Fish and Shellfish Immunology 91 (2019) 50–57

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

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*)

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ARTICLE INFO

Keywords:
Antioxidants
Caspian brown trout
Digestive enzymes
Hemato-immunological parameters
Pre-spawning stage
Soybean lecithin

ABSTRACT

A feeding experiment was conducted to evaluate the effects of dietary soybean lecithin (SBL) supplementation on performance, hemato-immunological parameters, lipid biochemistry, antioxidant status, digestive enzymes activity and intestinal histomorphometry of Caspian brown trout, *Salmo trutta caspius* in the pre-spawning stage. The basal diet was supplemented with 0% (control), 3%, 6%, 9% and 12% of SBL to obtain five experimental diets. Fish with an average weight of 350 ± 10 g were randomly distributed among five experimental groups and fed for 90 days. Dietary SBL resulted in better performance including specific growth rate (SGR), weight gain (WG) and feed conversion ratio (FCR) ($p < 0.05$). Among the different hemato-immunological parameters, white blood cell counts (WBC), lysozyme, alternative complement activity (ACH50) and total immunoglobulin (IgM) content of serum were significantly increased with dietary SBL inclusion ($p < 0.05$). For antioxidant enzymes, glutathione S-transferase (GST) and catalase (CAT) showed significant differences among various experimental diets ($p < 0.05$). Furthermore, digestive enzymes activity including alkaline protease, lipase and amylase were increased in those fish received SBL supplemented diets ($p < 0.05$). Our results revealed that the dietary SBL improved some physiological responses of the fish and indicate 6–9% dietary SBL supplementation would improve the physiological competence of the pre-spawning Caspian brown trout breeders.

1. Introduction

The beneficial effects of dietary phospholipids (PLs) including various structural (e.g., lipoproteins and digestion of lipids), metabolic (e.g., eicosanoids and inositol) and developmental/physiologic (e.g., embryonic and early larval development) roles have been extensively reviewed [1]. It has been known that aquatic animals (e.g., crustaceans and fish) cannot synthesize PLs at a rate adequate to fulfill requirements and will depend on exogenous PLs to meet the metabolic requirements [2].

The lecithin molecule, composed of choline, phosphate and fatty acids, plays important roles in hepatic lipid and carbohydrate metabolism of fish [3]. Soybean lecithin (SBL) is a natural-sourced, consistent and superior feed ingredient. It is also an excellent source of dietary PLs in aquafeeds. Furthermore, bio-surfactant property of SBL makes it suitable for use as an agent to improve luminal emulsification of dietary lipids [4].

The positive role of PLs in diet has been proved in several freshwater and marine fish species during different life stages such as Atlantic salmon (*Salmo salar*) juvenile by Hung et al. [5]; sea bream (*Sparus aurata*) larvae by Seiliez et al. [6]; Striped Knifejaw (*Oplegnathus fasciatus*) broodfish by Yong et al. [7]; pikeperch (*Sander lucioperca*) larvae by Hamza et al. [8]; Caspian brown trout (*Salmo trutta Caspius*) alevin by Kenari et al. [9]; Channel Catfish (*Ictalurus punctatus*) juvenile by Sink and Lochmann [10] and Common carp (*Cyprinus carpio*) fingerlings by Adel et al. [11].

The nutritional status of fish at various ovarian developmental stages greatly affects gonadal maturation, reproductive performance, and finally progeny quality [12]. Nutrition of fish species broodstock including salmonids, is poorly understood. Furthermore, the Caspian brown trout is an endangered species and currently being considered for conservation program in the southern part of the Caspian Sea [13]. To the best of our knowledge, there is no information regarding the species nutritional requirements, especially during pre-spawning stage

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https://doi.org/10.1016/j.fsi.2019.05.022
Received 9 December 2018; Received in revised form 5 May 2019; Accepted 10 May 2019
Available online 11 May 2019

of the fish. Therefore, the present study was conducted to examine the effects of varying dietary SBL levels on some important physiological parameters of pre-spawning Caspian brown trout.

2. Materials and methods

2.1. Experimental fish

The experiment was conducted at the Cold-Water Fishes Research Center (Tonekabon, Mazandaran province, Iran) for a period of 90 days. Hatchery reared individuals (with an average weight of 350 ± 10 g) were randomly assigned to five groups (30 fish per group) maintained in 1700L concrete tanks. During the experimental feeding period, physicochemical parameters of rearing water were routinely monitored. The natural photoperiod was used and the water quality parameters including pH, DO, temperature, salinity and water flow rate were 7.6–7.9, 8.5 ± 0.5, 12 ± 1.0 °C, < 1 g/l and 3 ± 0.2 l/min, respectively. Experimental diets were fed to fish at 2% of their respective body weight three times a day.

2.2. Experimental diets

Dietary formulation and proximate composition of the basal diet in the present study have been given in Table 1. Experimental diets were prepared via dietary inclusion of various amounts of SBL (Behpak Industrial Company, Behshahr, Mazandaran, Iran) to the basal diet (Table 2). In brief, SBL was added to the basal diet at different levels of 0% (control), 3%, 6%, 9% and 12%. According to NRC [14], present commercial diets for salmonids could contain 25–30% lipid, while the basal diet used in the present study only contained 16% lipid. Therefore, maximum soybean oil and/or SBL of 12% were included to the basal diet which yielded maximum of 28% dietary lipid level well within the recommended dietary lipid requirements for salmonids. One should notice that soybean oil were added to the experimental diets at the expense of SBL just to keep energy contents and fatty acid profiles of the diets constant since they both have similar fatty acid profiles (Table 3). Basal diet ingredients were weighed and after a thorough mixing distilled water were added and mixed to yield the desired consistency. Finally, dietary lipid sources (fish oil, soybean oil or SBL) were added to the dough and pelleted (strands with 6 mm of diameter). The strands were dried in a convection oven at 40 °C to the constant weight and stored refrigerated at 4 °C.

Table 1
Dietary formulation and proximate composition of the basal diet (%).

