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

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22 ABSTRACT

23 An eleven weeks feeding trial was conducted to determine the effects of different levels of dietary
24 soybean lecithin (SBL) on growth performance, blood chemistry and immunity in juvenile stellate
25 sturgeon (*Acipenser stellatus*). Fish were fed seven isoproteic (44% crude protein) and isolipidic (17%
26 crude fat) diets containing graded levels of SBL: 0 (control), 1, 2, 4, 6, 8 and 10%. Results showed that
27 dietary SBL supplementation significantly improved the final body weight (BW) and weight gain (WG).
28 Fish fed 6% SBL showed the highest BW and WG values in comparison to fish fed the control diet ($P <$
29 0.05), whereas increasing SBL levels above 6% had little practical benefit in terms of somatic growth
30 performance. The inclusion of SBL in diets significantly improved the immune response as data from
31 lysozyme, total Ig levels, alternative complement, phagocytic and bactericidal activities indicated ($P <$
32 0.05). The broken-line regression analysis of immunological variable revealed that depending on the
33 parameter considered, the optimal SBL levels in diets for stellate sturgeon juveniles varied. In particular,
34 dietary SBL levels requirements in stellate sturgeon when considering the phagocytic activity rate were
35 determined at 3.3%, whereas 4.1-4.2% were recommended when considering data from lysozyme,
36 alternative complement and bactericidal activities. In contrast, the highest minimum dietary SBL content
37 was estimated at 6.9% when data from total Ig levels were considered. These results indicated that dietary
38 PLs are required for boosting innate immunity in stellate sturgeon, although their minimal level changed
39 depending on the immunological parameter considered. Therefore, we assume that SBL levels comprised
40 between 3.3 to 6.9% may be used as a prophylactic measure to improve the health status in stellate
41 sturgeon. Red blood cell count, hemoglobin and hematocrit levels increased with increasing dietary SBL
42 levels, especially in those sturgeons fed the diet with 6% SBL ($P < 0.05$). In addition, white blood cell
43 counts significantly increased as dietary SBL levels increased from 4 to 8% in comparison to the control
44 group. Blood biochemistry was also affected by different dietary SBL levels. In particular, significantly
45 higher levels of glucose, cholesterol, HDL and triglycerides were detected in fish fed >6%, >4%, >2%
46 and 2% SBL, respectively ($P < 0.05$). Based on somatic growth parameters, blood chemistry and systemic
47 immunity parameters, diets containing *ca.* 6% SBL are recommended for juvenile stellate sturgeon.
48 Key words: Soybean lecithin, *Acipenser stellatus*, growth, immune response, blood biochemistry.

49

50 **1. Introduction**

51 It has been reported that lipids play an important role in the immune system [1, 2]. Among lipid
52 components, phospholipids (PL) are important components for maintaining the structure and function of
53 cellular membranes, emulsifying lipids in the gut and improving intestinal absorption of long chain fatty
54 acids [3]. Phospholipids are a source of fatty acids for the synthesis of eicosanoids, a wide range of
55 bioactive compounds with multiple functions. It has been reported that the composition of dietary fatty
56 acids influenced the non-specific immunity (e.g. phagocytosis, respiratory burst and serum lysozyme)
57 [4-6] and specific immunity (e.g. antibody production and resistance to pathogens) [7-10] and eicosanoid
58 production [9, 11]. The optimal level of dietary phospholipid supplementation depends on the species,
59 developmental stage, culture conditions, and PL source. In this regard, soybean lecithin (SBL) due to its
60 market availability and relatively stable composition has been commercially used as a convenient source
61 of PL in aquafeeds, although some studies dealing with larvae have used marine phospholipid sources
62 [3].

63 Among the fish species living in the Caspian Sea, sturgeons are of utmost interesting from an
64 economic perspective, not only for their caviar, but also for the meat. However, all sturgeon species
65 inhabiting the Caspian Sea are highly vulnerable and endangered, and the stellate sturgeon (*Acipenser*
66 *stellatus*) is not an exception. Based on catch data and the number of individuals migrating into the Volga
67 and Ural rivers, it is estimated that the species has undergone a population decline of at least 80%
68 (possibly close to 100%) in the past three generations, which is expected to continue. Consequently, this
69 species is classified in the IUCN Red List of Threatened Species as critically endangered, and it is
70 highlighted that its survival will only depend on restocking activities and effective fishery management
71 plans. Thus, during the last decades a lot of interest has been developed for sturgeon aquaculture,
72 regardless of the final purpose of this activity: restocking and conservation of wild population or
73 production for human consumption. Under culture conditions, the nutritional requirements on protein
74 [12], lipid [13], carbohydrate [12] and trace elements [14, 15] have been studied in various sturgeon
75 species. However, there is scarce information about the PL requirements in Acipenserides [16]. The only
76 available study on SBL requirements in this group of primitive fish reported that white sturgeon
77 (*Acipenser transmontanus*) had no requirements for lecithin, but there was a requirement for choline in

