Effects of dietary soybean lecithin on growth performance, blood chemistry and immunity in juvenile stellate sturgeon (*Acipenser stellatus*)

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ABSTRACT

An eleven weeks feeding trial was conducted to determine the effects of different levels of dietary soybean lecithin (SBL) on growth performance, blood chemistry and immunity in juvenile stellate sturgeon (*Acipenser stellatus*). Fish were fed seven isoproteic (44% crude protein) and isolipidic (17% crude fat) diets containing graded levels of SBL: 0 (control), 1, 2, 4, 6, 8 and 10%. Results showed that dietary SBL supplementation significantly improved the final body weight (BW) and weight gain (WG). Fish fed 6% SBL showed the highest BW and WG values in comparison to fish fed the control diet ($P < 0.05$), whereas increasing SBL levels above 6% had little practical benefit in terms of somatic growth performance. The inclusion of SBL in diets significantly improved the immune response as data from lysozyme, total Ig levels, alternative complement, phagocytic and bactericidal activities indicated ($P < 0.05$). The broken-line regression analysis of immunological variable revealed that depending on the parameter considered, the optimal SBL levels in diets for stellate sturgeon juveniles varied. In particular, dietary SBL levels requirements in stellate sturgeon when considering the phagocytic activity rate were determined at 3.3%, whereas 4.1-4.2% were recommended when considering data from lysozyme, alternative complement and bactericidal activities. In contrast, the highest minimum dietary SBL content was estimated at 6.9% when data from total Ig levels were considered. These results indicated that dietary PLs are required for boosting innate immunity in stellate sturgeon, although their minimal level changed depending on the immunological parameter considered. Therefore, we assume that SBL levels comprised between 3.3 to 6.9% may be used as a prophylactic measure to improve the health status in stellate sturgeon. Red blood cell count, hemoglobin and hematocrit levels increased with increasing dietary SBL levels, especially in those sturgeons fed the diet with 6% SBL ($P < 0.05$). In addition, white blood cell counts significantly increased as dietary SBL levels increased from 4 to 8% in comparison to the control group. Blood biochemistry was also affected by different dietary SBL levels. In particular, significantly higher levels of glucose, cholesterol, HDL and triglycerides were detected in fish fed >6%, >4%, >2% and 2% SBL, respectively ($P < 0.05$). Based on somatic growth parameters, blood chemistry and systemic immunity parameters, diets containing *ca.* 6% SBL are recommended for juvenile stellate sturgeon.

Key words: Soybean lecithin, *Acipenser stellatus*, growth, immune response, blood biochemistry.

1. Introduction

It has been reported that lipids play an important role in the immune system [1, 2]. Among lipid components, phospholipids (PL) are important components for maintaining the structure and function of cellular membranes, emulsifying lipids in the gut and improving intestinal absorption of long chain fatty acids [3]. Phospholipids are a source of fatty acids for the synthesis of eicosanoids, a wide range of bioactive compounds with multiple functions. It has been reported that the composition of dietary fatty acids influenced the non-specific immunity (e.g. phagocytosis, respiratory burst and serum lysozyme) [4-6] and specific immunity (e.g. antibody production and resistance to pathogens) [7-10] and eicosanoid production [9, 11]. The optimal level of dietary phospholipid supplementation depends on the species, developmental stage, culture conditions, and PL source. In this regard, soybean lecithin (SBL) due to its market availability and relatively stable composition has been commercially used as a convenient source of PL in aquafeeds, although some studies dealing with larvae have used marine phospholipid sources [3].

Among the fish species living in the Caspian Sea, sturgeons are of utmost interesting from an economic perspective, not only for their caviar, but also for the meat. However, all sturgeon species inhabiting the Caspian Sea are highly vulnerable and endangered, and the stellate sturgeon (*Acipenser stellatus*) is not an exception. Based on catch data and the number of individuals migrating into the Volga and Ural rivers, it is estimated that the species has undergone a population decline of at least 80% (possibly close to 100%) in the past three generations, which is expected to continue. Consequently, this species is classified in the IUCN Red List of Threatened Species as critically endangered, and it is highlighted that its survival will only depend on restocking activities and effective fishery management plans. Thus, during the last decades a lot of interest has been developed for sturgeon aquaculture, regardless of the final purpose of this activity: restocking and conservation of wild population or production for human consumption. Under culture conditions, the nutritional requirements on protein [12], lipid [13], carbohydrate [12] and trace elements [14, 15] have been studied in various sturgeon species. However, there is scarce information about the PL requirements in Acipenserides [16]. The only available study on SBL requirements in this group of primitive fish reported that white sturgeon (*Acipenser transmontanus*) had no requirements for lecithin, but there was a requirement for choline in

this species [17]. In this context, the former authors concluded that refined soybean lecithin (SBL) could be used to replace some of the oil mix in the white sturgeon diets as an alternative source of dietary lipid. However, knowledge about the effects of PL on the immune system, hematological parameters and blood chemistry in sturgeons are limited. The aim of this study was to investigate the impact of PL from SBL on growth performance, immune system and blood biochemistry in juvenile stellate sturgeon in order to determine the appropriate dietary lecithin level for diet formulation in sturgeons.

2. Materials and Methods

2.1. Experimental diets and experimental design

The formulation of the experimental diets was conducted by means of the WUFFDA software (Lindo© 1995, Release 6.1). Seven diets were formulated to be isonitrogenous (44% crude protein) and isolipidic (17% crude fat) (Table 1). Defatted fish meal and corn gluten were the main protein sources in the experimental diets, while lipid sources included soybean lecithin, fish oil and corn oil. Different PL levels in diets were achieved by adding SBL at different levels (0, 1, 2, 4, 6, 8 and 10%) at the expense of corn oil (Tables 1 and 2). Soybean lecithin contained: 19-21% phosphatidylcholine, 8-20% phosphatidylethanolamine, 20-21% phosphatidylinositol, 5-11% other phosphatides and 33-35% soy bean oil [18]. All dry ingredients were weighed and mixed for 30 min, then fish and corn oils and SBL were added, followed by addition of distilled water and mixed thoroughly. Once the desired consistency was reached, the mixture was then mechanically pelleted to obtain suitable sized pellets (3 mm). The pellets were dried in a convection oven at 35 °C and stored in re-sealable plastic bags at 4°C until use. Diets were tested by triplicate during 75 days. The fatty acid composition of diets was analyzed by gas chromatography (Agilent 7890A GC System, USA) using a BP×70 capillary glass column (0.32 mm × 50 m, SGE Analytical Science Australia) after esterification in acetyl-chloride/methanol mixture. Fatty acid methyl esters were prepared by the modified procedure of Lepage and Roy [19]. The phospholipid profile of the experimental feed were analyzed using Densitometer GS900 calibrated (Bio Rad, Germany).

Table 1. Ingredient list and proximate composition (%) of experimental diets containing graded levels of soybean lecithin.

Ingredient	Experimental diets containing different levels of dietary lecithin (%)						
	0 (Control)	1	2	4	6	8	10
Kilka fish meal[a] (defatted)	40	40	40	40	40	40	40
Wheat gluten	12	12	12	12	12	12	12
Wheat meal	20	20	20	20	20	20	20
Soybean lecithin	0	1	2	4	6	8	10
Corn oil	13.5	12.5	11.5	9.5	7.5	5.5	3.5
Fish oil[a]	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Methionine	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Lysine	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Betaine	1	1	1	1	1	1	1
Vitamin[b] and mineral[c] mixes	3	3	3	3	3	3	3
Yeast	2	2	2	2	2	2	2
Calcium Carbonate	2	2	2	2	2	2	2
Wheat bran	1	1	1	1	1	1	1
Proximate composition in dry basis (%)							
Crude protein (%)	43.66	44.08	44.05	44.07	44.06	44.09	43.55
Crude lipid (%)	17.65	17.36	17.65	17.16	17.07	17.77	16.38
Ash (%)	10.03	10.71	9.74	10.27	10.56	11.92	11.77
Gross energy (J kg^{-1})	2,130.13	2,112.9	2,130.7	2,110.7	2,098.5	2,092.6	2,062.5

[a] Ettehad Khazar Shomal Company, Babolsar, Mazandaran, Iran. [b] Composition of vitamin premix (IU or g/kg): Vit.A, 8,00,000 IU; Vit.D3, 300,000, IU; Vit.E, 2,500 mg ; Vit.K, 1,000 mg; Vit. B1, 1,200 mg; Vit.B2, 1,200 mg; Vit. B3, 2400 mg; Vit. B5, 3,500 mg; Vit.B6, 1,300 mg; Vit B9, 600 mg; Vit. B12, 750 µg; Vit. C, 35,000 mg; Vit. H2, 600 mg. ATA Company, Tabriz, Iran.
[c] Mineral premix (g kg^{-1} premix): Magnesium, 6,400mg; Copper, 2,000 mg; Iron, 11,000 mg; Zinc, 7,000 mg; Selenium, 100mg; Iodine, 300 mg; Cobalt, 50mg; Natrium, 5,000mg. ATA Company, Tabriz, Iran.