Ingredients	(%)
Fish meal [a]	37
Soybean protein concentrate	11
Wheat flour	10
Meat meal	8
Corn gluten	8
Fish oil [a]	9
Cellulose	5
Yeast, brewers dried	4
Soybean meal [b]	4
Vitamin premix [c]	2
Mineral premix [d]	2
Proximate composition (%)	
Moisture content	9.31
Crude protein	43.45
Crude lipid	16
Crude fiber	2.5
Ash	17.7
Gross energy (MJ kg^{-1})[e]	18.26

[a] Pars-Kilka Mazandaran, Iran.
[b] Behpak Industrial Company, Behshahr, Mazandaran, Iran.
[c] Composition of vitamin premix (IU, g or mg/kg): A (3600000 IU), D_3 (8000000 IU), E (14.4 g), K_3 (800 mg), B_1 (7 g), B_2 (2.64 g), Niacin (11.8 g), Calcium pantothenate (3.92 g), B_6 (1.17 g), B_9 (0.4 g), Biotin (40 mg), Choline chloride (100000 mg). Aras Bazar Pharmaceutical Company, Mazandaran, Iran.
[d] Composition of mineral premix (g/kg): Mn (39.68 g), Fe (20 g), Zn (33.88 g), Co (4 g), I (0.39 g), Se (0.08 g), Choline chloride (100 g). Aras Bazar Pharmaceutical Company, Mazandaran, Iran.
[e] Gross energy (MJ kg^{-1}) calculated according to 23.6 kJ g^{-1} for protein, 39.5 kJ g^{-1} for lipid and 17.0 kJ g^{-1} for NFE [14].

Table 2
Different experimental diets used in the present study.

Treatment	Diets specification
1	basal diet +12% soy oil
2	basal diet +9% soy oil +3% lecithin
3	basal diet +6% soy oil +6% lecithin
4	basal diet +3% soy oil +9% lecithin
5	basal diet +12% lecithin

Table 3
Fatty acid profiles of basal diet, soybean oil and SBL (% of total fatty acids).

Fatty acid	Basal diet	Soybean oil	SBL
C14:0	1.51	0.11	0.12
C16:0	16.04	11.86	21.63
C18:0	6.85	4.75	-
C20:0	0.25	0.23	0.20
C22:0	0.46	0.32	-
C24:0	0.09	0.05	-
Total Saturates	25.20	17.32	21.95
C14:1n-5	0.13	0.02	0.05
C16:1n-7	3.11	0.14	0.03
C18:1n-9	24.45	25.6	16.15
C18:1n-7	3.89	0.84	4.13
C20:1n-9	0.29	0.20	0.11
C22:1n-9	0.06	0.15	-
C24:1n-9	0.46	-	-
Total Monoens	32.39	26.95	20.47
C18:2n-6	10.91	48.25	48.85
C20:2n-6	0.25	-	-
C20:4n-6	0.67	-	-
Total n-6 PUFA	11.83	48.25	48.85
C18:3n-3	2.51	5.35	4.78
C20:3n-3	0.08	-	-
C20:5n-3	5.10	-	-
C22:6n-3	10.18	-	-
Total n-3 PUFA	17.87	5.35	4.78

2.3. Fish performance study

At the end of the experiment, fish were fasted for 24 h to determine their performance. The following equations were used to calculate fish growth performance [15]: weight gain (WG, %) = $(W_f - W_i)/W_i) \times 100$; feed conversion ratio (FCR) = dry feed intake (g)/weight gain (g) and specific growth rate (SGR) g day^{-1} = 100 (Ln W_f - Ln W_i)/t. W_f is final body weight (g), W_i is initial body weight (g) and t is experimental duration (days).

2.4. Sample preparation

At the end of experiment, six fish from each treatment were anesthetized with 200 mg/ml clove powder. Blood was collected from the

caudal vein using 5 ml heparinized (for hematology) and non-heparinized (for biochemical analyses) syringes. Sera were separated by centrifugation (Heraeus Labofuge 400) at 1600 g for 10 min and stored at −20 °C for further analysis. Then, the all six fish were humanly sacrificed by a sharp blow to the head and internal organs were dissected out on ice. Samples were placed in individually marked plastic test tubes and stored at −80 °C for later determination of antioxidant status (liver), digestive enzymes activity (pyloric caeca) and histological analyses (intestine) according to the methods described below.

2.5. Hematological and biochemical analysis

The red blood cell (RBC) and white blood cell (WBC) counts were determined using a Neubaeur hemocytometer [16]. Hematocrit percentage (Ht) was measured using the standard microhematocrit method. Hemoglobin (Hb) levels were obtained using the cyanomethemoglobin spectrophotometry method [17]. Monocyte, lymphocyte and neutrophil were determined using the Giemsa staining method and blood smears were detected under a light microscope [18]. Mean corpuscular volume (MCV), mean corpuscular Hb (MCH), and MCH concentration (MCHC) were determined as described by Seiverd [19].

Lipid biochemical parameters including very-low-density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride (TG) and cholesterol (CHO) were estimated using commercial kits (Pars Azmoon, Tehran, Iran) by means of a biochemical auto analyzer instrument (Labsystemphotic100, Japan).

2.6. Immunological analysis

Serum lysozyme activity was determined turbidimetrically using a slightly modified method described by Ellis [20]. Total immunoglobulin (IgM) levels were determined according to the method described by Siwicki and Anderson [21] and finally alternative complement activity (ACH50) was determined according to the method described by Sunyer and Tort [22] and based on the haemolysis of rabbit RBCs.

2.7. Antioxidants analysis

Prior to enzymes assay, liver tissues were cut into small pieces and homogenized in phosphate buffer (0.050 M, pH 7.4) to obtain a 10% (w/v) liver homogenate. The supernatant was obtained via centrifuging the homogenates at 10000 g for 20 min at 4 °C and kept frozen at −80 °C until analysis. Superoxide dismutase (SOD) activity (Unit/mg tissue protein), was determined as described by McCord and Fridovich [23]. In brief, aliquots of 100 μl of tissue supernatant were added to 2 ml of reaction mixture and incubated for 20 min at 37 °C. Then, the absorbance was measured with a spectrophotometer (at 560 nm). Catalase (CAT) activity (Unit/mg tissue protein) was determined as described by Aebi [24]. In brief, 100 μl of tissue supernatant was incubated for 1 min with 1 ml reaction mixture. The reaction was terminated by adding 0.5 ml of ammonium molybdate solution. Then, the absorbance was measured at 405 nm. Glutathione s-transferase (GST) activity (Unit/mg tissue protein) was determined According to Keen et al. [25]. In brief, 100 μl of tissue supernatant were incubated with 1 ml of the reaction mixture for 5 min at 25 °C. Finally, the absorbance was measured at 340 nm.

2.8. Digestive enzyme analysis

Pyloric caeca tissues were homogenized in 1:3 (w/v) cold 50 mM tris-HCl buffer, pH = 7.5. The supernatant was obtained centrifuging homogenates at 10000 g for 20 min at 4 °C and kept frozen at −80 °C until analysis. Alkaline protease activity was determined as described by Garcia-Carreno and Haard [26] using Azocasein 2% in Tris-HCl, pH = 7.5 as substrate. The lipase specific activity was determined as described by Iijima et al. [27] using nitrophenyl myristate as substrate. α-Amylase activity was determined according to Bernfeld [28] using starch as substrate. The activity of all enzymes was expressed as specific activity being micromole of substrate hydrolyzed per minute per milligram protein (U/mg).