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

84

85 ***2. Materials and Methods***

86 ***2.1. Experimental diets and experimental design***

87 The formulation of the experimental diets was conducted by means of the WUFFDA software (Lindo[®]
88 1995, Release 6.1). Seven diets were formulated to be isonitrogenous (44% crude protein) and isolipidic
89 (17% crude fat) (Table 1). Defatted fish meal and corn gluten were the main protein sources in the
90 experimental diets, while lipid sources included soybean lecithin, fish oil and corn oil. Different PL levels
91 in diets were achieved by adding SBL at different levels (0, 1, 2, 4, 6, 8 and 10%) at the expense of corn
92 oil (Tables 1 and 2). Soybean lecithin contained: 19-21% phosphatidylcholine, 8-20%
93 phosphatidylethanolamine, 20-21% phosphatidylinositol, 5-11% other phosphatides and 33-35% soy
94 bean oil [18]. All dry ingredients were weighed and mixed for 30 min, then fish and corn oils and SBL
95 were added, followed by addition of distilled water and mixed thoroughly. Once the desired consistency
96 was reached, the mixture was then mechanically pelleted to obtain suitable sized pellets (3 mm). The
97 pellets were dried in a convection oven at 35 °C and stored in re-sealable plastic bags at 4°C until use.
98 Diets were tested by triplicate during 75 days. The fatty acid composition of diets was analyzed by gas
99 chromatography (Agilent 7890A GC System, USA) using a BP×70 capillary glass column (0.32 mm ×
100 50 m, SGE Analytical Science Australia) after esterification in acetyl-chloride/methanol mixture. Fatty
101 acid methyl esters were prepared by the modified procedure of Lepage and Roy [19]. The phospholipid
102 profile of the experimental feed were analyzed using Densitometer GS900 calibrated (Bio Rad,
103 Germany).

104 Table 1. Ingredient list and proximate composition (%) of experimental diets containing graded levels of
 105 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

106
 107 ^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,
 108 300,000, 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
 109 mg; Vit B9, 600 mg; Vit. B12, 750 µg; Vit. C, 35,000 mg; Vit. H2, 600 mg. ATA Company, Tabriz, Iran.
 110 ^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,
 111 300 mg; Cobalt, 50mg; Natrium, 5,000mg. ATA Company, Tabriz, Iran.

112 Table 2. Fatty acid profile of experimental diets containing graded levels of soybean lecithin (g kg⁻¹ dry
 113 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

114 Nd: Not detected

115
 116
 117
 118
 119
 120

121 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

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

126

127 2.3. Fish and sampling procedures

128 Juvenile *A. stellatus* were obtained from Shahid Beheshti sturgeon fish hatchery in Rasht, located in
 129 Northern Province of Iran, Guilan. Prior to the feeding trials, all fish were acclimated to the indoor rearing
 130 conditions for 3 weeks fed a local commercial feed from Fradane Company, Esfahan (Iran) and live
 131 *Artemia* nauplii. Twenty-six fish with initial body weight (BW) of 11.3 ± 0.05 g were stocked in each
 132 90-L polycarbonate tank containing 80 L ground water at the flow rate of 1.0 L min^{-1} . Photoperiod of 12
 133 h light:12 h dark was maintained throughout the experiment. Water temperature, dissolved oxygen and
 134 pH were maintained at 18.9 ± 0.5 °C, $8.5 \pm 0.5 \text{ mg L}^{-1}$ and 8.02 ± 0.11 (mean \pm standard error of the
 135 mean, SEM), respectively throughout the experiment. Fish were fed experimental diets at apparent
 136 satiation at 08:00, 11:00, 14:00 and 17:00 h for 75 days.

137

138

139 **2.4. Growth performance**

140 At the end of the experiment, fish were fasted for 24 h and then weighed to the nearest 0.1 g and measured
141 to the nearest 1 mm (total length TL) to determine their somatic growth performance. The following
142 formulae were used to evaluate body growth performance: weight gain (WG, %) = $(BW_f - BW_i) / BW_i$
143 $\times 100$; specific growth rate (SGR; % day⁻¹) = $[(\ln BW_f - \ln BW_i) / t] \times 100$; where BW_f is the final body
144 weight, BW_i is the initial body weight and t is the length of the experimental period (42 days); survival
145 (S, %) = (number of fish in each group remaining at day 75 / initial number of fish) $\times 100$, and Fulton's
146 condition factor (K) = $(BW / TL^3) \times 100$.