Table 2. Fatty acid profile of experimental diets containing graded levels of soybean lecithin (g kg^{-1} dry weight).

Fatty acid	Experimental diets containing different levels of dietary lecithin (%)						
	0 (Control)	1	2	4	6	8	10
C14:0	0.59	0.65	0.73	0.78	0.75	0.99	0.80
C16:0	15.32	17.01	19.03	20.19	19.49	25.28	20.36
C18:0	2.90	3.00	3.32	3.63	3.57	4.59	3.75
C20: 0	0.28	0.05	0.07	0.41	0.39	0.45	0.32
C22:0	0.02	0.07	0.06	0.24	0.21	0.31	0.29
SFA	19.57	20.79	23.23	25.28	24.44	31.65	25.54
C14:1n5	0.06	0.02	0.02	0.06	0.08	0.12	0.09
C16:1n7	1.07	1.13	1.41	1.39	1.35	1.70	1.39
C18:1n9	31.32	33.46	35.56	34.47	30.14	33.91	24.57
C18: 1n7	0.97	1.02	0.96	1.09	1.24	1.59	1.25
C20:1n9	0.40	0.44	0.47	0.03	0.02	0.05	0.04
C22:1n9	0.13	0.16	0.14	0.04	Nd	Nd	Nd
MUFA	33.97	36.22	38.59	37.10	32.85	37.40	27.36
C18:2n6	50.78	55.67	59.51	57.91	51.17	58.79	53.24
C20:2n6	0.34	0.35	0.36	0.27	0.08	0.13	0.10
C20: 4n6	0.22	0.11	0.06	0.27	0.39	0.45	0.28
n-6 PUFA	51.34	56.13	59.93	58.45	51.64	59.37	53.62
C18:3n3	0.42	1.39	1.67	1.93	2.38	2. 95	2.65
C20:3n3	0.09	0.17	0.10	0.04	0.03	0.04	0.04
C20:5n3	0.99	0.97	1.22	1.26	1.29	1.61	1.37
C22:6n3	3.10	3.50	3.90	4. 10	4.00	4.87	4.04
n-3 HUFA	4.68	6.08	6.92	7.43	7.71	9.49	8.12

Nd: Not detected

Table 3. Phospholipid profile of the experimental diets

Class of lipids	Experimental diets containing different levels of dietary PL (%)						
	0	1	2	4	6	8	10
PC	0.6	2.5	3.6	6.3	8.5	11.2	13.5
PS/PI	-	1.7	1.2	2.6	3.6	5.5	5.6
PG+SQDG	-	-	0.6	1.5	2.5	3.3	3.4
PE	-	0.9	1.7	3.3	4.6	5.7	6.2
DGDG	-	-	-	-	0.9	1.0	1.2
Unknown	-	.	1.1	1.2	1.9	2.5	2.3
MGDG	-	-	-	-	0.9	1.0	1.2
Total PL	1.4	5.1	8.8	15.7	22.7	30.2	33.1
CHOL	3.9	3.3	1.9	1.8	1.3	1.4	2.1
FFA	6.5	5.8	6.4	6.9	7.4	8.4	6.7
TAG	78.8	76.4	77.9	70.0	63.34	54.6	52.5
SE+W	6.0	6.4	3.0	3.2	3.1	3.0	2.8
Total NL	98.63	94.9	91.3	84.3	77.29	69.8	66.9

Abbreviations: PC, phosphatidylcholine; PS+PI, phosphatidylserine and phosphatidylinositol; PG+SQDG, phosphatidylglycerol + sulphoquinovosyl diacylglycerols; PE, phosphatidylethanolamine; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; PL, polar lipids; CHOL, cholesterol; FFA, free fatty acids; TAG, triacylglycerols; SE+W, sterol esters + waxes; NL, neutral lipids, -, below detection limits.

2.3. Fish and sampling procedures

Juvenile *A. stellatus* were obtained from Shahid Beheshti sturgeon fish hatchery in Rasht, located in Northern Province of Iran, Guilan. Prior to the feeding trials, all fish were acclimated to the indoor rearing conditions for 3 weeks fed a local commercial feed from Fradane Company, Esfahan (Iran) and live *Artemia* nauplii. Twenty-six fish with initial body weight (BW) of 11.3 ± 0.05 g were stocked in each 90-L polycarbonate tank containing 80 L ground water at the flow rate of 1.0 L min^{-1}. Photoperiod of 12 h light:12 h dark was maintained throughout the experiment. Water temperature, dissolved oxygen and pH were maintained at 18.9 ± 0.5 °C, 8.5 ± 0.5 mg L^{-1} and 8.02 ± 0.11 (mean ± standard error of the mean, SEM), respectively throughout the experiment. Fish were fed experimental diets at apparent satiation at 08:00, 11:00, 14:00 and 17:00 h for 75 days.

2.4. Growth performance

At the end of the experiment, fish were fasted for 24 h and then weighed to the nearest 0.1 g and measured to the nearest 1 mm (total length TL) to determine their somatic growth performance. The following formulae were used to evaluate body growth performance: weight gain (WG, %) = (BWf – BWi) / BWi) x 100; specific growth rate (SGR; % day^{-1}) = [(ln BWf - ln BWi)/t] x 100; where BWf is the final body weight, BWi is the initial body weight and t is the length of the experimental period (42 days); survival (S, %) = (number of fish in each group remaining at day 75 / initial number of fish) x 100, and Fulton's condition factor (K) = (BW / TL^3) x 100.

2.5. Immunological analysis

Three specimens from each replicate were anaesthetized with 200 mg L^{-1} clove powder and blood was collected from the caudal vein with sterilized syringes, and transferred immediately into sterile tubes and allowed to clot at room temperature for 1 h. Supernatants were separated by centrifugation (3,000 × g for 5 min at 4°C) and stored at –80 °C until analysis.

2.5.1 Serum alternative complement

Alternative complement activity (ACH50) was assayed based on the hemolysis of rabbit red blood cells (RaRBC) as described by Willey et al. [20]. The RaRBC were washed three times in ethylene glycol tetra acetic acid magnesium-gelatin veronal buffer (0.01 M EGTA-Mg-GVB, pH 7) and the cell numbers were adjusted to 2×10^8 cells mL^{-1} in the same buffer. At first, the 100% lysis value was obtained by adding 100 mL of the above RaRBC to 3.4 mL distilled water. The hemolysate was centrifuged and the optical density (OD) of the supernatant was determined at λ = 414 nm using a spectrophotometer (Awareness, USA). Following, the serum was diluted (100 times), and different volumes ranging from 100 to 250 mL (total volume was adjusted to 250 mL with the buffer) were allowed to react with 100 mL of RaRBC in small test tubes. These mixtures were incubated at 20 °C for 90 min with intermittent mixing, and then

3.15 mL of 0.85% NaCl solution was added and tubes were centrifuged at 1,600 × g for 10 min at 4 °C. The OD of the supernatant was measured at λ = 414 nm. A lysis curve was obtained by plotting the percentage of haemolysis against the volume of serum added on a log-log graph. The volume yielding 50% haemolysis was used for determining the complement activity of the sample as follows: ACH50 (Units mL^{-1}) = K × [(reciprocal of the serum dilution) × 0.5], where K is the amount of serum (mL) giving 50% lysis and 0.5 is the correction factor since the assay was performed on half scale of the original method.

2.5.2 Serum total immunoglobulin

Total immunoglobulin was assayed following the method of Siwicki et al. [21]. Serum samples were diluted with 0.85% NaCl (100 times) and total protein content was determined by the Bradford method [22] . One hundred mL of total serum was mixed with an equal volume of 12% solution of polyethylene glycol (Sigma-Aldrich Corporation, St Louis, MI, USA) in wells of a 96-well micro titer plate. Following 2 h of incubation at room temperature, the microplate was centrifuged at 5000 × g at 4 °C. The supernatant was diluted 50 times with 0.85% of NaCl and the protein content was determined by Bradford method [22]. This value was subtracted from the total protein level and the result was equal to the total immunoglobulin concentration of the serum (mg mL^{-1}).

2.5.3 Lysozyme activity

Lysozyme activity in serum was measured according to Hultmark et al. [23]. Briefly, *Micrococcus lysodeikticus* (Sigma-Aldrich) was applied as the substrate in 0.01 M PBS buffer (pH 6.4) to form a suspension (OD ≈ 0.3). A volume of 50 μL of serum was added to 3 mL of the bacterial suspension on an ice-bath. The absorbance was recorded at λ = 570 nm, immediately (A1). The mixture was then incubated at 37 ˚C for 30 min, transferred to an ice-bath to stop the reaction and then the absorbance was recorded again (A2). Lysozyme activity was calculated according to the following formula: U = A1 – A2 / A1.

