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Phospholipids improve the performance, physiological, antioxidative responses and, *lpl* and *igf1* gene expressions in juvenile stellate sturgeon (*Acipenser stellatus*)

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Abstract

The effects of dietary phospholipids (PL) on the performance of juvenile stellate sturgeon (*Acipenser stellatus*) was evaluated in terms of growth and feed efficiency parameters, muscle and liver fatty acid profiles, activity of digestive and antioxidative stress enzymes, and expression of lipoprotein lipase (*lpl*) and insulin-like growth factor (*igf1*). For this purpose, seven isoproteic (44% crude protein) and isolipidic (17% crude fat) diets containing graded levels of soybean lecithin (0, 1, 2, 4, 6, 8 and 10%) were prepared, resulting in 0.3, 0.9, 1.6, 2.7, 3.9, 5.3 and 5.4% of dietary PLs, respectively. At the end of the nutritional study (75 days), we found that there was a positive quadratic polynomial response between growth performance parameters and dietary PLs; somatic growth increased with increasing dietary PL levels up to 3.9% when growth parameters remained stable. Dietary PLs reduced the accumulation of fat stores in the liver and up-regulated the expression of the lipoprotein lipase gene, confirming the important role of this enzyme in incorporating plasma lipids into tissues, whereas the activities of CAT and SOD showed a positive linear increase with dietary PL levels. Increasing dietary PLs from 0.9 to 3.9% promoted the activity of gastric (pepsin) and pancreatic (trypsin, chymotrypsin, bile salt-activated lipase and α-amylase) enzymes, whereas higher inclusion levels of PLs did not provide any advantage in terms of *A. stellatus* digestive capacities. The analysis of fatty acid profiles in diets and selected tissues (liver and muscle) showed the capacity of *A. stellatus* juveniles to desaturate and elongate linoleic (C18:2n–6) and alpha-linolenic (C18:3n–3) acids to arachidonic (C20:4n-6), eicosapentaenoic (C20:5n-3) and docosahexaenoic (C22:6n-3) acids. Based on the results of growth performance, feed efficiency and physiological parameters, the inclusion of 3.9% of PLs in compound diets for juvenile stellate sturgeon are recommended**.**

36 *Keywords:* sturgeon; phospholipids; soybean lecithin; growth performance; digestive enzymes;
37 oxidative stress; lipoprotein lipase.

38

39 **1. Introduction**

40 The aquaculture of sturgeon fish has attracted considerable attention worldwide due to the continuous
41 decrease in fishery yields (Bronzi et al., 2011; Agh et al., 2012; Kalbassi et al., 2013; Ruban et al.,
42 2019), being their artificial production the most logical and practical alternative to reduce the fishing
43 pressure on these group of species and promote their conservation. The Ponto-Caspian basin is one of
44 the most important areas of sturgeon fisheries in the world, where several countries have developed
45 large-scale stocking programs (Dabrovici and Patriche, 1999; Abdolhay and Tahori, 2006; Peterson et
46 al., 2007).

47 The culture of any organism for either conservation or meat production requires a deep
48 knowledge and understanding of its nutritional requirements. In particular, the macronutrient
49 requirements of different sturgeon species were recently reviewed by Hung (2017). In this sense, the
50 optimal dietary lipid levels for different sturgeon species were reported to range from 11 to 26% of
51 crude lipid, values that depended on the species, stage of development and diet formulation. However,
52 fish capacity to digest and utilize lipids is a function of the physical state of the lipid source, the
53 structural form of the lipid (*i.e.*, waxes, sterols, phospholipids and triglycerides) and its fatty acid
54 composition, as well as its digestive capacity (gut morphology and enzyme activities) (Trushenski and
55 Lochmann, 2009). Although lesser digestible than triglycerides, dietary phospholipids (PLs) are
56 nonetheless important for meeting dietary requirements for this nutrient class and for enhancing lipid
57 digestion and absorption via the emulsifying action of the byproducts of PL hydrolysis (Tocher et al.,