147

148 **2.5. Immunological analysis**

149 Three specimens from each replicate were anaesthetized with 200 mg L⁻¹ clove powder and blood was
150 collected from the caudal vein with sterilized syringes, and transferred immediately into sterile tubes and
151 allowed to clot at room temperature for 1 h. Supernatants were separated by centrifugation (3,000 \times g for
152 5 min at 4°C) and stored at -80 °C until analysis.

153

154 **2.5.1 Serum alternative complement**

155 Alternative complement activity (ACH50) was assayed based on the hemolysis of rabbit red blood cells
156 (RaRBC) as described by Willey et al. [20]. The RaRBC were washed three times in ethylene glycol tetra
157 acetic acid magnesium-gelatin veronal buffer (0.01 M EGTA-Mg-GVB, pH 7) and the cell numbers were
158 adjusted to 2×10^8 cells mL⁻¹ in the same buffer. At first, the 100% lysis value was obtained by adding
159 100 mL of the above RaRBC to 3.4 mL distilled water. The hemolysate was centrifuged and the optical
160 density (OD) of the supernatant was determined at $\lambda = 414$ nm using a spectrophotometer (Awareness,
161 USA). Following, the serum was diluted (100 times), and different volumes ranging from 100 to 250 mL
162 (total volume was adjusted to 250 mL with the buffer) were allowed to react with 100 mL of RaRBC in
163 small test tubes. These mixtures were incubated at 20 °C for 90 min with intermittent mixing, and then

164 3.15 mL of 0.85% NaCl solution was added and tubes were centrifuged at $1,600 \times g$ for 10 min at 4°C .
165 The OD of the supernatant was measured at $\lambda = 414$ nm. A lysis curve was obtained by plotting the
166 percentage of haemolysis against the volume of serum added on a log-log graph. The volume yielding
167 50% haemolysis was used for determining the complement activity of the sample as follows: ACH50
168 $(\text{Units mL}^{-1}) = K \times [(\text{reciprocal of the serum dilution}) \times 0.5]$, where K is the amount of serum (mL) giving
169 50% lysis and 0.5 is the correction factor since the assay was performed on half scale of the original
170 method.

171

172 **2.5.2 Serum total immunoglobulin**

173 Total immunoglobulin was assayed following the method of Siwicki et al. [21]. Serum samples were
174 diluted with 0.85% NaCl (100 times) and total protein content was determined by the Bradford method
175 [22]. One hundred mL of total serum was mixed with an equal volume of 12% solution of polyethylene
176 glycol (Sigma-Aldrich Corporation, St Louis, MI, USA) in wells of a 96-well micro titer plate. Following
177 2 h of incubation at room temperature, the microplate was centrifuged at $5000 \times g$ at 4°C . The supernatant
178 was diluted 50 times with 0.85% of NaCl and the protein content was determined by Bradford method
179 [22]. This value was subtracted from the total protein level and the result was equal to the total
180 immunoglobulin concentration of the serum (mg mL^{-1}).

181

182 **2.5.3 Lysozyme activity**

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

190

191 **2.5.4 Phagocytic activity**

192 Macrophage isolation was done as described by Secombes [24] with slight modifications. Briefly, 2 mL
193 blood samples were taken by a heparinized syringe from the caudal vein and gently mixed with 3 mL
194 ice-cold Leibovitz L15 medium (Sigma-Aldrich) containing 2% fetal calf serum (FCS, Sigma-Aldrich),
195 heparin (10 IU mL⁻¹, Sigma-Aldrich), and penicillin (100 IU mL⁻¹) / streptomycin (100 µg mL⁻¹) (Merck,
196 Germany). The cell suspension was layered over a 51% Percoll (Sigma-Aldrich) and centrifuged at 400
197 ×g for 25 minutes at 4°C to remove erythrocyte contamination and cell debris. The macrophages isolated
198 from the L15 medium/Percoll interface were washed twice by centrifugation at 400 ×g for 5 minutes in
199 L15 medium and adjusted to 5 ×10⁶ viable macrophages per mL of L15 medium supplemented with 5%
200 FCS and penicillin/streptomycin.