2.9. Intestinal histomorphometry

For this purpose, middle intestine tissue samples were saline (0.6%) washed and fixed in 10% buffered formalin for 24 h. After dehydration in graded ethanol concentrations (50–99.9%), the samples were cleared in xylene and embedded in paraffin wax. Histological sections were stained with haematoxylin and eosin (H & E) and documented photographically with a digital camera. Finally, the images were analyzed using LC software for villus height and muscular layer thickness measures [29].

2.10. Data analysis

Standard normality test of Kolmogorov-Smirnov was applied to determine normality of dataset. After ensuring homogeneity of variances using Levene's test, one-way analysis of variance (ANOVA) and Tukey's HSD test were used to identify significant differences. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp, Armonk, NY, USA) at the significance level of $p < 0.05$. Results were reported as Mean ± SE.

3. Results

3.1. Growth performance

At the end of the experiment, final weight was significantly higher in those fish fed diet supplemented with 9% SBL in comparison to other experimental groups ($P < 0.05$). The highest FCR was observed in fish fed on 12% SBL, which was significantly higher ($P < 0.05$) than the fish fed on other experimental diets. Similarly, SGR and WG of fish fed

Table 4
Growth performance of pre-spawning Caspian brown trout fed diets containing varying levels of soybean lecithin (n = 3).

	Lecithin (%) in diets				
	0	3	6	9	12
Performance					
W_i	331.7 ± 11.7^{a}	327.7 ± 11.1^{a}	348.7 ± 14.5^{a}	341.7 ± 13^{a}	320 ± 5.77^{a}
W_f	616.7 ± 20.5^{b}	713.3 ± 17.6^{a}	756.7 ± 13^{a}	766.7 ± 28.5^{a}	453.3 ± 16.9^{c}
SGR	0.579 ± 0.03^{b}	0.727 ± 0.02^{ab}	0.725 ± 0.02^{ab}	0.766 ± 0.02^{a}	0.324 ± 0.05^{c}
FCR	1.79 ± 0.09^{b}	1.44 ± 0.04^{b}	1.44 ± 0.04^{b}	1.39 ± 0.03^{b}	3.3 ± 0.5^{a}
WG	86.18 ± 6.41^{b}	117.98 ± 6.04^{a}	117.45 ± 5.25^{a}	124.54 ± 5.6^{a}	41.94 ± 7.68^{c}

Data are presented as mean ± SE. Means in each row with different superscripts show significant difference ($P < 0.05$).
Wi (g), initial body weight; W_f (g), final weight; SGR (%), specific growth rate; FCR, feed conversion ratio; WG (%), weight gain.

Table 5
Hematological parameters of Caspian brown trout fed diets containing varying levels of soy lecithin in the pre-spawning stage (n = 6).

	Lecithin (%) in diets				
	0	3	6	9	12
Parameters					
WBC	6533.3 ± 665.8[a]	9800 ± 916.5[b]	9233.3 ± 1537.3[b]	8966.6 ± 802.08[ab]	7966.6 ± 723.4[ab]
RBC	1733333 ± 76376	1640000 ± 87177	153666 ± 40414	168333 ± 68.68.5	1596666 ± 196044
HB	13.1 ± 0.51	12.6 ± 0.64	11.7 ± 0.25	12.87 ± 0.47	12.36 ± 1.15
HCT	57 ± 2	54.33 ± 3.21	50.66 ± 1.52	55.33 ± 1.52	53 ± 5
MCV	328.66 ± 3.8	331 ± 2	329.33 ± 3.51	328.33 ± 4.61	332.67 ± 10.69
MCH	75.33 ± 0.57	75.33 ± 0.58	76 ± 1	76.33 ± 0.57	78.33 ± 2.08
MCHC	22.9 ± 0.2	23.1 ± 0.17	23.16 ± 0.3	23.26 ± 0.2	23.33 ± 0.05
NEUT	12.66 ± 1.15	15.66 ± 0.57	13 ± 2.64	11.66 ± 1.52	12.33 ± 0.57
LYM	81.33 ± 1.15	78.66 ± 1.52	82.66 ± 4.16	84.33 ± 1.52	82.33 ± 1.15
MO	5.66 ± 0.57	4.66 ± 0.57	4 ± 1.73	3.66 ± 0.57	4.66 ± 0.57

Data are presented as mean ± SE. Means in each row with different superscripts show significant difference ($P < 0.05$).
WBC (cell/mL), white blood cell; RBC (cell/mL), red blood cell; Hb (g/dL), hemoglobin; Ht (%), hematocrit; MCV (fL), Mean corpuscular volume; MCH (pg), mean corpuscular Hb; MCHC (g/dL), MCH concentration; NEUT (%), neutrophils; LYM (%), lymphocytes; MO (%) monocytes.

diet containing 9% SBL were significantly higher ($P < 0.05$) than other experimental groups (Table 4).

3.2. Hematological and lipid biochemical analysis

At the end of the experiment, the WBC count was significantly higher in fish fed 3%–9% SBL compared to control group received basal diet without SBL supplementation. No significant differences ($P > 0.05$) were observed in other hematological indices among experimental groups (Table 5). Except for HDL and CHO, there were also no significant differences ($P > 0.05$) in VLDL, LDL and TG values among various experimental groups (Table 6).

3.3. Immunological analysis

Serum lysozyme activity of fish fed 12% SBL supplemented diet significantly differed from that of fish fed 3% SBL supplemented diet ($P < 0.05$). The ACH50 of serum was significantly ($P < 0.05$) higher in those fish fed 9% and 12% SBL supplemented diet compared to those fish received basal diet supplemented only with 12% soybean oil and the basal diet supplemented with 3% SBL. Serum IgM content was slightly higher in fish fed 6% and 9% SBL supplemented diet in comparison to other experimental groups (Table 7).

3.4. Antioxidant enzymes

The lowest CAT activity was observed in fish fed 9% SBL which significantly differed from control group received basal diet only supplemented with soybean oil ($P < 0.05$). GST activity of those fish fed control diet and also those fish received 9% SBL supplemented diet were significantly different in comparison to other experimental groups ($P < 0.05$). No significant difference ($P > 0.05$) were observed in SOD activity among various experimental groups (Table 8).

3.5. Digestive enzymes

Alkaline protease and lipase activities increased in concomitant with increasing dietary SBL supplementation level. For amylase, there was a significant difference ($P < 0.05$) between those fish fed control diet and those received 12% SBL supplemented diet, however, no significant differences ($P > 0.05$) were observed among fish fed on other experimental diets in this regard (Table 9).