2.5.4 Phagocytic activity

Macrophage isolation was done as described by Secombes [24] with slight modifications. Briefly, 2 mL blood samples were taken by a heparinized syringe from the caudal vein and gently mixed with 3 mL ice-cold Leibovitz L15 medium (Sigma-Aldrich) containing 2% fetal calf serum (FCS, Sigma-Aldrich), heparin (10 IU mL^{-1}, Sigma-Aldrich), and penicillin (100 IU mL^{-1}) / streptomycin (100 µg mL^{-1}) (Merck, Germany). The cell suspension was layered over a 51% Percoll (Sigma-Aldrich) and centrifuged at 400 ×g for 25 minutes at 4°C to remove erythrocyte contamination and cell debris. The macrophages isolated from the L15 medium/Percoll interface were washed twice by centrifugation at 400 × g for 5 minutes in L15 medium and adjusted to 5 $\times 10^6$ viable macrophages per mL of L15 medium supplemented with 5% FCS and penicillin/streptomycin.

Phagocytosis was measured according to the method of Mehrzad et al [25] following the isolation of blood macrophages from three fish per tank as described above. Blood samples were plated in 96-well flat-bottomed plates in RPMI 1640 medium supplemented with 10% fetal calf serum (1×10^5 cells 100 μL^{-1} per well) and stimulated with 50 µL PHA solution (1 mg mL^{-1}) or medium alone. After 72 hr of incubation, cultures were pulsed with 20 µL of the MTT solution (5 mg mL^{-1}) for 4 hr at 37°C. Then, 150 mL DMSO were added and shaken vigorously to dissolve Formosan crystals. Values of DO were measured at $\lambda = 550$ nm in a microplate reader (Dynatech, Denkendorf, Germany). Analyses were done in triplicate sets (methodological replicates). Results were expressed as the proliferation index according to the ratio of OD ($\lambda = 550$) of stimulated cells with MOG35-55 to OD ($\lambda = 550$) of non-stimulated cells.

2.6 Hematological parameters

Nine fish from each group were anaesthetized with clove powder (200 mg L^{-1}) and blood was collected by caudal vein puncture with heparinised syringes. Red blood cell (RBC) and white blood cells (WBC) were enumerated in an improved Neubaeur hemocytometer, using Hayem and Turck diluting fluids [26]. Hematocrit (Htc, %) was determined by the standard microhematocrit method [27]. The amount of

hemoglobin (Hb, g dL^{-1}) was determined according to the cyanomethemoglobin procedure [26]. The following hematologic indices: mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg) and MCH concentration (MCHC, g dL^{-1}) were calculated according to Seiverd [28]. Differential leukocyte counts were obtained by preparing panchromatically stained smears [29]; cells were identified on the basis of morphology and cell ultrastructure as documented in previous fish leukocyte studies [30].

2.7 Serum biochemical analysis

Total triglycerides (TG), cholesterol (CHO), glucose (GLU), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) in serum were analyzed using commercial kits (Pars Azmon, Iran) by an auto-analyzer (BT1500 Biotecnica Instruments S.p.A., Italy). Total soluble proteins were determined in the supernatant by the Bradford method [31], using bovine serum albumin as standard.

2.8. Statistical analyses

The value of each variable was expressed as mean ± SEM. Statistical analyses were performed using SPSS software (Ver 21.0, IBM, USA). All the data were tested for normality, homogeneity and independence of variance before the ANOVA tests. Arcsine transformations were conducted on data expressed as percentage in order to achieve homogeneity of variance before statistical analysis. Differences between experimental groups were evaluated by means of One-way ANOVA, followed by a post hoc Tukey test when significant differences were found ($P < 0.05$). The broken-line regression method considering data on immune parameters was used to quantify the minimum dietary SBL requirements in stellate sturgeon [32].

3. Results

3.1. Growth performance

At the end of the trial, growth performance in terms of BW, SGR and WG in sturgeon fed diets containing 4, 6, 8 and 10% SBL was higher compared to the control group and those fish fed diets with 1 and 2% SBL (Table 4, $P < 0.05$). The highest BW, SGR and WG were registered in the fish fed diets containing from 6 to 10% SBL. No statistically significant differences were found in survival nor K between experimental groups ($P > 0.05$).

Table 4. Growth performance in stellate sturgeon (*A. stellatus*) fed graded levels of soybean lecithin for 11 weeks.

	Dietary soybean lecithin levels (%)						
	Control (0)	1	2	4	6	8	10
BW i (g)	11.29 ± 0.04	11.29 ± 0.04	11.31 ± 0.03	11.31 ± 0.06	11.25 ± 0.00	11.25 ± 0.00	11.30 ± 0.05
BWf (g)	27.46 ± 1.5 ^{a}	32.86 ± 1.6 ^{a}	38.55 ± 1.7 ^{ab}	46.80 ± 2.1 ^{bc}	51.40 ± 3.39 ^{c}	47.01 ± 2.7 ^{bc}	46.42 ± 3.01 ^{bc}
SL (cm)	24.59 ± 0.42 ^{a}	26.00 ± 0.66 ^{a}	27.07 ± 0.92 ^{a}	30.94 ± 0.69 ^{b}	32.10 ± 0.48 ^{b}	30.04 ± 0.29 ^{b}	29.65 ± 0.41 ^{b}
WG (%)	143.2 ± 13.1 ^{a}	191.1 ± 14.8 ^{a}	240.8 ± 16.5 ^{ab}	314.0 ± 21.5 ^{b}	356.9 ± 30.2 ^{c}	317.9 ± 24.6 ^{bc}	310.4 ± 24.7 ^{bc}
SGR (% day^{-1})	0.16 ± 0.05 ^{a}	0.34 ± 0.05 ^{ab}	0.49 ± 0.04bc	0.69 ± 0.05 ^{cd}	0.79 ± 0.06 ^{cd}	0.70 ± 0.05 ^{d}	0.68 ± 0.05 ^{cd}
K factor	0.19 ± 0.002	0.19 ± 0.003	0.18 ± 0.01	0.18 ± 0.003	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.004
Survival (%)	89.6 ± 2.1	89.6 ± 4.1	87.5 ± 0.0	89.6 ± 5.5	87.4 ± 3.4	87.5 ± 3.6	85.4 ± 5.5

Values are mean ± SEM from triplicate groups. Means in each row with different letters are significantly different ($P < 0.05$). Absence of letters indicates no significant differences between dietary treatments.

3.2. Humoral immune parameters

Alternative complement activity was similar in fish fed the control diet and those diets containing 1 and 2% SBL, whereas higher dietary inclusion of SBL significantly increased ACH50 values (Table 5, $P < 0.05$). Considering the broken-line regression method, the optimal dietary SBL in relation to ACH50 values was 4.1% (Fig. 1a). Lysozyme activity was significantly higher in fish fed from 4 to 10% SBL compared to the control group, whereas the rest of dietary treatments showed intermediate values (Table 5, $P < 0.05$). Considering the broken-line regression method, the optimal dietary SBL in relation to lysozyme activity values was 4.2% (Fig. 1b). The highest levels of serum total antibody were found in sturgeon fed 4, 6 and 8% SBL; whereas the lowest values were recorded in fish fed 0, 1 and 2% SBL. Sturgeon fed 10% SBL showed intermediate levels between former groups (Table 5, $P < 0.05$). Considering the broken-line regression method, the optimal dietary SBL in relation to total antibody levels was 6.9% (Fig. 1c).