201 Phagocytosis was measured according to the method of Mehrzad et al [25] following the isolation
202 of blood macrophages from three fish per tank as described above. Blood samples were plated in 96-well
203 flat-bottomed plates in RPMI 1640 medium supplemented with 10% fetal calf serum (1×10⁵ cells 100
204 µL⁻¹ per well) and stimulated with 50 µL PHA solution (1 mg mL⁻¹) or medium alone. After 72 hr of
205 incubation, cultures were pulsed with 20 µL of the MTT solution (5 mg mL⁻¹) for 4 hr at 37°C. Then,
206 150 µL DMSO were added and shaken vigorously to dissolve Formosan crystals. Values of DO were
207 measured at λ = 550 nm in a microplate reader (Dynatech, Denkendorf, Germany). Analyses were done
208 in triplicate sets (methodological replicates). Results were expressed as the proliferation index according
209 to the ratio of OD (λ = 550) of stimulated cells with MOG35-55 to OD (λ = 550) of non-stimulated cells.

210

211 **2.6 Hematological parameters**

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

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

222

223 ***2.7 Serum biochemical analysis***

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

228

229 ***2.8. Statistical analyses***

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

238

239

240 **3. Results**

241 **3.1. Growth performance**

242 At the end of the trial, growth performance in terms of BW, SGR and WG in sturgeon fed diets containing
 243 4, 6, 8 and 10% SBL was higher compared to the control group and those fish fed diets with 1 and 2%
 244 SBL (Table 4, $P < 0.05$). The highest BW, SGR and WG were registered in the fish fed diets containing
 245 from 6 to 10% SBL. No statistically significant differences were found in survival nor K between
 246 experimental groups ($P > 0.05$).

247

248 Table 4. Growth performance in stellate sturgeon (*A. stellatus*) fed graded levels of soybean lecithin for
 249 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
BW _f (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 ± 0.04 ^{bc}	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

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

252

253

254

255 **3.2. Humoral immune parameters**

256 Alternative complement activity was similar in fish fed the control diet and those diets containing 1 and
 257 2% SBL, whereas higher dietary inclusion of SBL significantly increased ACH50 values (Table 5, $P <$
 258 0.05). Considering the broken-line regression method, the optimal dietary SBL in relation to ACH50
 259 values was 4.1% (Fig. 1a). Lysozyme activity was significantly higher in fish fed from 4 to 10% SBL
 260 compared to the control group, whereas the rest of dietary treatments showed intermediate values
 261 (Table 5, $P <$ 0.05). Considering the broken-line regression method, the optimal dietary SBL in relation
 262 to lysozyme activity values was 4.2% (Fig. 1b). The highest levels of serum total antibody were found
 263 in sturgeon fed 4, 6 and 8% SBL; whereas the lowest values were recorded in fish fed 0, 1 and 2%
 264 SBL. Sturgeon fed 10% SBL showed intermediate levels between former groups (Table 5, $P <$ 0.05).
 265 Considering the broken-line regression method, the optimal dietary SBL in relation to total antibody
 266 levels was 6.9% (Fig. 1c).

267

268 Table 5. Immune parameters (lysozyme, alternative complement, total immunoglobulin levels, and
 269 bactericidal and phagocytic activities) in stellate sturgeon (*A. stellatus*) fed graded levels of soybean
 270 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}	