3.6. Intestinal histomorphometry

There were significant differences among those fish received control diet and other experimental groups regarding epithelia thickness ($P < 0.05$). Statistically no significant changes ($P > 0.05$) were observed among various experimental groups in terms of villus height, villus diameter, inner muscle and outer muscle (Table 10).

4. Discussion

In the present study, the increase in dietary SBL levels up to 9% led to a significant increase in Caspian brown trout final weight and SGR. Similar results were found in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon, where the authors observe difference in growth between the fish fed on diet containing SBL and the fish fed on other PLs sources [30,31]. In another study by De Santis et al. [32] improved performance along with reducing intestinal tissue steatosis in Atlantic salmon fed on dietary SBL were observed. It has been hypothesized that intact PLs were required for lipoproteins formation, such as chylomicrons, involved in packing and transport of dietary lipid [33]. Thus, beneficial PLs effects could be related to an increased transport and

Table 6
Lipid biochemical parameters (mg/dL) of Caspian brown trout spawning fed diets containing varying levels of soy lecithin in the pre-spawning stage (n = 6).

	Lecithin (%) in diets				
	0	3	6	9	12
Parameters					
VLDL	42.66 ± 6.42	46 ± 9.84	36 ± 2.64	46.66 ± 4.16	46.33 ± 1.52
LDL	118 ± 22.53	74 ± 10.14	85 ± 13	118.66 ± 27.06	116 ± 20.51
HDL	186.66 ± 5.68[a]	183.33 ± 2.51[a]	194 ± 7.54[ab]	219.66 ± 13.79[c]	218.66 ± 11.93[bc]
TG	214.67 ± 32.71	230.33 ± 49.36	179.33 ± 10.21	232.66 ± 22.67	229 ± 7
CHO	394.66 ± 20.2[b]	318 ± 26[a]	349.66 ± 43.4[ab]	408.7 ± 17.15[b]	411 ± 16.52[b]

Data are presented as mean ± SE. Means in each row with different superscripts show significant difference ($P < 0.05$).
VLDL, very-low-density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglyceride; CHO, cholesterol.

Table 7
Innate immune parameters of Caspian brown trout fed diets containing varying levels of soy lecithin in the pre-spawning stage (n = 6).

	Lecithin (%) in diets				
	0	3	6	9	12
Parameters					
LYZ	51 ± 5.29^{ab}	46.33 ± 10.11^{a}	74 ± 15.71^{ab}	67.66 ± 8.08^{ab}	79.66 ± 15.14^{b}
IgM	29 ± 0.7^{ab}	23.23 ± 1.55^{a}	31.83 ± 4.66^{b}	31.36 ± 2.51^{b}	29 ± 1.15^{ab}
ACH50	133.6 ± 9.6^{a}	135.3 ± 7.63^{a}	158 ± 15.13^{ab}	169.7 ± 12.01^{b}	168.3 ± 9.45^{b}

Data are presented as mean ± SE. Means in each row with different superscripts show significant difference ($P < 0.05$).
LYZ (μg/mL), lysozyme; IgM (mg/mL), total immunoglobulin; ACH50 (U/mL), alternative complement activity.

Table 8
Antioxidant status of Caspian brown trout fed diets containing varying levels of soy lecithin in the pre-spawning stage (n = 6).

	Lecithin (%) in diets				
	0	3	6	9	12
Parameters					
SOD	129.66 ± 12.5	138 ± 16.37	134.66 ± 11.06	114 ± 9.53	136.66 ± 3.78
CAT	32.33 ± 11.01^{b}	27.66 ± 9.86^{ab}	25.33 ± 3.51^{ab}	16.66 ± 1.52^{a}	20 ± 1^{ab}
GST	13.86 ± 0.4^{a}	17.16 ± 0.76^{c}	16.73 ± 1.01^{c}	14.73 ± 0.86^{ab}	16.16 ± 0.35^{bc}

Data are presented as mean ± SE. Means in each row with different superscripts show significant difference ($P < 0.05$).
SOD (unit/mg tissue protein), superoxide dismutase; CAT (unit/mg tissue protein), catalase; GST (unit/mg tissue protein), glutathione s-transferase activity.

lipid mobilization from intestine through circulation to other tissues or organs. Such phenomena result in enhanced lipid deposition and increased energy availability for growth [33,34]. Furthermore, lecithin has an interactive role in the intestinal absorption of cholesterol, which helps improve the growth and survival of animal [35]. In addition, dietary SBL reportedly improves growth performance of various life stages of finfish species [1]. For instance, increased growth performance has been reported in Atlantic salmon fry fed a diet containing 4% or 6% SBL/oil mixture [36], in rainbow trout fed a diet containing 4% SBL/oil mixture [37] and also in common carp fed a diet containing 1–5% SBL [11]. These studies suggested that an improved growth by dietary PLs might be as a result of increased feed intake and better efficiency in feed utilization. However, in the present study all experimental groups received a fixed ration and such inference might not be reasonable. Meanwhile, according to digestive enzymes activity and feed conversion ratio of various experimental groups, improved feed digestion and utilization might make sense. In addition, providing various PLs via hydrolyzing SBL constituents in digestive tract of fish may provide the animal with required PLs and thereby sparing energy indispensable for *de novo* synthesis of PLs for somatic growth [14]. It should also be added that energy and essential fatty acids with PLs origin are better assimilated in comparison to triacylglycerols [35].

Blood parametric tests (hematology and immunology) have been adopted in aquaculture as important markers of fish health status. Previous studies revealed dietary SBL inclusion helps to improve fish immune response [38]. Our results indicated that inclusion of SBL improved immunity of fish via changes made in WBC, IgM, ACH50 and lysozyme activity. Similar results were reported for common carp [11] and stellate sturgeon, *Acipenser stellatus* [39] fed diets containing SBL. In contrast, the innate immune parameters of channel catfish were not altered by dietary inclusion of SBL [10]. Moreover, up and down-regulation of inflammatory cytokines may affect innate immune responsiveness of animals [40]. For instance, improved immune response of fish fed on different PL levels might be attributable to down-regulation and/or up-regulation of inflammatory and anti-inflammatory cytokines such TNF-α and TGF-β1, respectively [41,42]. Since there were no differences in dietary fatty acid profiles of various experimental feeds in the present study, it might be reasonable to postulate that differences in immune indices of fish amongst various experimental groups might be associated with lipid class (i.e., dietary PL content) but not with dietary fatty acid composition. In addition, better availability of essential fatty acids from PLs in comparison to neutral lipids (i.e., triacylglycerols) [35] might result in modified cell membrane fatty acid profile of the experimental fish, which has been reportedly shown that it would improve fish immune performance via stimulating eicosanoids production [39]. However clarification of such speculations requires further research at least in Caspian brown trout.

