feed intake (FI) without affecting FCR values in fish fed the SBL-supplemented diets in comparison with the control group might have resulted in better growth performance in these groups. In this sense, it has been proved that the trimethyl group of the choline base of phosphatidylcholine, as well as as well as inositol group of the phosphatidylinositol can stimulate the gustatory response of fish (Izquierdo and Koven 2010; La et al. 2018). Similar results were also reported in juveniles of other marine and freshwater fish species such as Japanese flounder (*Paralichthys olivaceus,* Uyan et al. 2007), rainbow trout (*Oncorhynchus mykiss*, Rinchard et al. 2007), amberjack (*Seriola dumerilli*, Uyan et al. 2009), Atlantic salmon (*Salmo salar*, De Santis et al. 2015) and yellowtail *Seriola quinqueradiata* (La et al. 2018). Secondly, dietary lecithin as the major source of phosphatidylcholine can be hydrolysed in the digestive tract to the form of lysophosphatidylcholine, an important precursor of PLs, which may save some energy for their biosynthesis (Tocher et al. 2008), energy that may be derived to other metabolic processes, including somatic growth. In addition, dietary lecithin can increase the digestibility of diets and stimulate the synthesis and secretion of lipoproteins, and the utilization of dietary lipids (Tocher et al. 2008), improving somatic growth (Seiliez et al. 2006). Under current experimental conditions, the increased HSI values in the control group may be due to a higher accumulation of fat stores in the liver, which may be attributed to an insufficient dietary PLs, thus, affecting the normal lipid transportation in the body as also reported in common carp (*Cyprinus carpio* L.) larvae fed PL deficient diets (Fontagné et al. 1998). In addition, the slight increase in HSI level with increasing dietary SBL inclusion might be as a result of lipid accumulation due to the high percentage of linoleic acid in SBL, which promoted lipid accumulation in the liver (Piedecausa et al. 2007).

In the current study, the FA profile and lipid classes of the fillet of fish generally reflected those of experimental diets. The concentrations of OA as well as linoleic acid (18:2n-6, LA) in the fillet from different experimental groups were significantly lower than their levels in respective diets, indicating that these FAs were mainly catabolized for energy purposes, which was in agreement with other studies in different fish species (Bell et al. 2003; Regost et al. 2003; Benedito-Palos et al. 2008; Wassef et al. 2009; Tocher et al. 2010; Mozanzadeh et al. 2015, 2016a). The amount of polyunsaturated fatty acids (PUFA), especially LA and α-linolenic acid (18:3n-3, α-LNA),

**Table 7.** Hematological profile of *S. hasta* juveniles fed experimental diets differing in their content in soybean lecithin (SBL) (mean ± SEM, $n = 3$).

| | SBL (g $kg^{-1}$) | | | | |
|---|---|---|---|---|---|
| Parameter | 0 | 30 | 60 | 90 | *P*-value |
| RBC (×$10^6$ $\mu L^{-1}$) | 2.2 ± 0.1 | 2.3 ± 0.1 | 2.4 ± 0.1 | 2.3 ± 0.1 | 0.200 |
| Hb (g $dL^{-1}$) | 7.3 ± 0.2 | 7.8 ± 0.2 | 7.9 ± 0.2 | 7.4 ± 0.2 | 0.155 |
| Hct (%) | 50.8 ± 1.5 | 54.3 ± 1.7 | 55.2 ± 1.5 | 51.7 ± 1.1 | 0.139 |
| MCV (Fl) | 230.3 ± 2.1 | 231.5 ± 1.5 | 230.0 ± 0.5 | 231.3 ± 0.8 | 0.833 |
| MCH (pg)[a] | 32.8 ± 0.3 | 33.2 ± 0.3 | 32.7 ± 0.4 | 33.3 ± 0.2 | 0.292 |
| MCHC (g $dL^{-1}$)[b] | 14.3 ± 0.2 | 14.0 ± 0.0 | 14.2 ± 0.2 | 14.0 ± 0.0 | 0.269 |
| WBC (×$10^3$ $\mu L^{-1}$)[c] | 4.5 ± 0.2 | 4.9 ± 0.1 | 5.0 ± 0.6 | 5.0 ± 0.2 | 0.570 |
| Neutrophilis (%) | 23.8 ± 1.5 | 24.2 ± 1.6 | 27.2 ± 2.5 | 22.8 ± 0.8 | 0.286 |
| Lymphoctes (%) | 72.2 ± 2.0 | 71.8 ± 1.2 | 68.7 ± 3.2 | 74.5 ± 1.1 | 0.286 |
| Monocytes (%) | 4.0 ± 0.6 | 4.0 ± 0.3 | 4.2 ± 0.7 | 2.7 ± 0.3 | 0.155 |