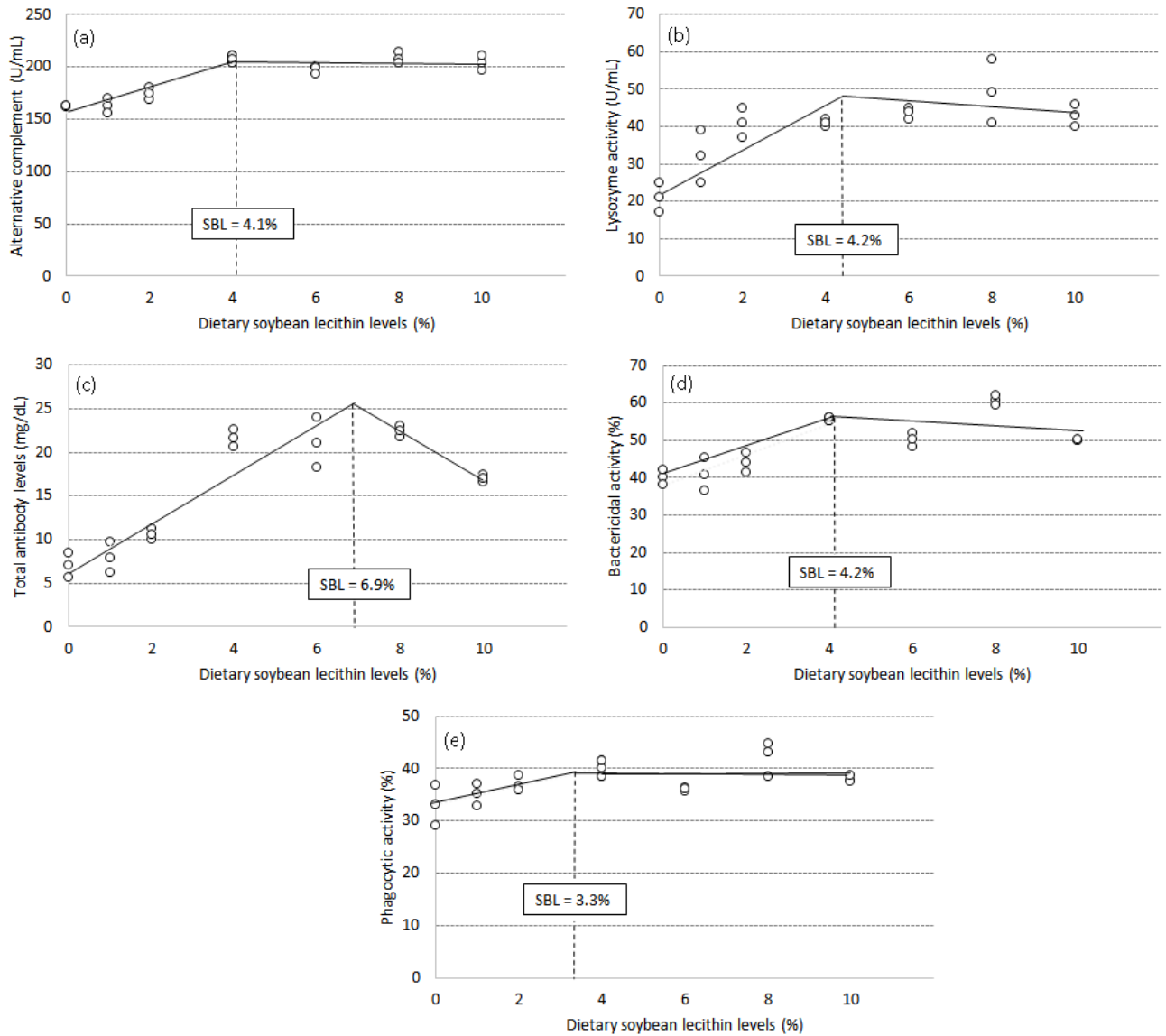
271 Values are mean ± SEM from triplicate groups. Means in each row with different letters are significantly different ($P <$ 0.05).

272

273 **3.3 Phagocytic and bactericidal activity**

274 The phagocytic and bacterial killing rates were significantly higher in sturgeon fed 4 and 8% SBL
275 compared to control, whereas the rest of dietary treatments showed intermediate values (Table 5, $P <$
276 0.05). Taking into consideration the results obtained from the broken-line regression analysis, the
277 optimal dietary SBL regarding phagocytic and bactericidal activities were 3.3 and 4.2%, respectively
278 (Fig. 1d, e)

279



280

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.

284

285

286 3.4. Blood haematology and biochemistry

287 Hematological parameters were significantly affected by dietary SBL ($P < 0.05$, Table 6). Red blood
288 cells increased in sturgeon fed SBL, but the highest RBC were found in fish fed the 6% SBL diet ($P <$
289 0.05). White blood cells increased significantly in fish fed 4, 6 and 8% SBL in comparison to the control
290 group, whereas the rest of dietary treatments showed intermediate values of WBC between the above-
291 mentioned groups ($P < 0.05$). Hemoglobin (Hb) and Hematocrit (HCT) were significantly higher in fish
292 fed 6% SBL in comparison to the control group that showed the lowest levels, whereas the rest of dietary
293 groups showed intermediate levels ($P < 0.05$). Dietary SBL levels did not affect the mean corpuscular
294 hemoglobin concentration (MCHC) and the mean corpuscular volume (MCV) levels ($P > 0.05$).
295 However, the mean corpuscular hemoglobin levels (MCH) were significantly influenced by the dietary
296 SBL levels, being the lowest MCH values found in fish fed 6% SBL and the highest ones in the control
297 and 10% SBL groups ($P < 0.05$). Monocyte counts were not statistically significant different among fish
298 fed different SBL levels, but neutrophils and lymphocytes were significantly higher in sturgeon h fed 8
299 % SBL compared to the control devoid of SBL (Table 6, $P < 0.05$).