In the present study, with the exception of HDL and CHO, there were no differences in lipid biochemistry of bloodstream. Our results are in agreement with results reported by Sink and Lochmann [10] on channel catfish. This might be resulted from fasting fish prior to blood collection, a common procedure in fish nutrition studies for sampling, as lipid absorption and transport from the digestive system were interrupted [10]. CHO, the predominant sterol in animals [43], is transported via HDL [44] and LDL [45]. The latter molecules play a pivotal role in TG clearance and CHO removal from animal tissues [44]. According to Niu et al. [46], plasma CHO was elevated and plasma triglycerides were decreased in non-fasted cobia, *Rachycentron canadum*,

Table 9
Digestive enzyme activities (U/mg protein) of Caspian brown trout fed diets containing varying levels of soy lecithin in the pre-spawning stage (n = 6).

	Lecithin (%) in diets				
	0	3	6	9	12
Parameters					
Protease	24.8 ± 0.36^{a}	35.13 ± 3.5^{ab}	43.33 ± 5.9^{bc}	48.46 ± 2.32^{c}	52.83 ± 2.96^{c}
Amylase	71.33 ± 6.5^{a}	116 ± 26.5^{ab}	136.33 ± 55.01^{ab}	135.66 ± 11.33^{ab}	151.33 ± 6.5^{b}
Lipase	181.66 ± 11.37^{a}	248 ± 33.88^{ab}	318 ± 28.84^{bc}	347.66 ± 18.82^{c}	386.66 ± 45.09^{c}

Data are presented as mean ± SE. Means in each row with different superscripts show significant difference ($P < 0.05$).

Table 10
Intestinal histomorphometry (μm) of Caspian brown trout fed diets containing varying levels of soy lecithin in the pre-spawning stage (n = 6).

	Lecithin (%) in diets				
	0	3	6	9	12
Parameters					
Villus height	2594. ± 582	2917 ± 199.1	2840 ± 360.1	2830 ± 471.01	2488 ± 338.2
Villus diameter	138.5 ± 52.5	132.5 ± 64.5	169.70 ± 83.1	131.52 ± 93	97.58 ± 69.1
Epithelium	1128.6 ± 106.4^{c}	936 ± 189.9^{b}	526.68 ± 331.36^{a}	919.6 ± 144.79^{b}	916.6 ± 1761^{b}
Inner muscle	2675 ± 405.4	2257 ± 542.5	2658 ± 319.2	1421 ± 285.2	1772 ± 202.7
Outer muscle	2023 ± 810.8	2140 ± 848.7	2610 ± 725.3	1605 ± 100.3	1872 ± 438.2

Data are presented as mean ± SE. Means in each row with different superscripts show significant difference ($P < 0.05$).

fed diets supplemented with up to 8% PL. In another research, Taggart et al. [47] demonstrated that genes of hepatic CHO biosynthesis pathway were up-regulated in Atlantic salmon fed a vegetable oil blend compared to fish fed a diet containing fish oil. Possibly serum CHO might be increased in those fish fed SBL-supplemented diets due to higher hepatic synthesis of CHO [39] or improved dietary lipids transport including CHO by dietary SBL supplementation [14,35].

An imbalance between the production of reactive oxygen species (ROS) and the antioxidant enzymes (e.g. SOD, catalase, and GST) of an organism causes oxidative stress, leading to tissue damage [48]. SOD catalysis dismutation of superoxide radicals to hydrogen peroxide and oxygen, while catalase catalysis breakdown of hydrogen peroxide to water and molecular oxygen and GST decomposes peroxides [49]. Their activities are commonly used as indicators to evaluate antioxidant defense system of fish [50]. Some antioxidants contributing to fish antioxidant capacity may originate from feed; especially those cannot be synthesized by animals *de novo* [51]. In the present study, the antioxidant enzymes activity (CAT and GST) were affected by dietary SBL supplementation. Our results were in good accordance with Adel et al. [11] reported that dietary SBL supplementation significantly increased CAT, GST and SOD activities of common carp and Cai et al. [52] found that antioxidant activities were significantly increased following dietary inclusion of PLs in large yellow croaker (*Larimichthys crocea*). The plausible mechanisms involved are not understood yet. However, higher dietary PLs contents may result in lipid peroxidation and provide an explanation for the higher activities of such antioxidant enzymes [53]. In addition, the antioxidant capacity improving effect of dietary SBL might result in increased cellular competence against oxidative stress with subsequent decreased lipid peroxidation and therefore higher cellular viability.

Digestive enzymes are responsible for nutrient digestion and availability [54] so that evaluation of such enzymes activity might provide reliable information about precocious fish nutritional status and performance evaluation [55]. Digestive enzyme activities following feeding on diets containing PL during different life stages of fish species have been reported [56–58]. Lipase plays an important role in breaking down dietary fats, especially triacylglycerols [52]. In the present study, the specific activities of lipase in SBL supplemented groups were significantly higher compared to the control fish, which might contribute to better lipid digestion and absorption. In agreement with the present study, the lipase activity in Caspian brown trout alevins was influenced by diet containing phosphatidylcholine (PtCho) [9]. Amylase obviously involves in dietary carbohydrates disintegration and its activity depends on the nature of the diet in fish species [59]. In the present study, the amylase activity also showed an increasing trend in fish fed on diet containing SBL in comparison to the control. Similar to our results, Adel et al. [11] found higher amylase activity in common carp fed different dietary SBL levels. Protein digestion occurs mainly by the action of alkaline proteases such as trypsin and chymotrypsin in combination with intestinal cytosolic peptidases [60]. In the present study, protease activity was elevated with increasing dietary SBL levels. Similar findings were also reported in tilapia, *Oreochromis niloticus* [61] and pejerrey, *Odontesthes bonariensis* [62] fed on diets containing soybean meal (SBM) and oil, respectively. In addition, increased activity of these three enzymes during pre-spawning stage of salmon confirmed their important digestive role in relation to diet supplementations. Meanwhile, to draw a thorough conclusion regarding the effect of dietary SBL supplementation on fish reproductive competence further research is required since there is paucity of information regarding the effect of dietary lecithin supplementation on fish digestive enzyme synthesis/secretion therefore activity in literature [56].