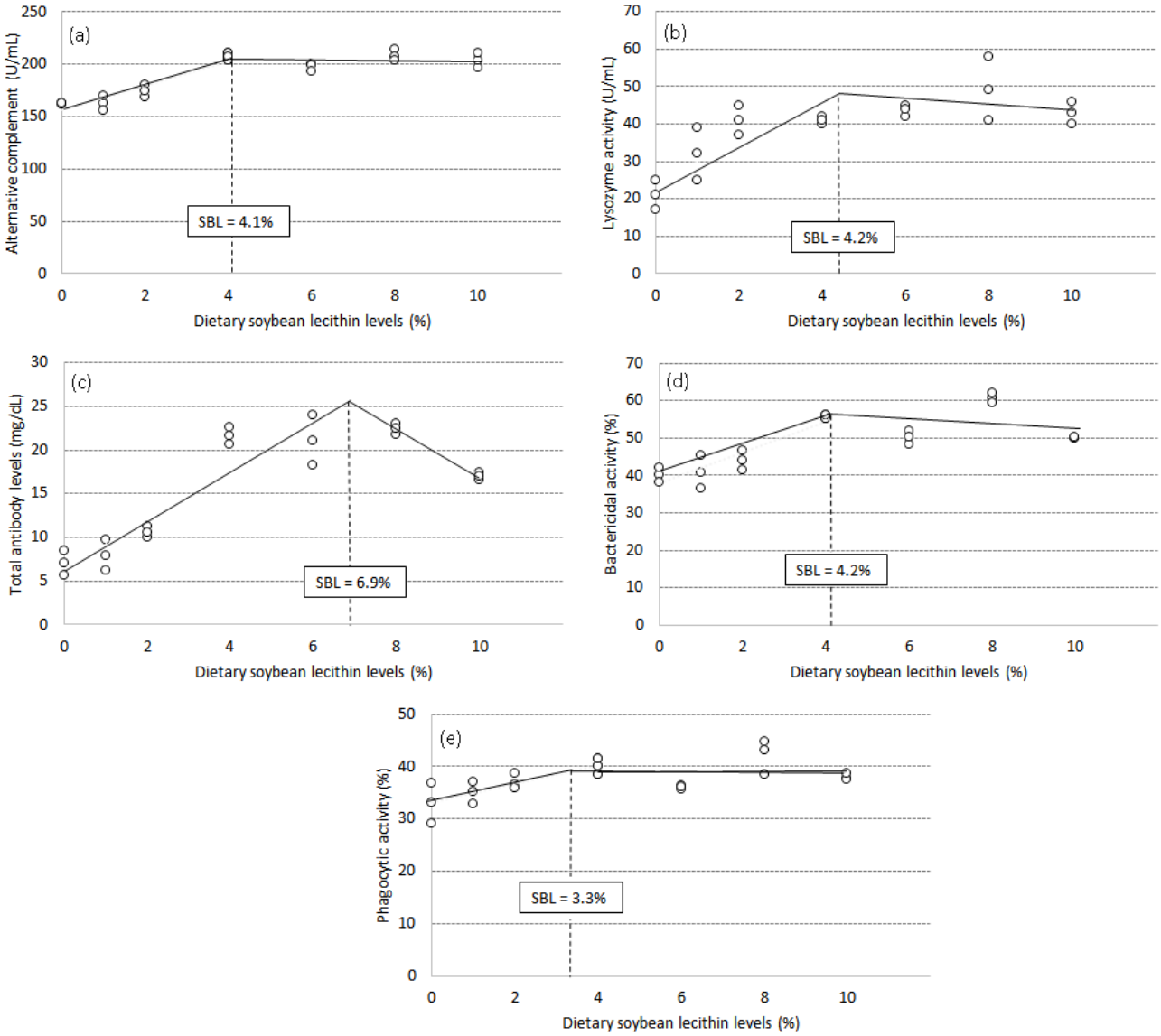
Table 5. Immune parameters (lysozyme, alternative complement, total immunoglobulin levels, and bactericidal and phagocytic activities) in stellate sturgeon (*A. stellatus*) fed graded levels of soybean lecithin for 11 weeks.

	Dietary soybean lecithin levels (%)						
	Control (0)	1	2	4	6	8	10
Alternative complement (U mL^{-1})	162.6 ± 0.4 ^{a}	162.8 ± 4.3 ^{a}	174.7 ± 3.3 ^{a}	207.2 ± 2.1 ^{b}	197.7 ± 2.0 ^{b}	208.6 ± 3.0 ^{b}	203.9 ± 3.9 ^{b}
Lysozyme (U mL^{-1})	21.0 ± 2.3 ^{a}	32.0 ± 4.0 ^{ab}	41.0 ± 2.3 ^{ab}	41.0 ± 0.6 ^{bc}	43.7 ± 0.9 ^{bc}	49.3 ± 4.9 ^{c}	43.0 ± 1.7 ^{bc}
Total Ig (mg mL^{-1})	7.0 ± 0.8 ^{a}	8.0 ± 1.0 ^{a}	10.6 ± 0.4 ^{a}	21.6 ± 0.6 ^{c}	21.1 ± 1.7 ^{c}	22.4 ± 0.4 ^{c}	17.0 ± 0.2 ^{b}
Bactericidal activity (%)	40.1 ± 1.1^{a}	41.0 ± 2.6 ^{a}	44.0 ± 1.5 ^{ab}	55.6 ± 0.3 ^{cd}	50.1 ± 1.1 ^{bc}	60.7 ± 0.7 ^{d}	50.0 ± 0.4 ^{bc}
Phagocytic activity (%)	33.1 ± 2.2 ^{a}	35.0 ± 1.1 ^{ab}	37.0 ± 0.8 ^{abc}	40.0 ± 0.8 ^{bc}	36.0 ± 0.1 ^{ab}	42.0 ± 1.9 ^{c}	38.0 ± 0.4 ^{abc}

Values are mean ± SEM from triplicate groups. Means in each row with different letters are significantly different ($P < 0.05$).

3.3 Phagocytic and bactericidal activity

The phagocytic and bacterial killing rates were significantly higher in sturgeon fed 4 and 8% SBL compared to control, whereas the rest of dietary treatments showed intermediate values (Table 5, $P < 0.05$). Taking into consideration the results obtained from the broken-line regression analysis, the optimal dietary SBL regarding phagocytic and bactericidal activities were 3.3 and 4.2%, respectively (Fig. 1d, e)



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281 Figure 1. Estimation of the minimum nutritional requirement in soybean lecithin (SBL) for stellate
282 sturgeon (*A. stellatus*) juveniles by means of broken-line regression analysis using the data from
283 different immune parameters.

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285

3.4. Blood haematology and biochemistry

Hematological parameters were significantly affected by dietary SBL ($P < 0.05$, Table 6). Red blood cells increased in sturgeon fed SBL, but the highest RBC were found in fish fed the 6% SBL diet ($P < 0.05$). White blood cells increased significantly in fish fed 4, 6 and 8% SBL in comparison to the control group, whereas the rest of dietary treatments showed intermediate values of WBC between the above-mentioned groups ($P < 0.05$). Hemoglobin (Hb) and Hematocrit (HCT) were significantly higher in fish fed 6% SBL in comparison to the control group that showed the lowest levels, whereas the rest of dietary groups showed intermediate levels ($P < 0.05$). Dietary SBL levels did not affect the mean corpuscular hemoglobin concentration (MCHC) and the mean corpuscular volume (MCV) levels ($P > 0.05$). However, the mean corpuscular hemoglobin levels (MCH) were significantly influenced by the dietary SBL levels, being the lowest MCH values found in fish fed 6% SBL and the highest ones in the control and 10% SBL groups ($P < 0.05$). Monocyte counts were not statistically significant different among fish fed different SBL levels, but neutrophils and lymphocytes were significantly higher in sturgeon h fed 8 % SBL compared to the control devoid of SBL (Table 6, $P < 0.05$).

Serum biochemistry was significantly affected by different dietary SBL levels (Table 7, $P < 0.05$). Sturgeon fed the diets containing 10% SBL showed the highest blood glucose levels, whereas fish fed the control diet showed the lowest one. The rest of dietary treatments showed intermediate values ($P < 0.05$). The highest cholesterol and LDL levels were found in sturgeon fed 10% SBL, whereas cholesterol levels decreased as the level of SBL inclusion decreased in experimental diets ($P < 0.05$). In addition, fish fed 8 and 10% SBL showed the highest content of triglycerides in blood in comparison to the control diet ($P < 0.05$). Fish fed 8% SBL had the highest HDL content, whereas the lowest HDL level was found in fish fed control diet ($P < 0.05$). Lowest plasma total protein levels were found in sturgeon fed 0, 1 and 2% SBL, whereas sturgeon fed from 4 to 10% SBL showed higher protein levels in plasma ($P < 0.05$).

Table 6. Hematological parameters in stellate sturgeon (*A. stellatus)* fed diets containing graded levels of soybean lecithin levels for 11 weeks.