300 Serum biochemistry was significantly affected by different dietary SBL levels (Table 7, $P <$
301 0.05). Sturgeon fed the diets containing 10% SBL showed the highest blood glucose levels, whereas fish
302 fed the control diet showed the lowest one. The rest of dietary treatments showed intermediate values (P
303 < 0.05). The highest cholesterol and LDL levels were found in sturgeon fed 10% SBL, whereas
304 cholesterol levels decreased as the level of SBL inclusion decreased in experimental diets ($P < 0.05$). In
305 addition, fish fed 8 and 10% SBL showed the highest content of triglycerides in blood in comparison to
306 the control diet ($P < 0.05$). Fish fed 8% SBL had the highest HDL content, whereas the lowest HDL level
307 was found in fish fed control diet ($P < 0.05$). Lowest plasma total protein levels were found in sturgeon
308 fed 0, 1 and 2% SBL, whereas sturgeon fed from 4 to 10% SBL showed higher protein levels in plasma
309 ($P < 0.05$).

310

311

312

313 Table 6. Hematological parameters in stellate sturgeon (*A. stellatus*) fed diets containing graded levels of
 314 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

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

316 Absence of letters indicates no significant difference between treatments.

317

318

319 Table 7. Blood biochemical parameters in stellate sturgeon (*A. stellatus*) fed diets containing graded
 320 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

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

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328 4. Discussion

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

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

358 The analysis of immunological data by means of the broken-line regression analysis that was
359 conducted to determine the minimum dietary SBL levels needed for boosting the immune function in
360 stellate juveniles, revealed that depending on the parameter considered, dietary SBL levels varied. In
361 particular, dietary SBL levels requirements in stellate sturgeon when considering the phagocytic activity
362 rate were determined at 3.3%, whereas 4.1-4.2% were recommended when considering data from
363 lysozyme, alternative complement and bactericidal activities. In contrast, the highest minimum content
364 of SBL in diets for stellate sturgeon was estimated at 6.9% when data from total Ig levels were considered.
365 These results indicated that high PL contents are required for boosting innate immunity in this species,
366 although their minimal dietary level changed depending on the immunological parameter considered.
367 Therefore, we assume that SBL levels comprised between 3.3 to 6.9% may be used as a prophylactic
368 measure to improve the health status in stellate sturgeon. In this context, Zhao et al. [44] reported that
369 dietary choline supplementation significantly improved the lysozyme and ACP activities, C3 content,
370 and upregulated antimicrobial peptides in the gills of grass carp (*Ctenopharyngodon idella*). Another
371 research also emphasized that acetylcholine, the metabolite of choline, could regulate the expression level
372 of lysozyme in Zhikong scallop *Chlamys farreri* [45]. These data were in agreement with our results on
373 the enhancement of lysozyme and ACP activities in fish fed higher dietary choline levels. Immune related
374 effects of lecithin may be also attributed to its fatty acid levels and composition [46]. Soybean lecithin
375 used in this study contained very high levels of linoleic acid (LA, 18:2n-6) and a gradual increase was
376 observed in linolenic acid (LNA, 18:3n-3) levels as a result of increasing feed SBL content. In brackish
377 and freshwater fish, it is known that both n-3 and n-6 fatty acid PUFA are important nutrients as LA and
378 LNA can be converted to the long chain n-6 and n-3 fatty acids, respectively. The synthesis of ARA is
379 achieved by delta6 desaturation of LA. Synthesis of EPA from LNA requires the same enzymes and
380 pathway as for ARA. Phospholipids are the source of the substrate fatty acids for the formation of
381 eicosanoids, a range of highly bioactive derivatives of, in particular C20 highly unsaturated fatty acids
382 (HUFA), especially arachidonic acid (ARA, 20:4n-6) and eicosapentaenoic acid (EPA, 20:5n-3). Fatty
383 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 1 β (IL-1 β), 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-1 β 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- β 1
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-1 β expression levels and up-regulating the TGF- β 1 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