Notes: A different superscript in the same row denotes statistically significant differences ($P < .05$). Abbreviations: RBC: red blood cell; Hb: haemoglobin; Hct: haematocrit; MCV: mean cell volume; MCH: mean cell haemoglobin; MCHC: mean cell haemoglobin concentration; WBC: white blood cell.

[a]Mean cell volume (MCV) = Hct (%)/RBC × $10^6$ (μL) × 10.

[b]Mean cell haemoglobin (MCH) = Hb (g/dL)/RBC × $10^6$ (μL) × 10.

[c]Mean cell haemoglobin concentration (MCHC) = (g/dL) = Hb (g/dL)/Hct (%).

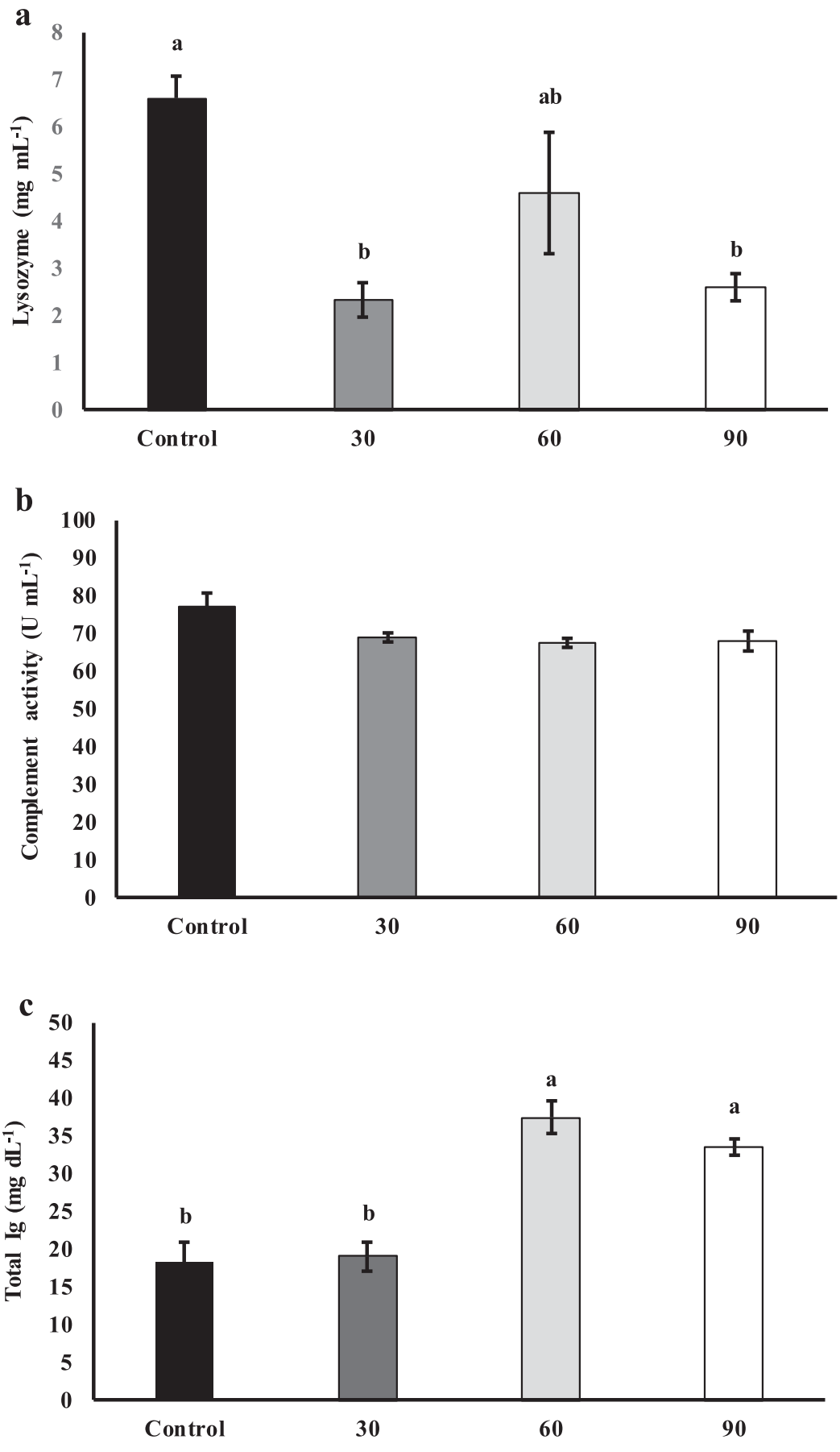


**Figure 1.** Plasma humoral immune parameters including lysozyme level (a) haemolytic activity (b) total Ig (c), and in *S. hasta* fed different experimental diets.

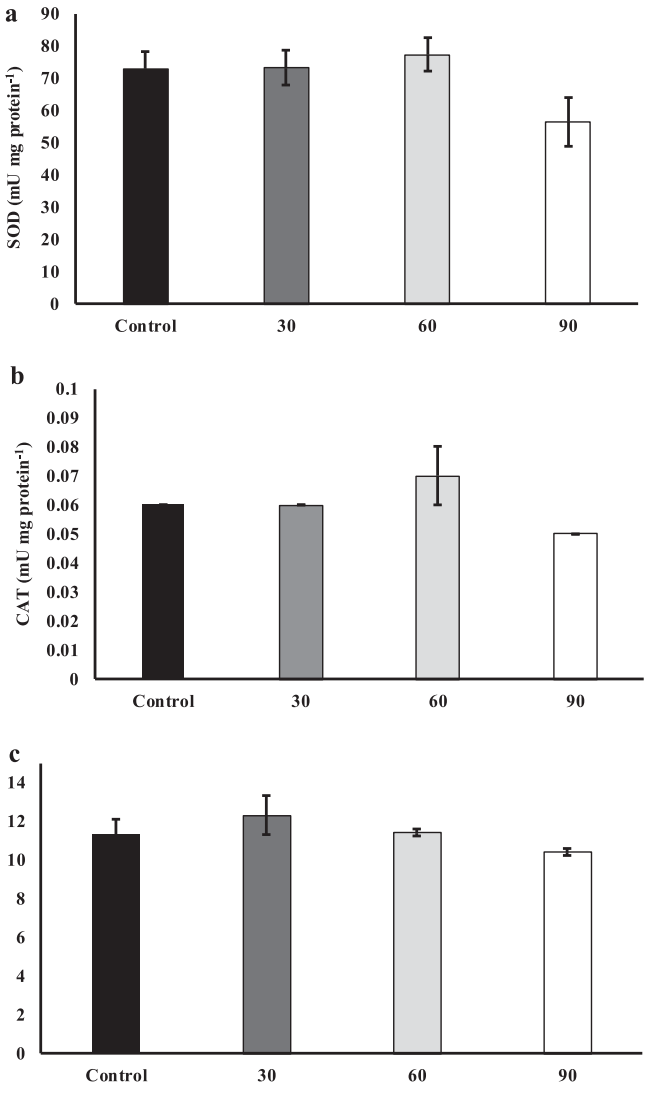


**Figure 2.** Liver SOD (a) and catalase (b) activities and total antioxidant capacity (c) in *S. hasta* fed different experimental diets.