	Dietary soybean lecithin levels (%)						
	Control (0)	1	2	4	6	8	10
RBC (10^5 mL^{-1})	7.0 ± 0.33 ^{a}	7.4 ± 0.39 ^{ab}	7.7 ± 0.23 ^{ab}	7.7 ± 0.49 ^{ab}	9.2 ± 0.41^{b}	7.9 ± 0.35 ^{ab}	8.0 ± 0.31 ^{ab}
WBC (10^3 mL^{-1})	9.2 ± 0.37 ^{a}	10.8 ± 0.27 ^{ab}	11.5 ± 0.12 ^{ab}	14.6 ± 0.120 ^{b}	14.8 ± 0.82 ^{bc}	18.8 ± 0.80 ^{c}	10.3 ± 0.60 ^{a}
Hb (g dL^{-1})	5.1 ± 0.66 ^{a}	5.4 ± 0.28 ^{ab}	5.9 ± 0.20 ^{ab}	5.9 ± 0.33 ^{ab}	6.8 ± 0.23 ^{b}	6.0 ± 0.30 ^{ab}	6.3 ± 0.15 ^{ab}
MCV (fL)	341.0 ± 0.57	340.0 ± 1.50	343.0 ± 0.88	342.0 ± 0.66	346.0 ± 3.10	337.0 ± 4.50	342.0 ± 2.60
HCT (%)	24.0 ± 2.10 ^{a}	25.0 ± 1.85 ^{ab}	26.0 ± 0.88 ^{ab}	26.0 ± 1.66^{ab}	32.0 ± 1.70 ^{b}	26.0 ± 0.88 ^{ab}	27.0 ± 0.88 ^{ab}
MCH (pg)	77.0 ± 0.66 ^{b}	76.0 ± 0.88 ^{ab}	75.0 ± 0.33 ^{ab}	76.0 ± 0.57 ^{ab}	73.0 ± 0.86 ^{a}	75.0 ± 1.20 ^{ab}	78.0 ± 1.15 ^{b}
MCHC (g dL^{-1})	21.66 ± 0.33	22.33 ± 0.66	22.0 ± 0.00	22.0 ± 0.00	21.5 ± 0.28	22.6 ± 0.33	22.3 ± 0.33
Lymphocytes (%)	74.0 ± 1.1 ^{b}	68.0 ± 1.5 ^{ab}	67.3 ± 2.3 ^{ab}	68.0 ± 2.5 ^{ab}	68.3 ± 0.9 ^{ab}	65.3 ± 0.7 ^{b}	67.3 ± 2.1 ^{ab}
Neutrophils (%)	21.6 ± 1.33 ^{a}	25.3 ± 0.88 ^{ab}	27.0 ± 2.08 ^{ab}	26.6 ± 1.85 ^{ab}	27.0 ± 1.15 ^{ab}	29.3 ± 0.33 ^{b}	27.6 ± 1.45 ^{ab}
Monocytes (%)	4.0 ± 0.33	5.0 ± 0.33	5.0 ± 0.57	4.0 ± 0.33	4.0 ± 0.57	5.0 ± 0.57	4.0 ± 0.88

Values are means ± SEM from triplicate groups. Means in each row with different letters are significantly different (ANOVA, $P < 0.05$). Absence of letters indicates no significant difference between treatments.

Table 7. Blood biochemical parameters in stellate sturgeon (*A. stellatus)* fed diets containing graded levels of soybean lecithin levels for 11 weeks.

	Dietary lecithin levels (%)						
	Control (0)	1	2	4	6	8	10
Glucose (mg dL-1)	39.5 ± 3.1 ^{a}	47.3 ± 0.88 ^{ab}	46.3 ± 2.6 ^{ab}	40.6 ± 1.2 ^{a}	58.0 ± 2.08 ^{bc}	50.3 ± 4.6^{b}	64.3 ± 6.2 ^{c}
Triglyceride (mg dL^{-1})	312 ± 12 ^{a}	419 ± 14 ^{ab}	544 ± 15 ^{bc}	602 ± 100 ^{bc}	587 ± 59 ^{bc}	749 ± 10 ^{c}	734 ± 37 ^{c}
Cholesterol(mg dL^{-1})	30.6 ± 6.6 ^{a}	40.2 ± 2.1 ^{ab}	40.0 ± 11.3 ^{ab}	64.0 ± 11.5 ^{bc}	78.6 ± 2.4 ^{bc}	93.0 ± 7.09 ^{c}	97.6 ± 11.6 ^{d}
HDL cholesterol (mg dL^{-1})	2.6 ± 0.33 ^{a}	5.3 ± 0.88 ^{ab}	6.6 ± 0.66^{bc}	7.0 ± 0.57 ^{bc}	9.0 ± 00 ^{c}	15.5 ± 0.05^{e}	12.0 ± 0.57^{d}
LDL cholesterol (mg dL^{-1})	13.5 ± 1.44^{a}	15 ± 1^{a}	16.6 ± 3.8^{ab}	23.5 ± 3. 7 ^{ab}	26.33 ± 1.4 ^{ab}	23.6 ± 5.3 ^{ab}	30.6 ± 2.7 ^{b}
Total protein (mg mL^{-1})	11.9 ± 0.37^{a}	11.9 ± 0.15^{a}	14.2 ± 1.4^{ab}	17.6 ± 0.26 ^{c}	19.0 ± 0.23 ^{c}	16.3 ± 0.59 ^{bc}	18.4 ± 0.66 ^{c}

Values are means ± SEM from triplicate groups. Values in each row with different letters are significantly different ($P > 0.05$). Absence sof letters indicates no significant difference between treatments). *Abbreviations*: HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol.

4. **Discussion**

Phospholipids are widely used as nutritional supplements in animal feed formulations, and these compounds are essential for the optimal growth and health of animals. Soybean is the main source of natural PLs [33]. Soybean lecithin is composed of a mixture of glycerophospholipids, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), other phosphatides and soybean oil [18, 34]. Analyses of experimental diets showed that highest percentage of PLs were PC followed by PE and PS/PI. In addition, the content of polar lipids increased with higher percentage SBL supplementation, a change that was concomitant with a reduction in the amount of dietary neutral lipids. Contrary to the results reported by Hung and Lutes [17] indicating that sturgeons had no requirements for lecithin; our findings clearly demonstrated the importance of dietary lecithin for optimal growth in juvenile stellate sturgeon fed diets with high levels of vegetal oil sources containing higher PLs levels. Somatic growth performance was poor in fish fed the control diet devoid of SBL, while a significant growth enhancement was observed in fish when corn oil, the main source of fat in compound diets, was substituted with SBL. In particular, final BW, WG and SGR significantly increased at SBL levels higher than 4%, whereas the highest somatic growth was observed in sturgeon fed the diet containing 6% SBL, which might suggest that this value was the optimum SBL inclusion level for stellate sturgeon. Similar trends were also reported in other freshwater and marine fish species fed diets supplemented with PL, including large yellow croaker *Larimichthys crocea* [35], amberjack *Seriola dumerili* [36], rainbow trout *Oncorhynchus mykiss* [37, 38], pikeperch *Sander lucioperca* [39], ayu *Plecoglossus altivelis* [40] and common carp *Cyprinus carpio* [41]. These studies suggested that an improved growth by dietary PLs might be a result of increased feed intake and better efficiency in feed utilization. The poor growth performance observed in the control group could have probably resulted from metabolic disturbances that may be affected by changes in nutrient and metabolic concentrations occurring in the blood [42], as a result of the low inclusion of fish oil (2.5%) in experimental diets. In this context, Mozanzadeh et al. [43] reported significant reduction in SGR and WG when 50% of diet fish oil was replaced by tallow in silvery-black porgy (*Sparidentex hasta*) indicating the importance of fish oil in the diet. Metabolic disturbances in the control fish could have resulted due to receiving feed containing minimal amount of phospholipids and HUFAs. The fatty acid analysis of experimental feeds

showed a minimum HUFA value in the control diet with gradually increasing HUFA levels in feed containing higher inclusions of SBL, which completely supported the above-mentioned hypothesis.

The analysis of immunological data by means of the broken-line regression analysis that was conducted to determine the minimum dietary SBL levels needed for boosting the immune function in stellate juveniles, revealed that depending on the parameter considered, dietary SBL levels varied. In particular, dietary SBL levels requirements in stellate sturgeon when considering the phagocytic activity rate were determined at 3.3%, whereas 4.1-4.2% were recommended when considering data from lysozyme, alternative complement and bactericidal activities. In contrast, the highest minimum content of SBL in diets for stellate sturgeon was estimated at 6.9% when data from total Ig levels were considered. These results indicated that high PL contents are required for boosting innate immunity in this species, although their minimal dietary level changed depending on the immunological parameter considered. Therefore, we assume that SBL levels comprised between 3.3 to 6.9% may be used as a prophylactic measure to improve the health status in stellate sturgeon. In this context, Zhao et al. [44] reported that dietary choline supplementation significantly improved the lysozyme and ACP activities, C3 content, and upregulated antimicrobial peptides in the gills of grass carp (*Ctenopharyngodo nidella)*. Another research also emphasized that acetylcholine, the metabolite of choline, could regulate the expression level of lysozyme in Zhikong scallop *Chlamys farreri* [45]. These data were in agreement with our results on the enhancement of lysozyme and ACP activities in fish fed higher dietary choline levels. Immune related effects of lecithin may be also attributed to its fatty acid levels and composition [46]. Soybean lecithin used in this study contained very high levels of linoleic acid (LA, 18:2n-6) and a gradual increase was observed in linolenic acid (LNA, 18:3n-3) levels as a result of increasing feed SBL content. In brackish and freshwater fish, it is known that both n-3 and n-6 fatty acid PUFA are important nutrients as LA and LNA can be converted to the long chain n-6 and n-3 fatty acids, respectively. The synthesis of ARA is achieved by delta6 desaturation of LA. Synthesis of EPA from LNA requires the same enzymes and pathway as for ARA . Phospholipids are the source of the substrate fatty acids for the formation of eicosanoids, a range of highly bioactive derivatives of, in particular C20 highly unsaturated fatty acids (HUFA), especially arachidonic acid (ARA, 20:4n-6) and eicosapentaenoic acid (EPA, 20:5n-3). Fatty acids released from membrane phospholipids by the action of phospholipase A2 are converted by either