Histological observations along with other physiological parameters are valuable tools to provide further insights on tissue alterations caused by various dietary supplements [63–65]. In the present study, we investigated the intestinal histomorphometry of fish fed on diet with various SBL supplementation level. Based on the results, epithelial thickness of fish fed on diet containing SBL significantly differed from that of those fish fed basal diet containing soybean oil instead of SBL supplement. The intestinal morphology such as villus height or epithelial thickness is indicative of the gut health in animals including fish [66]. Intestinal epithelium is an important environment-organism interface with various physiological functions include nutrient transport, osmoregulation and protection from environmental stressors, such as toxicants [67]. An important function of intestinal epithelial layer is the expression of Na^+/K^+-ATPase (NKA), which plays a key role for several physiological processes including nutrient uptake [68,69]. In a study Iwashita et al. [29] found that soybean lecithin has similar effects on normalizing the hepatic and intestinal morphologies and effectively prevents morphological abnormalities in the distal intestine of rainbow trout. Furthermore, Yamamoto et al. [70] reported that supplementation of crude SBL to SBM-based diets prevents morphological abnormalities in rainbow trout. In another study, dietary protein and lipid sources did not affect intestinal morphology of meagre, *Argyrosomus regius* [55]. In addition, the histomorphological changes in the intestine of pre-spawning salmon reported in the present study can be provide new information regarding the potential for using the diets containing vegetable oil as alternative for broodstocks management in this endangered species. Meanwhile the possibly and also the extent of such dietary changes especially on reproductive competence of the species pends clarification in future studies.

5. Conclusion

The present study revealed that the growth performance of pre-spawning Caspian brown trout could be positively affected by dietary SBL supplementation. Furthermore, dietary SBL also affected serum immune indices, digestive enzymes activity and hepatic antioxidative status of Caspian brown trout. However, we did not see significant changes in intestinal histomorphometry (except for epithelium) among various treatments. In conclusion, higher (6–9%) inclusion levels of SBL, depending on the parameter of interest, showed promising results. Therefore, it is conceivable that dietary SBL inclusion would improve the physiological competence of the pre-spawning breeders.

Acknowledgements

The authors are grateful to Cold-Water Fishes Research Center (Tonekabon, Mazandaran province, Iran) employees for their assistance in fish husbandry. Financial support of Urmia University Research Council is also greatly acknowledged.