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

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

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

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

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

470 In conclusion, the optimum SBL inclusion for stellate sturgeon juveniles fed diets containing
471 low levels of fish oil was 6% when somatic growth parameters were considered. Increasing SBL levels
472 above 6% had little practical benefit in terms of growth. The broken-line regression analysis of
473 immunological variable revealed that depending on the parameter considered, the optimal SBL levels in
474 diets for stellate sturgeon juveniles varied. In particular, dietary SBL levels requirements in stellate
475 sturgeon when considering the phagocytic activity rate were determined at 3.3%, whereas 4.1-4.2%
476 were recommended when considering data from lysozyme, alternative complement and bactericidal
477 activities. In contrast, the highest minimum content of SBL in diets for stellate sturgeon was estimated
478 at 6.9% when data from total Ig levels were considered. These results indicated that dietary PLs are
479 required for boosting innate immunity in this species, although their minimal level changed depending
480 on the immunological parameter considered. Therefore, we assume that SBL levels comprised between
481 3.3 to 6.9% may be used as a prophylactic measure to improve the health status in stellate sturgeon. In
482 addition, hematological parameters indicated that higher dietary levels than 4% SBL promoted the
483 innate immune response in this primitive fish species. Thus, considering data on growth performance
484 and, serological and hematological parameters, it is recommended to include SBL at *ca.* 6% in diets for
485 sturgeon containing low levels of fish oil, being a sound strategy for promoting growth and health
486 resistance in aquafeeds for this group of species.

487

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494

495 **References**

- 496 1. Calder PC. The relationship between the fatty acid composition of immune cells and their function.
497 Prostaglandins, Leukotrienes and Essential Fatty Acids. 2008 79:101-8.
- 498 2. MEADE CJ, MERTIN J. Fatty acids and immunity. *Advances in lipid research*; Elsevier; 1978, p. 127-65.
- 499 3. Tocher DR, Bendiksen EÅ, Campbell PJ, Bell JG. The role of phospholipids in nutrition and metabolism of
500 teleost fish. *Aquaculture*. 2008 280:21-34.
- 501 4. Kiron V, Thawonsuwan J, Panigrahi A, Scharsack J, Satoh S. Antioxidant and immune defences of rainbow
502 trout (*Oncorhynchus mykiss*) offered plant oils differing in fatty acid profiles from early stages. *Aquaculture*
503 *nutrition*. 2011 17:130-40.
- 504 5. Sun S, Ye J, Chen J, Wang Y, Chen L. Effect of dietary fish oil replacement by rapeseed oil on the growth,
505 fatty acid composition and serum non-specific immunity response of fingerling black carp, *Mylopharyngodon*
506 *piceus*. *Aquaculture Nutrition*. 2011 17:441-50.
- 507 6. Xu H, Ai Q, Mai K, Xu W, Wang J, Ma H, et al. Effects of dietary arachidonic acid on growth performance,
508 survival, immune response and tissue fatty acid composition of juvenile Japanese seabass, *Lateolabrax japonicus*.
509 *Aquaculture*. 2010 307:75-82.
- 510 7. Li E, Lim C, Klesius PH, Welker TL. Growth, body fatty acid composition, immune response, and resistance
511 to *Streptococcus iniae* of hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus*, fed diets containing various
512 levels of linoleic and linolenic acids. *Journal of the World Aquaculture Society*. 2013 44:42-55.
- 513 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
514 growth, nonspecific immunity, expression of some immune related genes and disease resistance of large yellow
515 croaker (*Larimichthys crocea*) following natural infestation of parasites (*Cryptocaryon irritans*). *Fish & shellfish*
516 *immunology*. 2012 32:249-58.
- 517 9. Montero D, Mathlouthi F, Tort L, Afonso J, Torrecillas S, Fernández-Vaquero A, et al. Replacement of
518 dietary fish oil by vegetable oils affects humoral immunity and expression of pro-inflammatory cytokines genes
519 in gilthead sea bream *Sparus aurata*. *Fish & shellfish immunology*. 2010 29:1073-81.
- 520 10. Thompson K, Tatner M, Henderson R. Effects of dietary (n-3) and (n-6) polyunsaturated fatty acid ratio
521 on the immune response of Atlantic salmon, *Salmo salar* L. *Aquaculture Nutrition*. 1996 2:21-31.
- 522 11. Mourente G, Good JE, Thompson KD, Bell JG. Effects of partial substitution of dietary fish oil with blends
523 of vegetable oils, on blood leucocyte fatty acid compositions, immune function and histology in European sea
524 bass (*Dicentrarchus labrax* L.). *British Journal of Nutrition*. 2007 98:770-9.
- 525 12. Stuart JS, Hung SS. Growth of juvenile white sturgeon (*Acipenser transmontanus*) fed different proteins.
526 *Aquaculture*. 1989 76:303-16.
- 527 13. Şener E, Yildiz M, Savaş E. Effects of dietary lipids on growth and fatty acid composition in Russian
528 sturgeon (*Acipenser gueldenstaedtii*) juveniles. *Turkish Journal of Veterinary and Animal Sciences*. 2005 29:1101-
529 7.
- 530 14. Wen H, Yan A, Gao Q, Jiang M, Wei Q. Dietary vitamin A requirement of juvenile Amur sturgeon
531 (*Acipenser schrenckii*). *Journal of Applied Ichthyology*. 2008 24:534-8.
- 532 15. Xu Q, Xu H, Wang C, Zheng Q, Sun D. Studies on dietary phosphorus requirement of juvenile Siberian
533 sturgeon *Acipenser baerii*. *Journal of Applied Ichthyology*. 2011 27:709-14.
- 534 16. Hung SS. Recent advances in sturgeon nutrition. *Animal Nutrition*. 2017.