384 cyclooxygenase enzymes, which produces cyclic oxygenated derivatives, collectively called prostanoids,
385 including prostaglandins, prostacyclins and thromboxanes, or lipoxygenase enzymes which produce
386 linear oxygenated derivatives including hydroperoxy- and hydroxyl fatty acids, leukotrienes and lipoxins.
387 Eicosanoids are implicated in many physiological processes including immune and inflammatory
388 responses. The distribution and production of eicosanoids in fish species and tissues and their possible
389 roles have been reviewed previously[47-49]). Based on these facts and the improved fatty acid profile
390 of the experimental diets, we may assume that higher levels of LA, LNA and HUFA in feed containing higher
391 levels of SBL could have stimulated production of higher levels of eicosanoids resulting in improved immune
392 responses in these groups. Similar results were recently reported in different fish species fed different levels of
393 LA [50-52]. Another study found that supplementation of 3.29% PL significantly improved the lysozyme, acid
394 phosphatase activities and complement component 3 contents in all intestinal segments of juvenile grass carp,
395 proving the PL (choline) contributed enhancement of innate immunity in the intestine of fish [53]. Documented
396 literature has confirmed the role of inflammation as a key element in the response of the innate immune system
397 mediated by cytokines [54]. In teleost fish, the pro-inflammatory cytokines, such as tumor necrosis factor α (TNF-
398 α) and interleukin 1β (IL-1β), could initiate and accelerate additional inflammatory processes[55] .The anti-
399 inflammatory cytokines interleukin 10 (IL-10) and transforming growth factor-β (TGF-β) are produced to inhibit
400 the excessive activation of the inflammatory response[55] .According to the findings of Chen et al., 2015[53] the
401 mRNA levels of TNF-α and IL-1β in all intestinal segments of juvenile grass carp significantly down-regulated as
402 the dietary PL levels increased up to 3.29%, whereas 3.29% PL significantly up-regulated the IL-10 and TGF-β1
403 mRNA levels. Based on their findings, we assume that the improved immune responses in juvenile fish
404 including the stellate sturgeon fed optimal PL levels may be partly through down-regulating the TNF-α
405 and IL-1β expression levels and up-regulating the TGF-β1 and IL-10 expression levels. Based on our
406 findings, higher SBL levels containing higher concentration of PLs significantly increased phagocytic
407 activities in blood macrophages. However, we did not observe any significant changes in number of
408 lymphocytes and monocytes among treatments, but the number of neutrophils were significantly
409 increased in fish fed 8% SBL compared to the control group. Similar studies on higher vertebrates
410 (humans and rats) showed a significant improvement in phagocytic activity as a result of dietary soybean
411 PLs [56, 57], which may be attributed to the role of PLs as a source of HUFA for eicosanoid synthesis
412 [58]. In addition, Adel et al. [59] reported significantly higher antibacterial activity against different

pathogenic bacteria like *Streptococcus iniae*, *Yersinia ruckeri*, *Aeromonas hydrophila*, *Lactococcus garviae*, in common carp fed a diet enriched with 3% SBL compared to a control group that was fed lower levels of lecithin. Our results are in agreement to the above-mentioned study, supporting our findings with improved immune system and phagocytic activity in fish fed >3.3% SBL. Differences between the optimal dietary SBL inclusion for enhancing the immune function among different species existed, which may be related to species-specific differences, as well as differences in the nutritional trials, diet formulation, and SBL source and quality. Nevertheless, not all species necessarily respond equally to dietary FO replacements and, although these generalizations may be used as a benchmark, effects of dietary alternative lipid sources should be evaluated on a case-by-case basis.

Blood analysis is a useful, rapid, non-lethal and inexpensive tool for fish monitoring, reliable information on metabolic disorders, deficiencies, adaptation processes to various environmental influences and chronic stress status [60, 61]. Many factors significantly alter haematological parameters in fish, including diet, strain, age, sex, season, method of capture and state of sexual maturity among others [62, 63]. Present results revealed that there was a general trend of increased complete blood count (CBC) values with increasing dietary PL levels; in particular, the highest values of RBC, HTC and Hb were observed in fish fed the diet containing 6% SBL. The higher amount of RBC and Hb concentration could be in response to increased metabolic demand of the body, which was confirmed by the significantly higher somatic growth parameters in sturgeon fed diets containing higher 4% SBL levels. No significant changes were observed in the MCHC, MCV and MCH between experimental and control treatments. However, WBC values were significantly higher in sturgeon fed 6 and 8% SBL compared to the control diet, which also reflected a higher immune condition in these fish groups compared to those fish fed a diet deprived of SBL. These results may be explained as dietary PLs and their unsaturated fatty acids can improve fluidity and permeability of cell membranes and enhance fish immunity [64] .

In the present study, fish fed 4-6% PL levels showed higher glucose, cholesterol and triglyceride levels compared to the control group. Triglycerides (TG) constitute the major class of neutral lipid and they are the primary class for lipid storage and energy provision [48]. The levels of TG are considered to be major indices of the health status of teleost fish [65]. In current work, TG levels increased significantly in fish received those diets containing SBL at higher levels than 2% compared to the control diet. In

addition, our results showed a trend of increase in the CHO levels with increasing dietary SBL levels, being CHO levels higher than in the control group in sturgeon fed >4% SBL. Cholesterol is transported in the circulatory system by means of HDL [66-68] and LDL [69], playing an important role in TG clearance and CHO removal from animal tissues [66, 67]. In this study, fish fed 8% SBL diet had the highest plasma HDL and cholesterol, which may be also in agreement with the higher TG levels found in these groups. The ratio of HDL to total CHO followed a similar trend. In this study, although the LDL levels tended to increase with increasing SBL levels, this increment was only significant in fish fed 10% SBL in comparison to the control group. A possible explanation for the high plasma LDL levels may be related with the effect of acyl-coenzyme A: cholesterol acyltransferase (ACAT), a key hepatic enzyme involved in the esterification of free CHO to cholesterol esters with a preference for unsaturated rather than saturated fatty acids [70]. Juvenile shrimp (*Litopenaeus vannamei*) fed on the 3% SBL diets showed higher triglyceride concentration in serum than those fed on the other experimental diets [71], which goes in the same direction as our findings. Zhou et al [72] reported 50% replacement of fish meal with soybean meal in diet containing 1.5% SBL significantly increased TG levels. Some other studies reported that plasma TG and CHO contents in juvenile yellow drum *Nibea albiflora* increased with the increasing dietary lipid level indicating a more active endogenous lipid transport in response to the higher dietary lipid level [73, 74]. Qin et al. [75] reported a tendency of incremental TG values in orange-spotted grouper (*Epinephelus coioides*) with increasing dietary choline levels. The increase in serum TG and CHO may be due to the fact that the increasing levels of dietary choline can facilitate the synthesis of CHO and TG in the liver and accelerate their transport, resulting in an elevation of their content in the serum. Similar results were also found by Craig and Gatlin [76] in juvenile red drum (*Sciaenops ocellatus*). According to the Sink et al. [77], 2 and 4% SBL inclusion did not affect TG concentration in juvenile channel catfish. However, total lipid content in diets of Sink et al [77] is about 50% of lipid in current work with a different feed formula. Total serum protein (TSP) is considered as a good signal for fish increased immunity [78]. In current study, fish fed diet containing 4-10% SBL showed significantly higher TSP compared to the control and those receiving lower SBL levels, confirming the results of improved immunity in sturgeons from these groups. These results are in agreement with those reported

by Aničić et al. [79], these authors reported a considerable increase in TSP levels in brown bullhead *Ameiurus nebulosus* fed 2.5% SBL.