References

[1] D.R. Tocher, E.A. Bendiksen, P.J. Campbell, J.G. Bell, The role of phospholipids in nutrition and metabolism of teleost fish, Aquaculture 280 (2008) 21–34.
[2] S. Fontagné, L. Burtaire, G. Corraze, P. Bergot, Effects of dietary medium-chain triacylglycerols (tricaprylin and tricaproin) and phospholipid supply on survival, growth and lipid metabolism in common carp (*Cyprinus carpio* L.) larvae, Aquaculture 190 (2000) 289–303.
[3] J.R. Colares, E. Schemitt, R.M. Hartmann, R.M. Mouram, M.I. Morgan-Martins, H.S. Fillmann, Effect of lecithin on oxidative stress in an experimental model of rats colitis induced by acetic acid, J. Coloproctol. 36 (2016) 97–103 2016.
[4] J.W. Hertrampe, F. Piedad-Pascual, Handbook on Ingredients for Aquaculture Feeds, Kluwer Academic Publishers, Dordrencht, The Netherlands, 2000, p. 573.
[5] S.S.O. Hung, G.M. Berge, T. Storebakken, Growth and digestibility of soya lecithin and choline chloride in juvenile Atlantic salmon, Aquacult. Nutr. 3 (1997) 141–144.
[6] I. Seiliez, J.S. Bruant, J. Zambonino-Infante, S. Kaushik, P. Bergot, Effect of dietary phospholipid level on the development of gilthead sea bream (*Sparus aurata*) larvae fed a compound diet, Aquacult. Nutr. 12 (2006) 372–378.
[7] S.K. Yong, A. Seoka, M.O. Takaoka, S.C. Ji, A.K. Biswas, K. Takii, H. Kumai, Effect of dietary docosahexaenoic acid and soybean lecithin on spawning performance, egg and broodfish fatty acid and lipid class of striped knifejaw, *Oplegnathus fasciatus*. Aquaculture Sci. 55 (2007) 449–458.
[8] N. Hamza, M. Mhetli, I.B. Khemis, C. Cahu, P. Kestemont, Effect of dietary phospholipids levels on performance, enzyme activities and fatty acid composition of pikeperch (*Sander lucioperca*) larvae, Aquaculture 275 (2008) 274–282.
[9] A.A. Kenari, E. Sotoudeh, M.H. Rezaei, Dietary soybean phosphatidylcholine affects growth performance and lipolytic enzyme activity in Caspian brown trout (*Salmo trutta Caspius*) alevin, Aquacult. Res. 42 (2011) 655–663.
[10] T.D. Sink, R.T. Lochmann, The effects of soybean secithin supplementation to a practical diet formulation on juvenile channel catfish, *Ictalurus punctatus*: growth, survival, hematology, innate immune activity, and lipid biochemistry, J. World Aquac. Soc. 45 (2014) 163–172.
[11] M. Adel, M. Gholaghaie, P. Khanjany, T. Citarasu, Effect of dietary soybean lecithin on growth parameters, digestive enzyme activity, antioxidative status and mucosal immune responses of common carp (*Cyprinus carpio*), Aquacult. Nutr. 23 (2017) 1145–1152.
[12] P. Lavens, P. Sorgeloos, Experiences on importance of diet for shrimp postlarval quality, Aquaculture 191 (2000) 169–176.
[13] B.H. Kiabi, A. Abdoli, M. Naderi, Status of fish fauna in the south Caspian basin of Iran, Zool. Middle East 18 (1999) 57–65.
[14] NRC. National Research Council, Nutrient Requirements of Fish and Shrimp, National Academy Press, Washington, DC, USA, 2011, p. 376.
[15] M. Farhangi, C.G. Carter, Growth physiological and immunological responses of rainbow trout (*Oncorhynchus mykiss*) to different dietary inclusion levels of dehulled lupin (*Lupins angustifolius)*, Aquacult. Res. 32 (2001) 329–340.
[16] M.L. Martins, M. Tavares-Dias, R.Y. Fujimoto, E.M. Onaka, D. T Nomura, Haematological alterations of *Leporinus macrocephalus* (Osteichtyes: anostomidae) naturally infected by Goezia leporine (Nematoda: anisakidae) in fishponds, Arq. Bras. Med. Vet. Zootec. 56 (2004) 640–646.
[17] H.B. Collier, The standardization of blood hemoglobin determinations, Can. Med. Assoc. J. 50 (1994) 550–552.
[18] F. Ghiasi, S.S. Mirzargar, H. Badakhshan, S. Shamsi, Effects of low concentration of cadmium on the level of lysozyme in serum, leukocyte count and phagocytic index in *Cyprinus carpio* under the wintering conditions, J. Fish. Aquat. Sci. 5 (2010) 113–119.
[19] C.E. Seiverd, Hematology for Medical Technologists, Lea and Febiger, Philadelphia, PA, 1964, p. 946.
[20] T.A. Ellis, Lysozyme assays, in: J.S. Stolen, T.C. Fletcher, D.P. Anderson, B.S. Roberson, W.B. Van Muiswinkel (Eds.), Techniques in Fish Immunology, SOS Publications, Fair Haven, 1990, pp. 101–103.
[21] A.K. Siwicki, D.P. Anderson, Nonspecific Defense Mechanisms Assay in Fish: II. Potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and Total immunoglobulin level in serum, Fish Dis. Diagn. Prev. Methods (1993) 105–112 (Olsztyn, Poland).
[22] J. Sunyer, L. Tort, Natural hemolytic and bactericidal activities of sea bream *Sparus aurata* serum are affected by the alternative complement pathway, Vet. Immunol. Immunopathol. 45 (1995) 333–345.
[23] J.M. McCorrd, I. Fridovich, Superoxide dismutase an enzymic function for erythrocuprein (hemocuprein), J. Biol. Chem. 244 (1969) 6049–6055.
[24] H. Aebi, Catalase *in vitro*, Methods in Enzymology, 105 Academic Press, 1984, pp. 121–126.
[25] J.H. Keen, W.H. Habig, W.B. Jakoby, Mechanism for the several activities of the glutathione S-transferases, J. Biol. Chem. 251 (1976) 6183–6188.
[26] F.L. Garcia-Carreno, N.F. Haard, Characterization of proteinase classes in *Langostilla pleuroncodesplanipes* and crayfish *Pacifastacus astacus* extracts, J. Food Biochem. 17 (1993) 97–113.
[27] N. Iijima, S. Tanaka, Y. Ota, Purification and characterization of bile salt activated lipase from hepatopancreas of red sea bream *Pagarus major*, Fish Physiol. Biochem. 18 (1998) 59–69.
[28] P. Bernfeld, Amylase, in: S.P. Colowick, N.O. Kaplan (Eds.), Methods in Enzymology, Academic Press, New York, 1955, pp. 149–158.
[29] Y. Iwashita, N. Suzuki, T. Yamamoto, J. Shibata, K. Isokawa, A.H. Soon, et al., Supplemental effect of cholyltaurine and soybean lecithin to a soybean meal-based fish meal-free diet on the hepatic and intestinal morphology of rainbow trout *Oncorhynchus mykiss*, Fish. Sci. 74 (2008) 1083–1095.
[30] L. Palacios, T. Wang, Egg-yolk lipid fractionation and lecithin characterization, J. Am. Oil Chem. Soc. 82 (2005) 571–578.
[31] H.M. Azarm, A.A. Kenari, M. Hedayati, Effect of dietary phospholipid sources and levels on growth performance, enzymes activity, cholecystokinin and lipoprotein fractions of rainbow trout (*Oncorhynchus mykiss)* fry, Aquacult. Res. 44 (2013) 634–644.
[32] C. De Santis, J.F. Taylor, L. Martinez-Rubio, S. Boltana, D.R. Tocher, Influence of development and dietary phospholipid content and composition on intestinal transcriptome of atlantic salmon (*Salmo salar*), PLoS One 10 (2015) e0140964.
[33] S. Teshima, Phospholipids and sterols, in: L.R. D'Abramo, D.E. Conklin, D.M. Akiyama (Eds.), The World Aquaculture Society, Baton Rouge, LA, USA Crustacean Nutrition: Advances in World Aquaculture, 1997, pp. 85–107.
[34] S. Teshima, A. Kanazawa Y. Kakuta, Effects of dietary phospholipids on growth and body composition of the juvenile prawn, Bull. Jpn. Soc. Sci. Fish. 52 (1986) 155–158.
[35] P.I. Coutteau, M.R. Geurden, P. Camara, P. Sorgeloos, Review of the dietary effects of phospholipids in fish and crustacean larviculture, Aquaculture 155 (1997) 149–164.
[36] H.A. Poston, Effect of body size on growth, survival and chemical composition of Atlantic salmon fed soy lecithin and choline, Progressive Fish-Culturist 52 (1991) 226–230.
[37] H.A. Poston, Response of rainbow trout to soy lecithin, choline, and autoclaved isolated soy protein, Prog. Fish-Cult. 53 (1991) 85–90.
[38] Y. Dersjant-Li, The use of soy protein in aquafeeds, in: L.E. Cruz- Suárez, D. Ricque-Marie, M. Tapia-Salazar, M.G. Gaxiola-Cortés, N. Simoes (Eds.), Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola. 3 –6 de Septiembre del, Cancún, Quintana Roo, México, 2002, pp. 541–558.
[39] F. Jafari, N. Agh, F. Noori, A. Tokmechi, E. Gisbert, Effects of dietary soybean lecithin on growth performance, blood chemistry and immunity in juvenile stellate sturgeon (*Acipenser stellatus*), Fish Shellfish Immunol. 80 (2018) 487–496.
[40] J. Han, R.J. Ulevitch, Limiting inflammatory responses during activation of innate immunity, Nat. Immunol. 6 (2005) 1198.
[41] R.E. Voll, M. Herrmann, E.A. Roth, C. Stach, J.R. Kalden, I. Girkontaite, Immunosuppressive effects of apoptotic cells, Nature 390 (1997) 350–351.
[42] M. Lucas, L.M. Stuart, A. Zhang, K. Hodivala-Dilke, M. Febbraio, R. Silverstein, et al., Requirements for apoptotic cell contact in regulation of macrophage responses, J. Immunol. 177 (2006) 4047–4054.
[43] F.B. Padley, F.D. Gunstone, J.L. Harwood, Occurrence and characteristics of oils and fats, in: F.D. Gunstone, J.L. Harwood, F.B. Padley (Eds.), The Lipid Handbook, Chapman Hall, London, 1986pp. 49–170.
[44] S. Eisenberg, High density lipoprotein metabolism, J. Lipid Res. (1984) 1017–1058.
[45] T. Hevonoja, M.O. Pentikäinen, M.T. Hyvönen, P.T. Kovanen, M. Ala-Korpela, Structure of low density lipoprotein (LDL) particles: basis for understanding molecular changes in modified LDL, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1488 (2000) 189–210.
[46] J. Niu, Y.J. Liu, L.X. Tian, K.S. Mai, H.J. Yang, C.X. Ye, et al., Effects of dietary phospholipid level in cobia (*Rachycentron canadum*) larvae: growth, survival, plasma lipids and enzymes of lipid metabolism, Fish Physiol. Biochem. 34 (2008) 9–17.
[47] J.B. Taggart, J.E. Bron, S.A.M. Martin, P.J. Seear, B. Hoyheim, R. Talbot, et al., A description of the origins, design and performance of the TRAITS/SGP Atlantic salmon (*Salmo salar* L.) cDNA microarray, J. Fish Biol. 72 (2008) 2071–2094.
[48] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, 3ª ed., Oxford University Press, Oxford, 2000.
[49] D. Tovar-Ramırez, D. Mazurais, J.E. Gatesoupe, P. Quazuguel, C.L. Cahu, J.L. Zambonino-Infante, Dietary probiotic live yeast modulates antioxidant enzyme activities and gene expression of seabass (*Dicentrarchus labrax*) larvae, Aquaculture 300 (2010) 142–147.
[50] S. Sun, J. Ye, J. Chen, Y. Wang, L. Chen, Effect of dietary fish oil replacement by rapeseed oil on the growth, fatty acid composition and serum non-specific immunity response of fingerling black carp, *Mylopharyngodon piceus*. Aquac. Nutr. 17 (2011) 441–450.
[51] T.W. Goodwin, The Biochemistry of the Carotenoids, Chapman and Hall, London, 1984.
[52] Z. Cai, S. Feng, X. Xiang, K. Mai, Q. Ai, Effects of dietary phospholipid on lipase activity, antioxidant capacity and lipid metabolism-related gene expression in large yellow croaker larvae (*Larimichthys crocea*), Comp. Biochem. Physiol., B 201 (2016) 46–52.
[53] J.R. Sargent, J.G. Bell, M.V. Bell, R.J. Henderson, D.R. Tocher, The metabolism of phospholipids and polyunsaturated fatty acids in fish, in: B. Lahlou, P. Vitello (Eds.), Aquaculture: Fundamental and Applied Research, Coastal and Estuarine Studies, 43 American Geophysical Union, Washington, 1999, pp. 103–124.
[54] E. Awad, B. Austin, A. Lyndon, Effect of dietary supplements on digestive enzymes and growth performance of rainbow trout (*Oncorhynchus mykiss*, Walbaum), J. Am. Sci. 8 (2012) 858–864.
[55] L. Ribeiro, J. Moura, M. Santos, R. Colen, V. Rodrigues, N. Bandarra, et al., Effect of