58 2008) among other important functions. In particular, dietary PLs are important elements of aquafeeds,
59 especially at larval and juvenile stages when there is limited capacity of PL *de novo* synthesis (Tocher
60 et al., 2008). Furthermore, PLs have been also associated to improvements in growth performance and
61 survival, incidence of skeletal deformities, digestive physiology and resistance to stress at early life
62 stages (Cahu et al., 2009). As Tocher et al. (2008) reviewed; PL requirements in fish larvae are lower
63 in freshwater species like carp (*Cyprinus carpio*) (2%) and ayu (*Plecoglossus altivelus*) (3-5%) in
64 comparison to marine ones such as Japanese flounder (*Paralichthys olivaceus*) (7%), red bream
65 (*Pagrus major*) and knife jaw (*Oplegnathus fasciatus*) (5–7%). In juveniles, the values ranged from
66 around 1.5–7% of diet, including 1.5% for striped jack (*Pseudocaranx dentex*), 2–3% for European
67 seabass (*Dicentrarchus labrax*) and around 4–6% for Atlantic salmon (*Salmo salar*), and 7% for *P.*
68 *olivaceus.* Regardless of their nutritional importance, there exist contradictory results when evaluating
69 the effects of different PL levels on sturgeon performance (Hung and Lutes, 1988; Jafari et al., 2018).
70 For instance, whereas Hung and Lutes (1988) reported that white sturgeon (*Acipenser transmontanus*)
71 fry had no requirements on PLs, recent data from *Acipenser stellatus* juveniles showed a clear
72 relationship between dietary PL levels and innate immunity (Jafari et al., 2018). In this sense,
73 physiological and molecular responses to dietary PLs were reported to vary between fry and early
74 juvenile stages in rainbow trout (*Oncorhynchus mykiss*) (Daprà et al., 2011), which may potentially
75 explain the former disagreement between results found in *A. transmontanus* and *A. stellatus* among
76 other factors like diet composition, rearing conditions among others. Thus, further research is needed
77 in order to proper evaluate the effects of dietary PLs on key performance indicators in fish.

78 The efficiency of a given diet on growth performance and feed efficiency depends on the
79 capacity of the organism to digest and absorb dietary nutrients, although other factors may also
80 influence the processes related to uptake, digestion and nutrition absorption (Furné et al., 2008).

81 Regarding PLs, lipoprotein lipase (LPL) plays an important role in triglyceride hydrolysis and
82 chylomicrons and very low-density lipoproteins synthesis and transport (Mead and Ramji, 2002;
83 Saera-Vila et al., 2005), which directly influences the accumulation of lipids in tissues (Gisbert et al.,
84 2005). This is of special relevance since dietary lipid composition influences tissue composition and,
85 in turn, the nutritional value of the resultant fillets to the human consumer (Trushenski and Lochmann,
86 2009). In addition, fish fed PL-deficient diets may have impaired lipid transport from the intestine
87 and/or liver to other tissues and consequently, result in steatosis in those tissues where dietary lipids
88 are absorbed or accumulated (Caballero et al., 2002; 2004; Gisbert et al., 2005; Morais et al., 2006).
89 Additionally, the antioxidant system involves several enzymes like catalase (CAT) and superoxide
90 dismutases (SODs) among others, that act in detoxifying the reactive oxygen species (ROS), as well as
91 reduce the levels of lipid peroxidation in tissues (Mourente et al., 2002).

92 Considering the limited information on the physiological effects of dietary PLs on fish
93 performance, as well as the contradictory results regarding the dietary requirements on PLs in different
94 sturgeon species; this study is focused on evaluating the effects of dietary graded levels of PLs
95 (soybean lecithin) in terms of growth and feed efficiency performance, body proximate composition
96 and fatty acid profile of target tissues (muscle and liver), as well as changes in lipid accumulation in
97 the liver and intestine, digestive enzyme activities and modulation of *lpl* expression in *A. stellatus*
98 juveniles.