significantly increased with increasing dietary SBL levels, as it has also been reported in other fish species fed diets supplemented with SBL (Benedito-Palos et al. 2008; Alves Martins et al. 2010; Sotoudeh et al. 2011; Azarm et al. 2013; Saleh et al. 2015; Salini et al. 2016). Fish oil is the main source of the LC-PUFA, including arachidonic (20:4n-6, ARA), eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) (Glencross 2009; Turchini et al. 2009). Thus, the replacement of FO with SBL led to a decrease in LC-PUFA including ARA, EPA and DHA, as it has also been reported in different fish species such as gilthead seabream larvae (*Sparus aurata*, Alves Martins et al. 2010) and juveniles at commercial size (Benedito-Palos et al. 2008), Caspian brown trout (*Salmo trutta caspius*, Sotoudeh et al. 2011) and juvenile barramundi (*Lates calcarifer*; Salini et al. 2016). The selective retention of DHA in *S. hasta* tissues has been proved in previous studies (Mozanzadeh et al. 2015, 2016a, 2016b), and it has a similar pattern that was demonstrated in other sparid species (Benedito-Palos et al. 2008; Peng et al. 2008). In this study, the incorporation of gradient levels of SBL at the expense of FO, increased the PL concentration in the diet and modified its lipid classes. In this sense, fish fed with SBL-supplemented diets had higher fillet PC content than those fed other experimental diets. Similar to our results, Teshima et al. (1986) reported that the concentrations of PLs such as PC slightly higher in the shrimp larvae (*Penaeus japonicus*) receiving SBL-PC than in those receiving other PL classes. In contrast to the result of the present study, Geurden et al. (1998) reported higher deposition of neutral lipids in the whole body of turbot post-larvae (*Scophthalmus maximus*) fed with PL- supplemented diet in comparison with fish fed a PL-free one. These differences may be attributed to the differences in lipid metabolism between different developmental stages (juvenile *vs*. post-larvae) (Tocher et al. 2008).

Because of their potent antioxidant capacity due to the side-chain moiety that contains amine/hydroxyl groups (Saito and Ishihara 1997), PLs might maintain the fluidity and stability of the RBC membranes and protect them against oxygen free radicals. Results of present study showed that complete blood count indices were not affected by different diets. In contrast, it has been reported that lecithin (10 g $kg^{-1}$ diet) tended to stimulate erythropoiesis in rainbow trout (*O. mykiss*), which resulted in higher red blood cell, haemoglobin concentration and haematocrit levels than in fish fed non-supplemented lecithin diet (Réhulka and Minarik 2003). These differences between studies may be attributed to differences in fish species, diet formulations and purity of SBL tested in different nutritional studies.

In the current study, the replacement of dietary FO with SBL resulted in decreasing body n-3/n-6 PUFAs ratio in *S. hasta*, which may have influenced fish immune responses. It has been reported that dietary SBL supplementation increased mucosal antibacterial activity in common carp (*C. carpio*, Adel et al. 2017). Moreover, supplementation of dietary PLs increased the stress resistance in different fish species such as *Labeo rohita* fingerlings (Kumar et al. 2012), large yellow croaker (*Larmichthys crocea*, Zhao et al. 2013), milkfish (*Chanos chanos*, Kumar et al. 2014) and stellate sturgeon (*Acipenser stellatus*, Jafari et al. 2018). Our study showed that plasma lysozyme activity values decreased, whereas plasma total Ig levels showed the opposed trend in fish fed SBL-supplemented diets. Similar to our result, Jafari et al. (2018) reported that increasing dietary SBL from 4 to 8 g SBL $kg^{-1}$ significantly increased serum total Ig in juvenile stellate sturgeon (*A. stellatus*) compared to fish fed dies supplemented with 0 and 2 g SBL $kg^{-1}$ and fish fed 10 g SBL $kg^{-1}$ showed intermediate values. The results of our study indicate that more immunological (cellular and mucosal) analyses need to be conducted in order to provide a more precise result of the effects of dietary PL on fish immunity. It is well documented that lecithin acts in synergy with other antioxidants preventing the oxidation of vitamins A, C and E, as well as enhance their utilization (ADM 2003). Using butylated hydroxyl toluene (3 g $kg^{-1}$) as an antioxidant in the experimental diets might be masked the antioxidant properties of SBL in the present study. In contrast, it has been reported that dietary SBL supplementation (1–3%) increased antioxidant enzyme activities (CAT, SOD, glutathione-S-transferase and glutathione peroxidase) in milkfish (Kumar et al. 2014) and common carp (Adel et al. 2017).