vegetable based diets on growth, intestinal morphology, activity of intestinal enzymes and haematological stress indicators in meagre (*Argyrosomus regius*), Aquaculture 447 (2015) 116–128.
[56] C.L. Cahu, J.L. Zambonino Infante, V. Barbosa, Effect of dietary phospholipid level and phospholipid/neutral lipid ratio on development of Sea bass (*Dicentrarchus labrax*) fed compound diet, Br. J. Nutr. 90 (2003) 21–28.
[57] Y. Li, J.Y. Wang, Z.D. Song, L.M. Zhang, H. Zhang, X.X. Li, et al., Evaluation of soy protein concentrate as a substitute for fishmeal in diets for juvenile starry flounder (*Platichthys stellatus*), Aquaculture 448 (2015) 578–585.
[58] I. Lund, N. El Kertaoui, M.S. Izquierdo, D. Dominguez, The importance of phospholipids combined with long-chain PUFA in formulated diets for pikeperch (*Sander lucioperca*) larvae, Br. J. Nutr. 120 (2018) 628–644.
[59] S. Kolkovsk, Digestive enzymes in fish larvae and juveniles—implications and applications to formulated diets, Aquaculture 200 (2001) 181–201.
[60] J. Zambonino-Infante, E. Gisbert, C. Sarasquete, I. Navarro, J. Gutiérrez, C.L. Cahu, Ontogeny and physiology of the digestive system of marine fish larvae, in: J.E.O. Cyrino, D. Bureau, B.G. Kapoor (Eds.), Feeding and Digestive Functions of Fish, Science Publishers, Inc, Enfield, USA, 2009, pp. 277–344.
[61] S. Lin, L. Luo, Effects of different levels of soybean meal inclusion in replacement for fish meal on growth, digestive enzymes and transaminase activities in practical diets for juvenile tilapia, *Oreochromis niloticus* × *O. aureus*, Anim. Feed Sci. Technol. 168 (2011) 80–87.
[62] P. Gomez-Requeni, F. Bedolla-Cazares, C. Montecchia, J. Zorrilla, M. Villian, E.M. Toledo-Cuevas, et al., Effects of increasing the dietary lipid levels on the growth performance, body composition and digestive enzyme activities of the teleost pejerrey (*Odontesthes bonariensis*), Aquaculture 416 (2013) 15–22.
[63] L. Benedito-Palos, J.C. Navarro, A. Sitjá-Bobadilla, J.G. Bell, S. Kaushik, J. Pérez-Sánchez, High levels of vegetable oils in plan protein-rich diets fed to gilthead sea bream (*Sparus aurata* L.): growth performance, muscle fatty acid profiles and histological alterations of target tissues, Br. J. Nutr. 100 (2008) 992–1003.
[64] S. Martínez-Llorens, R. Baeza-Ariño, S. Nogales-Mérida, M.J. Cerdá, A. Tomás-Vidal, Carob seed germ meal as a partial substitute in gilthead sea bream (*Sparus aurata*) diets: amino acid retention, digestibility, gut and liver histology, Aquaculture 338–341 (2012) 124–133.
[65] E. Santigosa, J. Sánchez, F. Médale, S. Kaushik, J. Pérez-Sánchez, M.A. Gallardo, Modifications of digestive enzymes in trout (*Oncorhynchus mykiss*) and sea bream (*Sparus aurata*) in response to dietary fish meal replacement by plant protein sources, Aquaculture 282 (2008) 68–74.
[66] B. Ruyter, C. Moya-Falcón, G. Rosenlund, A. Vegusdal, Fat content and morphology of liver and intestine of Atlantic salmon (*Salmo salar*): effects of temperature and dietary soybean oil, Aquaculture 252 (2006) 441–452.
[67] M. Grosell, M. Grosell, A.P. Farrell, C.J. Brauner, The Multifunctional Gut of Fish, Elsevier, Amsterdam, 2011.
[68] M. Grosell, K.M. Gilmour, S.F. Perry, Intestinal carbonic anhydrase, bicarbonate, and proton carriers play a role in the acclimation of rainbow trout to seawater, Am. J. Physiol. Regul. Integr. Comp. Physiol. 293 (2007) 2099–2111.
[69] W.S. Marshall, M. Grosell, Ion transport, osmoregulation, and acid–base balance, in: D.H. Evans, J.B. Claiborne (Eds.), The Physiology of Fishes, CRC Press Inc., Boca Raton, 2006, pp. 177–230.
[70] T. Yamamoto, N. Suzuki, H. Furuita, T. Sugita, N. Tanaka, T. Goto, Supplemental effect of bile salts to soybean meal-based diet on growth and feed utilization of rainbow trout *Oncorhynchus mykiss*, Fish. Sci. 73 (2007) 123–131.