In conclusion, the optimum SBL inclusion for stellate sturgeon juveniles fed diets containing low levels of fish oil was 6% when somatic growth parameters were considered. Increasing SBL levels above 6% had little practical benefit in terms of growth. The broken-line regression analysis of immunological variable revealed that depending on the parameter considered, the optimal SBL levels in diets for stellate sturgeon juveniles varied. In particular, dietary SBL levels requirements in stellate sturgeon when considering the phagocytic activity rate were determined at 3.3%, whereas 4.1-4.2% were recommended when considering data from lysozyme, alternative complement and bactericidal activities. In contrast, the highest minimum content of SBL in diets for stellate sturgeon was estimated at 6.9% when data from total Ig levels were considered. These results indicated that dietary PLs are required for boosting innate immunity in this species, although their minimal level changed depending on the immunological parameter considered. Therefore, we assume that SBL levels comprised between 3.3 to 6.9% may be used as a prophylactic measure to improve the health status in stellate sturgeon. In addition, hematological parameters indicated that higher dietary levels than 4% SBL promoted the innate immune response in this primitive fish species. Thus, considering data on growth performance and, serological and hematological parameters, it is recommended to include SBL at *ca.* 6% in diets for sturgeon containing low levels of fish oil, being a sound strategy for promoting growth and health resistance in aquafeeds for this group of species.

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References

1. Calder PC. The relationship between the fatty acid composition of immune cells and their function. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2008 79:101-8.
2. MEADE CJ, MERTIN J. Fatty acids and immunity. Advances in lipid research: Elsevier; 1978, p. 127-65.
3. Tocher DR, Bendiksen EÅ, Campbell PJ, Bell JG. The role of phospholipids in nutrition and metabolism of teleost fish. Aquaculture. 2008 280:21-34.
4. Kiron V, Thawonsuwan J, Panigrahi A, Scharsack J, Satoh S. Antioxidant and immune defences of rainbow trout (Oncorhynchus mykiss) offered plant oils differing in fatty acid profiles from early stages. Aquaculture nutrition. 2011 17:130-40.
5. Sun S, Ye J, Chen J, Wang Y, Chen L. 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. Aquaculture Nutrition. 2011 17:441-50.
6. Xu H, Ai Q, Mai K, Xu W, Wang J, Ma H, et al. Effects of dietary arachidonic acid on growth performance, survival, immune response and tissue fatty acid composition of juvenile Japanese seabass, Lateolabrax japonicus. Aquaculture. 2010 307:75-82.
7. Li E, Lim C, Klesius PH, Welker TL. Growth, body fatty acid composition, immune response, and resistance to Streptococcus iniae of hybrid tilapia, Oreochromis niloticus× Oreochromis aureus, fed diets containing various levels of linoleic and linolenic acids. Journal of the World Aquaculture Society. 2013 44:42-55.
8. Zuo R, Ai Q, Mai K, Xu W, Wang J, Xu H, et al. Effects of dietary n-3 highly unsaturated fatty acids on growth, nonspecific immunity, expression of some immune related genes and disease resistance of large yellow croaker (Larmichthys crocea) following natural infestation of parasites (Cryptocaryon irritans). Fish & shellfish immunology. 2012 32:249-58.
9. Montero D, Mathlouthi F, Tort L, Afonso J, Torrecillas S, Fernández-Vaquero A, et al. 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 & shellfish immunology. 2010 29:1073-81.
10. Thompson K, Tatner M, Henderson R. Effects of dietary (n-3) and (n-6) polyunsaturated fatty acid ratio on the immune response of Atlantic salmon, Salmo salar L. Aquaculture Nutrition. 1996 2:21-31.
11. Mourente G, Good JE, Thompson KD, Bell JG. 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. 2007 98:770-9.
12. Stuart JS, Hung SS. Growth of juvenile white sturgeon (Acipenser transmontanus) fed different proteins. Aquaculture. 1989 76:303-16.
13. Şener E, Yildiz M, Savaş E. Effects of dietary lipids on growth and fatty acid composition in Russian sturgeon (Acipenser gueldenstaedtii) juveniles. Turkish Journal of Veterinary and Animal Sciences. 2005 29:1101-7.
14. Wen H, Yan A, Gao Q, Jiang M, Wei Q. Dietary vitamin A requirement of juvenile Amur sturgeon (Acipenser schrenckii). Journal of Applied Ichthyology. 2008 24:534-8.
15. Xu Q, Xu H, Wang C, Zheng Q, Sun D. Studies on dietary phosphorus requirement of juvenile Siberian sturgeon Acipenser baerii. Journal of Applied Ichthyology. 2011 27:709-14.
16. Hung SS. Recent advances in sturgeon nutrition. Animal Nutrition. 2017.

17. Hung SS, Lutes PB. A preliminary study on the non-essentiality of lecithin for hatchery-produced juvenile white sturgeon (Acipenser transmontanus). Aquaculture. 1988 68:353-60.
18. Scholfield C. Composition of soybean lecithin. Journal of the American Oil Chemists' Society. 1981 58:889-92.
19. Lepage G, Roy CC. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. Journal of Lipid research. 1984 25:1391-6.
20. Whaley K, North J. Haemolytic assays for whole complement activity and individual components. AW Dodds, and RB Sim, eds. Complement, A Practical Approach 1st. Oxford Univ. Press, Oxford; 1997.
21. Siwicki AK, Anderson DP, Rumsey GL. Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. Veterinary immunology and immunopathology. 1994 41:125-39.
22. Kruger N. The bradford method forproten quantitation In: WalkerJM, ed. The Protein Protocols Handbook NewJersey: Human PressInc. 1996 20.
23. Hultmark D, STEINER H, Rasmuson T, Boman HG. Insect immunity. Purification and properties of three inducible bactericidal proteins from hemolymph of immunized pupae of Hyalophora cecropia. European Journal of Biochemistry. 1980 106:7-16.
24. Secombes CJ. Isolation of salmonid macrophages and analysis of their killing activity. Techniques in fish immunology. 1990 1:137-54.
25. Mehrzad J, Duchateau L, Burvenich C. Phagocytic and bactericidal activity of blood and milk-resident neutrophils against Staphylococcus aureus in primiparous and multiparous cows during early lactation. Veterinary microbiology. 2009 134:106-12.
26. Blaxhall P, Daisley K. Routine haematological methods for use with fish blood. Journal of fish biology. 1973 5:771-81.
27. Snieszko S. Microhaematocrit as a tool in fisheries management. Special Scientific Report, no. 314. US Department of the Interior. Fish and Fisheries Wildlife, Washington, DC. 1960.
28. Seiverd CE. Hematology for Medical Technologists. Academic Medicine. 1964 39:867.
29. Klontz G. Fish hematology. Techniques in fish immunology. 1994 3:121-31.
30. Rowley AF. Collection, separation and identification of fish leucocytes. Techniques in fish immunology. 1990 1:113-36.
31. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry. 1976 72:248-54.
32. Lamberson W, Firman J. A comparison of quadratic versus segmented regression procedures for estimating nutrient requirements. Poultry science. 2002 81:481-4.
33. Joachim WH, Felicitas P-P. Handbook on ingredients for aquaculture feeds. Dordrecht, The Natherlands: Kluwer academics publishers. 2000.
34. Smith J, Hong-Shum L. Food additives data book: John Wiley & Sons; 2011.
35. Zhao J, Ai Q, Mai K, Zuo R, Luo Y. Effects of dietary phospholipids on survival, growth, digestive enzymes and stress resistance of large yellow croaker, Larmichthys crocea larvae. Aquaculture. 2013 410:122-8.
36. Uyan O, Koshio S, Ishikawa M, Yokoyama S, Uyan S, Ren T, et al. The influence of dietary phospholipid level on the performances of juvenile amberjack, Seriola dumerili, fed non-fishmeal diets. Aquaculture nutrition. 2009 15:550-7.
37. Poston HA. Performance of rainbow trout fry fed supplemental soy lecithin and choline. The Progressive Fish-Culturist. 1990 52:218-25.