Literature regarding the effect of dietary PLs on the activity of digestive enzymes in juvenile fish is scarce. The results of

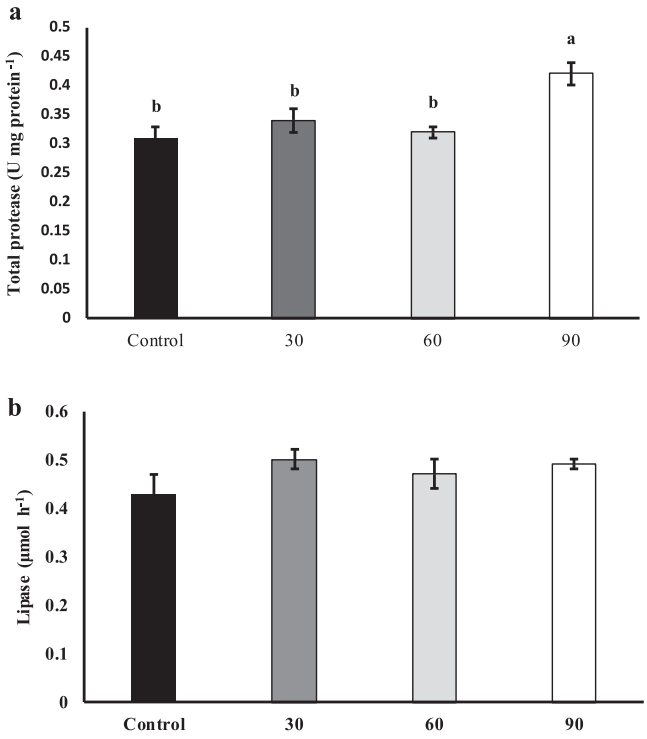


**Figure 3.** Digestive enzyme activities including total protease (a) and lipase (b) in *S. hasta* fed different experimental diets.

this study showed that total protease activity was increased in fish fed the 90 g SLB $kg^{-1}$ diet. Several studies have reported the beneficial effects of dietary PLs on the digestive function in larval stages of different marine (Cahu et al. 2003; Gisbert et al. 2005; Wold et al. 2007; Cai et al. 2016) and freshwater (Hamza et al. 2008) fish species. It has been reported that dietary PLs enhance the secretion of pancreatic digestive enzymes by increasing lysophospholipids, which act as supplementary emulsifiers in the intestinal lumen (Cahu et al. 2003). Moreover, PLs indirectly increase the levels of cholecystokinin that mediated in stimulating the pancreatic secretion (Gisbert et al. 2005; Azarm et al. 2013).

Concluding, the results of this study showed the replacement of dietary FO with SBL can improve somatic growth performance in *S. hasta* juveniles. Regarding to the results of WG and FI, substitution of dietary FO with SBL diet up to 67% (60 g SBL $kg^{-1}$) diet suggested as an optimum level in *S. hasta* juvenile. Moreover, increasing dietary SBL led to a significant decrease in fillet n-3 LC-PUFA (mainly EPA and DHA) and n-3/n-6 ratios in *S. hasta*, whereas increased the concentrations of different phospholipid classes in the fillet. A finishing feeding trial with FO diet is recommended for the restoration of the n-3 LC-PUFA in the fillet of fish fed SBL-supplemented diets.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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