- 535 17. Hung SS, Lutes PB. A preliminary study on the non-essentiality of lecithin for hatchery-produced juvenile
536 white sturgeon (*Acipenser transmontanus*). *Aquaculture*. 1988 68:353-60.
- 537 18. Scholfield C. Composition of soybean lecithin. *Journal of the American Oil Chemists' Society*. 1981 58:889-
538 92.
- 539 19. Lepage G, Roy CC. Improved recovery of fatty acid through direct transesterification without prior
540 extraction or purification. *Journal of Lipid research*. 1984 25:1391-6.
- 541 20. Whaley K, North J. Haemolytic assays for whole complement activity and individual components. AW
542 Dodds, and RB Sim, eds. *Complement, A Practical Approach* 1st. Oxford Univ. Press, Oxford; 1997.
- 543 21. Siwicki AK, Anderson DP, Rumsey GL. Dietary intake of immunostimulants by rainbow trout affects non-
544 specific immunity and protection against furunculosis. *Veterinary immunology and immunopathology*. 1994
545 41:125-39.
- 546 22. Kruger N. The Bradford method for protein quantitation In: Walker JM, ed. *The Protein Protocols*
547 *Handbook* New Jersey: Human Press Inc. 1996 20.
- 548 23. Hultmark D, STEINER H, Rasmuson T, Boman HG. Insect immunity. Purification and properties of three
549 inducible bactericidal proteins from hemolymph of immunized pupae of *Hyalophora cecropia*. *European Journal*
550 *of Biochemistry*. 1980 106:7-16.
- 551 24. Secombes CJ. Isolation of salmonid macrophages and analysis of their killing activity. *Techniques in fish*
552 *immunology*. 1990 1:137-54.
- 553 25. Mehrzad J, Duchateau L, Burvenich C. Phagocytic and bactericidal activity of blood and milk-resident
554 neutrophils against *Staphylococcus aureus* in primiparous and multiparous cows during early lactation.
555 *Veterinary microbiology*. 2009 134:106-12.
- 556 26. Blaxhall P, Daisley K. Routine haematological methods for use with fish blood. *Journal of fish biology*.
557 1973 5:771-81.
- 558 27. Snieszko S. Microhaematocrit as a tool in fisheries management. Special Scientific Report, no. 314. US
559 Department of the Interior. Fish and Fisheries Wildlife, Washington, DC. 1960.
- 560 28. Seiverd CE. Hematology for Medical Technologists. *Academic Medicine*. 1964 39:867.
- 561 29. Klontz G. Fish hematology. *Techniques in fish immunology*. 1994 3:121-31.
- 562 30. Rowley AF. Collection, separation and identification of fish leucocytes. *Techniques in fish immunology*.
563 1990 1:113-36.
- 564 31. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein
565 utilizing the principle of protein-dye binding. *Analytical biochemistry*. 1976 72:248-54.
- 566 32. Lamberson W, Firman J. A comparison of quadratic versus segmented regression procedures for
567 estimating nutrient requirements. *Poultry science*. 2002 81:481-4.
- 568 33. Joachim WH, Felicitas P-P. *Handbook on ingredients for aquaculture feeds*. Dordrecht, The Netherlands:
569 Kluwer academics publishers. 2000.
- 570 34. Smith J, Hong-Shum L. *Food additives data book*: John Wiley & Sons; 2011.
- 571 35. Zhao J, Ai Q, Mai K, Zuo R, Luo Y. Effects of dietary phospholipids on survival, growth, digestive enzymes
572 and stress resistance of large yellow croaker, *Larimichthys crocea* larvae. *Aquaculture*. 2013 410:122-8.
- 573 36. Uyan O, Koshio S, Ishikawa M, Yokoyama S, Uyan S, Ren T, et al. The influence of dietary phospholipid
574 level on the performances of juvenile amberjack, *Seriola dumerili*, fed non-fishmeal diets. *Aquaculture nutrition*.
575 2009 15:550-7.
- 576 37. Poston HA. Performance of rainbow trout fry fed supplemental soy lecithin and choline. *The Progressive*
577 *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- κ B 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- κ B, 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.
- 679
- 680