99

100 2. Materials and methods

101 *2.1. Experimental fish*

102 Juvenile *A. stellatus* specimens (*N* = 167) weighting 11.3 ± 0.05 g (mean ± standard deviation) in body

103 weight (BW) were obtained from the Central Sturgeon Hatchery in Rasht (Iran), and once in the

104 research facilities of the Artemia and Aquaculture Research Institute (Urmia, Iran), they were stocked

105 in 21 polyethylene circular tanks (functional volume: 80 L). Tanks were supplied with of ground

106 freshwater (flow rate = 1.0 L min^{-1}). Fish were reared under a photoperiod of 12 h light:12 h darkness,

107 whereas water temperature, pH and dissolved oxygen levels were kept at 18.9 ± 0.5 °C, 8.02 ± 0.11

108 and 8.5 ± 0.5 mg L^{-1} respectively, as tanks were connected to an open-flow water system. Fish were

109 hand-fed at apparent satiation four times a day at 08:00, 11:00, 14:00 and 17:00 h during the 75 days

110 that the nutritional trial lasted. Before each meal, uneaten feed pellets in the bottom of the tanks were

111 counted and their weight subtracted from the daily feed ration values in order to calculate the daily

112 feed intake of fish fed different experimental diets.

113

114 *2.2. Experimental diets*

115 Seven experimental diets were formulated to be isonitrogenous (*ca.* 44% protein) and isolipidic (*ca.* 17

116 % fat) (Table 1). Fishmeal, defatted with organic solvents like *n*-hexane and ethanol, and corn gluten

117 were used as the main protein sources, while soybean lecithin (SBL), fish oil and corn oil were used as

118 lipid sources in experimental diets. Graded dietary PL levels in experimental diets were obtained by

119 replacing corn oil by SBL at 0, 1, 2, 4, 6, 8 and 10%, which resulted in final PL content of 0.3, 0.9, 1.6,

120 2.7, 3.9, 5.3 and 5.4%, respectively. Unexpectedly, the inclusion of the highest level of SBL did not

121 result in the expected crude lipid and PL levels in the SBL10 diet (Table 1). Such disagreement

122 between the theoretical and real values of these ingredients might have occurred during diet

123 preparation when mixng the blend of fish and corn oils, which also resulted in changes in fatty acids

124 profile in the SBL10 diet. The composition of the SBL used in this study was 74.4% of PLs and 25.6%
125 of neutral lipids, as indicated in Table 1. Diets (3 mm diameter of pellet size) were prepared as
126 previously described by Jafari et al. (2018). The pellets were dried at 35 °C for 4-6 hours and stored at
127 4 °C until use. Fatty acid profiles of the experimental diets (Table 2) and target tissues were analyzed
128 using gas chromatography (Agilent 7890A GC System, USA) equipped with a FID detector and a
129 cyanopropyl-phenyl capillary column (DB-225MS, Agilent, USA) following the direct methyl
130 esterification method as described in Lepage and Roy (1984). A GLC-D mixed fatty acid methyl esters
131 was used as a standard for fatty acid identification and quantification.

132

133 *2.3. Fish performance*

134 At the end of the experimental period (75 days), fish were fasted for 24 h before sampling for final BW
135 and dissection of different organs for analytical purposes. Fish were anaesthetized with 200 mg L^{-1}
136 clove powder and their BW measured to the nearest 0.1 g and then, they were sacrificed with an
137 overdose of the anesthetic for tissue sampling purposes. The following equations were used to
138 calculate growth and feed performances, as well as body condition:

139 Body weight gain (WGR, %) = $(BW_f - BW_i) / BW_i \times 100$;

140 Specific growth rate SGR (%) = $[(\ln BW_f - \ln BW_i) / t] \times 100$, where t = 75 days;

141 Feed conversion ratio (FCR) = feed intake (g) / weight gain (g);

142 Feed intake (FI, g $fish^{-1}$) = (total feed intake per tank (g) / number of fish);

143 Hepatosomatic index (HSI, %) = (liver weight (g) / BW_f (g) $\times$ 100;

144 Viscerosomatic index (VSI, %) = (visceral weight (g) / BW_f (g)) × 100.

145 Protein efficiency ratio (PER) = Gain in body mass (g) / total protein intake (g)

146 Lipid efficiency ratio (LER) = Gain in body mass g) / total lipid intake (g)

147

148 *2.4. Diet and body composition analyses*

149 In order to evaluate the effects of dietary treatments on juvenile *A. stellatus* body proximate
150 composition in terms of crude proteins, lipids and ash, three fish per tank were pooled and dried at 105
151 °C in an electric oven for 24 h until constant weight for determining their moisture content, and then,
152 ground for further biochemical analyses. Ash content was determined with a muffle furnace (Iran
153 Khodsaz, Iran) at 600 °C for 6 h; crude protein (N × 6.25) was estimated using an automatic Kjeldahl
154 analyser (Behrotest WD 40, Germany). Crude lipid content in whole body, liver and muscle was
155 extracted by the diethyl ether method and determined according to AOAC (1990). The fatty acid
156 profiles of the liver and muscle were analyzed as previously described for feed samples. Lipid class
157 composition was determined by high-performance thin-layer chromatography as described in Olsen
158 and Henderson (1989). In brief, a wet weight of 10 µg of lipid was applied as a 2 mm streak and the
159 plate developed to two-thirds distance with methyl acetate/isopropanol/chloroform/methanol/0.25%
160 aqueous KCl (25:25:25:10:9, volume/volume, v/v), to separate polar lipid classes, and then fully
161 developed with isohexane/diethyl ether/acetic acid (85:15:1, v/v). Lipid classes were visualized by
162 charring at 160 ºC for 15 min after spraying with 3% (w/v) aqueous cupric acetate containing 8% (v/v)
163 phosphoric acid and quantified by densitometry using a GS-800 Densitometer (Bio-Rad Laboratories,
164 Spain).The identities of individual lipid classes were confirmed by comparison with lipid standards.

165

166 *2.5. Analysis of digestive and oxidative stress enzymes*

167 Once sacrificed, the whole digestive tract (*N* = 9 per diet) was dissected on an ice block (0-4 °C), and
168 the mid-posterior intestine, stomach, pyloric caeca and liver were separated from the rest of digestive
169 system, rinsed in distilled water and homogenized (15,000 rpm, 3 × 30 sec) in ice cold 50 mM Tris-
170 HCl buffer, pH 7.5 (1:3 weight to volume) using Polytron PT 1300 D homogenizer (Kinematica AG,
171 Littau-Lucerne, Switzerland). The homogenate was centrifuged at 10,000 × *g* for 20 min at 4 ºC and
172 the supernatant collected and used as crude extract to analyze the activity of different digestive
173 enzymes (Chong et al., 2002). Homogenates were prepared and analyzed following the
174 recommendations from Solovyev and Gisbert (2016) in order to avoid the potential loss of enzyme
175 activities. Moreover, the stomach was homogenized in five volumes of 10 mM HCl and centrifuged at
176 15,000 × *g* and 4 ºC for 60 min. The crude enzyme extract was collected and stored at -80 ºC for
177 enzymatic determinations. Alpha-amylase activity was measured following (Bernfeld, 1955); this
178 method measures the rate of maltose releasing from starch by its ability to reduce 3,5-dinitrosalicylic
179 acid (DNS). In particular, enzyme homogenates were incubated with a starch solution (1% w/v)
180 dissolved in 0.02 M phosphate buffer containing 0.006M NaCl (incubation time = 4 min; temperature
181 = 25 ºC; pH = 6.9). Then, 0.5 mL of 1% DNS solution was added to the crude enzyme extract and the
182 sample boiled for 5 min, cooled and the absorbance of the solution measured (λ = 540 nm). The
183 amount of maltose produced was measured using maltose standard curve. The activity of bile salt-
184 activated lipase was measured following the method described by Ijima et al. (1988). In brief, the
185 enzyme homogenate was incubated in a 250 mM Tris-HCl buffer (pH = 9) containing 5.2 mM sodium
186 cholate during 15 min at room temperature (25 ºC) in 20 mM p-nitrophenyle myristate as substrate.
187 Enzyme activity (U) was defined as the μmol of substrate hydrolyzed per min and mL of enzyme