578 38. Poston HA. Response of Atlantic salmon fry to feed-grade lecithin and choline. The Progressive Fish-
579 Culturist. 1991 53:224-8.
580 39. Hamza N, Mhetli M, Khemis IB, Cahu C, Kestemont P. Effect of dietary phospholipid levels on
581 performance, enzyme activities and fatty acid composition of pikeperch (Sander lucioperca) larvae. Aquaculture.
582 2008 275:274-82.
583 40. Kanazawa A, Teshima S-I, Sakamoto M. Effects of dietary bonito-egg phospholipids and some
584 phospholipids on growth and survival of the larval ayu, Plecoglossus altivelis. Zeitschrift für angewandte
585 Ichthyologie. 1985 1:165-70.
586 41. Geurden I, Radünz-Neto J, Bergot P. Essentiality of dietary phospholipids for carp (Cyprinus carpio L.)
587 larvae. Aquaculture. 1995 131:303-14.
588 42. Lee SM. Review of the lipid and essential fatty acid requirements of rockfish (Sebastes schlegeli).
589 Aquaculture Research. 2001 32:8-17.
590 43. Mozanzadeh MT, Agh N, Yavari V, Marammazi JG, Mohammadian T, Gisbert E. Partial or total
591 replacement of dietary fish oil with alternative lipid sources in silvery-black porgy (Sparidentex hasta).
592 Aquaculture. 2016 451:232-40.
593 44. Zhao H-F, Jiang W-D, Liu Y, Jiang J, Wu P, Kuang S-Y, et al. Dietary choline regulates antibacterial activity,
594 inflammatory response and barrier function in the gills of grass carp (Ctenopharyngodon idella). Fish & shellfish
595 immunology. 2016 52:139-50.
596 45. Wu P, Jiang J, Liu Y, Hu K, Jiang W-D, Li S-H, et al. Dietary choline modulates immune responses, and gene
597 expressions of TOR and eIF4E-binding protein2 in immune organs of juvenile Jian carp (Cyprinus carpio var. Jian).
598 Fish & shellfish immunology. 2013 35:697-706.
599 46. Fracalossi DM, Craig-Schmidt MC, Lovell RT. Effect of dietary lipid sources on production of leukotriene
600 B by head kidney of channel catfish held at different water temperatures. Journal of Aquatic Animal Health. 1994
601 6:242-50.
602 47. Sargent JR. Ether-linked glycerides in marine animals. Marine biogenic lipids, fats and oils. 1989 1:175-
603 97.
604 48. Tocher DR. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in fisheries science.
605 2003 11:107-84.
606 49. Tocher DR. Glycerophospholipid metabolism. Biochemistry and molecular biology of fishes: Elsevier;
607 1995, p. 119-57.
608 50. Li M, Chen L, Li E, Yu N, Ding Z, Chen Y, et al. Growth, immune response and resistance to Aeromonas
609 hydrophila of darkbarbel catfish, Pelteobagrus vachelli (Richardson), fed diets with different linolenic acid levels.
610 Aquaculture Research. 2015 46:789-800.
611 51. Zhang W, Sun S, Ge X, Xia S, Zhu J, Miao L, et al. Effects of dietary linolenic acid on growth, fatty acid
612 composition, immune function and antioxidant status of juvenile blunt snout bream, Megalobrama
613 amblycephala. Aquaculture Research. 2017 48:5430-8.
614 52. Zeng Y-Y, Jiang W-D, Liu Y, Wu P, Zhao J, Jiang J, et al. Dietary alpha-linolenic acid/linoleic acid ratios
615 modulate intestinal immunity, tight junctions, anti-oxidant status and mRNA levels of NF-κB p65, MLCK and Nrf2
616 in juvenile grass carp (Ctenopharyngodon idella). Fish & shellfish immunology. 2016 51:351-64.
617 53. Chen Y-P, Jiang W-D, Liu Y, Jiang J, Wu P, Zhao J, et al. Exogenous phospholipids supplementation
618 improves growth and modulates immune response and physical barrier referring to NF-κB, TOR, MLCK and Nrf2
619 signaling factors in the intestine of juvenile grass carp (Ctenopharyngodon idella). Fish & shellfish immunology.
620 2015 47:46-62.

621 54. Han J, Ulevitch RJ. Limiting inflammatory responses during activation of innate immunity. Nature
622 immunology. 2005 6:1198.
623 55. Verburg-Van Kemenade BL, Stolte EH, Metz JR, Chadzinska M. Neuroendocrine–immune interactions in
624 teleost fish. Fish physiology. 2009 28:313-64.
625 56. Jannace PW, Lerman RH, Santos JI, Vitale JJ. Effects of oral soy phosphatidylcholine on phagocytosis,
626 arachidonate concentrations, and killing by human polymorphonuclear leukocytes. The American journal of
627 clinical nutrition. 1992 56:599-603.
628 57. Miranda DT, Batista VG, Grando FC, Paula FM, Felício CA, Rubbo GF, et al. Soy lecithin supplementation
629 alters macrophage phagocytosis and lymphocyte response to concanavalin A: a study in alloxan-induced diabetic
630 rats. Cell biochemistry and function. 2008 26:859-65.
631 58. Smith WL. The eicosanoids and their biochemical mechanisms of action. Biochemical Journal. 1989
632 259:315.
633 59. Adel M, Gholaghaie M, Khanjany P, Citarasu T. Effect of dietary soybean lecithin on growth parameters,
634 digestive enzyme activity, antioxidative status and mucosal immune responses of common carp (Cyprinus
635 carpio). Aquaculture Nutrition. 2017.
636 60. Maita M. Fish health assessment. Dietary supplements for the health and quality of cultured fish CAB
637 International, Washington. 2007:10-34.
638 61. Peres H, Santos S, Oliva-Teles A. Blood chemistry profile as indicator of nutritional status in European
639 seabass (Dicentrarchus labrax). Fish physiology and biochemistry. 2014 40:1339-47.
640 62. McCarthy D, Stevenson J, Roberts M. Some blood parameters of the rainbow trout (Salmo gairdneri
641 Richardson). Journal of Fish Biology. 1973 5:1-8.
642 63. Kori-Siakpere O, Ake J, Idoge E. Haematological characteristics of the African snakehead, Parachanna
643 obscura. African Journal of Biotechnology. 2005 4:527-30.
644 64. Balfry SK, Higgs DA. Influence of dietary lipid composition on the immune system and disease resistance
645 of finfish. Nutrition and fish health. 2001:213-34.
646 65. Wagner T, Congleton JL. Blood chemistry correlates of nutritional condition, tissue damage, and stress in
647 migrating juvenile chinook salmon (Oncorhynchus tshawytscha). Canadian Journal of Fisheries and Aquatic
648 Sciences. 2004 61:1066-74.
649 66. Blum CB, Levy RI, Eisenberg S, Hall III M, Goebel RH, Berman M. High density lipoprotein metabolism in
650 man. Journal of Clinical Investigation. 1977 60:795.
651 67. Eisenberg S. High density lipoprotein metabolism. Journal of lipid research. 1984 25:1017-58.
652 68. Suzukawa M, Abbey M, Howe P, Nestel PJ. Effects of fish oil fatty acids on low density lipoprotein size,
653 oxidizability, and uptake by macrophages. Journal of lipid research. 1995 36:473-84.
654 69. Hevonoja T, Pentikäinen MO, Hyvönen MT, Kovanen PT, Ala-Korpela M. Structure of low density
655 lipoprotein (LDL) particles: basis for understanding molecular changes in modified LDL. Biochimica et Biophysica
656 Acta (BBA)-Molecular and cell biology of lipids. 2000 1488:189-210.
657 70. Levy E, Mehran M, Seidman E. Caco-2 cells as a model for intestinal lipoprotein synthesis and secretion.
658 The FASEB Journal. 1995 9:626-35.
659 71. Hu Y, Tan B, Mai K, Ai Q, Zhang L, Zheng S. Effects of dietary menhaden oil, soybean oil and soybean
660 lecithin oil at different ratios on growth, body composition and blood chemistry of juvenile Litopenaeus
661 vannamei. Aquaculture International. 2011 19:459-73.
662 72. ZHOU QC, MAI KS, TAN BP, LIU YJ. Partial replacement of fishmeal by soybean meal in diets for juvenile
663 cobia (Rachycentron canadum). Aquaculture Nutrition. 2005 11:175-82.

664 73. DU ZY, LIU YJ, TIAN LX, WANG JT, Wang Y, LIANG GY. Effect of dietary lipid level on growth, feed utilization
665 and body composition by juvenile grass carp (Ctenopharyngodon idella). Aquaculture Nutrition. 2005 11:139-46.
666 74. Babin PJ, Vernier J. Plasma lipoproteins in fish. Journal of Lipid Research. 1989 30:467-89.
667 75. Qin D, Dong X, Tan B, Yang Q, Chi S, Liu H, et al. Effects of dietary choline on growth performance, lipid
668 deposition and hepatic lipid transport of grouper (Epinephelus coioides). Aquaculture Nutrition. 2017 23:453-9.
669 76. Craig SR, Gatlin III DM. Dietary choline requirement of juvenile red drum (Sciaenops ocellatus). The
670 Journal of nutrition. 1996 126:1696.
671 77. Sink TD, Lochmann RT. The Effects of Soybean Lecithin Supplementation to a Practical Diet Formulation
672 on Juvenile Channel Catfish, Ictalurus punctatus: Growth, Survival, Hematology, Innate Immune Activity, and
673 Lipid Biochemistry. Journal of the World Aquaculture Society. 2014 45:163-72.
674 78. Coeurdacier J-L, Dutto G, Gasset E, Blancheton J-P. Is total serum protein a good indicator for welfare in
675 reared sea bass (Dicentrarchus labrax)? Aquatic Living Resources. 2011 24:121-7.
676 79. Aničić I, Treer T, Matulić D, Safner R, Tomljanović T, Piria M, et al. Effects of dietary vitamin C and soybean
677 lecithin in the nutrition of brown bullhead (Ameiurus nebulosus L.) fingerlings. Italian Journal of Animal Science.
678 2013 12:e27.

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