188 extract measured at λ = 405. The activity of the serine protease trypsin was measured using
189 benzoylarginine-p-nitroanilide (BAPNA, Sigma-Aldrich Chemie Gmbn, Munich, Germany) as a
190 substrate in 50 mM Tris–HCl containing 20mM$CaCl_2$ buffer (incubation time = 15 min; temperature =
191 25 ºC; pH = 7.8) and changes in absorbance measured at λ = 410 nm (Erlanger et al., 1961).
192 Chymotrypsin activity was assayed using 0.1 mM succinyl-(Ala)2-pro-phe-p-nitroanilide (SAPNA,
193 Sigma-Aldrich) as substrate in 50 mM Tris-HCl buffer containing 20 mM $CaCl_2$ (incubation time = 3
194 min; temperature = 25 ºC; pH = 8.5). The activity of this serine endopeptidase corresponded to the
195 μmol SAPNA hydrolyzed per min and mL (Erlanger et al. 1961). The activity of the acid protease
196 pepsin was determined following the method of Rungruangsak and Utne (1981). In brief, 1% casein
197 dissolved in 60 mM HCl was used as a substrate and the mixture with the enzyme homogenate
198 incubated at 37 ºC for 10 min. Then, the reaction was stopped by adding 1mL of 5% TCA; the mixture
199 centrifuged at 5,000 × *g* for 20 min, and 1 mL of 0.5 M NaOH added to 0.5 mL of the supernatant,
200 including 0.3 mL of Folin-Ciocalteu reagent (1:3 dilution). After 10 min at room temperature, the
201 absorbance of the mixture was read at λ = 720 nm and compared with a standard curve of L-tyrosine.
202 Pepsin specific activity (U) was expressed as mmol L-tyrosine h^{-1} mg^{-1} protein. The activity of all
203 assayed enzymes was expressed as specific activity (U mg $protein^{-1}$). Soluble protein in enzyme
204 extracts was quantified by means of the Bradford's method (Bradford, 1976), using bovine serum
205 albumin as standard.

206 For blood sample collection, nine fish from each treatment (three fish for each replicate) were
207 anesthetized using clove powder (200 mg L^{-1}). Blood was taken from the caudal vein by means of 1
208 mL syringes, transferred into non-heparinized tubes and centrifuged (10 min at 3000 × *g*) at 4 °C.
209 Then, serum samples were stored at -80 ^{O}C until they were used for evaluating lipid peroxidation
210 levels and the activity of selected oxidative stress enzymes (Jafari et al., 2018). Lipid peroxidation

211 levels were measured using the thiobarbituric acid (TBA) assay kit (ZellBio GmbH, Germany), which
212 determines the quantity of malondialdehyde in serum samples. In addition, catalase (CAT) levels in
213 serum were determined according to Goth (1991). In particular, serum samples (0.2 mL) were
214 incubated in 65 μM hydrogen peroxide in 60 mM phosphate-buffered saline at 37 °C and stopped by the
215 addition of 1 mL of 32 mM ammonium molybdate the optical density of the solution read at λ = 405 nm (Goth,
216 1991). The levels of superoxide dismutase (SOD) in serum samples were determined by measuring the
217 inhibition rates of pyrogallol auto-oxidation in presence of hydrogen peroxide at λ = 420 nm. One unit
218 of SOD activity was expressed as the mg protein causing 50% inhibition of pyrogallol oxidation under
219 the experimental conditions (Marklund and Marklund, 1974). All enzyme (digestive and oxidative
220 stress) and lipid peroxidation measurements were done in triplicate (methodological replicates) using a
221 microplate reader (Biotek Synergy HT, USA).

222

223 *2.5 Histological analysis of target tissues*

224 The histological description of the intestine and liver were used to describe nutritionally-induced
225 changes in tissue organization or accumulation of lipids, as these tissues respond rapidly to qualitative
226 and quantitative changes in the diet (Gisbert et al., 2008). For this purpose, intestine and liver samples
227 collected from six fish from each treatment and fixed in Bouin's solution. Tissues were embedded in
228 paraffin and thin sections (5–6 μm) were cut with a microtome (Leitz 1212 rotary microtome, Leyca
229 Biosystems) and stained with hematoxylin–eosin. All sections were photographed by a digital camera
230 (Olympus DP70, Olympus) and the images (300 dpi) were processed using image analysis software
231 (ANALYSIS; Soft Imaging Systems GmbH), and intestinal and fat deposits were identified as
232 unstained vacuoles within hepatocytes (Gisbert et al., 2017).

233

234 *2.6 Gene expression analyses*

235 Primers used for lipoprotein lipase (*lpl*) and insulin growth factor 1 (*igf1*) gene quantification in livers
236 of *A. stellatus* were designed using Primer3 software (Rozen and Skaletsky, 2000). In particular,
237 forward and reverse primers for *lpl* were designed using homolog sequences from Siberian sturgeon
238 (*A. baerii*; KJ720972), Russian sturgeon (*A. gueldenstaedtii*; KT207937.1) and Chinese sturgeon (*A.*
239 *sinensis*; FJ436088.1), whereas primers for *igf1* were designed using sequences from Persian sturgeon
240 (*A. persicus*; GU3256229.2), *A. ruthenus* (XM_034032301.1) and *A. baerii* (DQ329352.1). Elongation
241 factor 1a (*efla*) was used as a house-keeping gene in order to normalize gene expression values
242 (Akbarzadeh et al., 2013), since it did not exhibit any significant variation in expression levels among
243 the samples. Primer sequences are shown in Supplementary Table 1.

244 Total RNA from livers ($N = 9$ per dietary treatment) were isolated using the RNA extraction kit
245 (CinnaGen, Iran) according to the instructions provided by the manufacturer. First-strand cDNA was
246 synthesized from 1 µg of DNase I-treated total RNA and random hexamer primers using a
247 RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, K1622, USA). Extracted RNA was
248 quantified using Nanodrop spectrophotometer (Pico200, Picodrop Co., UK) and RNA integrity was
249 evaluated by electrophoresis on 1% agarose gel. Quantification of gene expression by means of real-
250 time PCR was done using SYBR Green Real-time PCR Master Mix (CinnaGen, Iran) in a Rotor-
251 Gen3000 real-time PCR Detection system (Corbett Research, Australia). Each sample was analyzed in
252 triplicate (methodological replicates) in a final well volume of 20 µL containing 1 µL cDNA and 10
253 µL of the SYBR Green reaction mix (Cinagene, Iran), 1 µL of each primer (10 mmol L^{-1}) and 7 µL
254 RNase/DNase-free water. Negative controls (non-template control) were systematically included in

255 each plate. The thermal conditions used were 3 min at 95 °C of preincubation followed by 40 cycles at
256 95 °C for 20 s and 60 °C for 30 s; an additional temperature ramping step from 65 to 95 °C was
257 included to produce melting curves. Melting curve analysis of the PCR products was performed for
258 validating the specific amplification for each amplicon, whereas the efficiency of the primer pairs were
259 calculated using the slope of a standard curve over 4-fold serial dilutions (1:10) of the pooled cDNA
260 samples. The threshold cycle (CT) was analyzed using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak,
261 2008).

262

263 *2.7 Statistical analysis*

264 Differences in key performance indicators (*e.g.*, somatic growth performance, FCR, body condition
265 indices, proximate composition and fatty acid profiles, as well as activity of digestive and oxidative
266 stress enzymes) among experimental diets differing in the PL content were analyzed with one-way
267 analysis of variance (ANOVA). The significant variation among experimental groups was decomposed
268 in a linear relationship with the PL content of the diet and a residual component (deviation) with
269 polynomial orthogonal contrasts (Sokal and Rohlf, 1995) and the SPSS "metric" option (SPSS Inc.,
270 2019). By default, polynomial contrasts assume equally-spaced levels, whereas with the metric option
271 unequal spacing for the factor levels (*i.e.*, real distances between phospholipid contents) may be
272 specified (SPSS Inc., 2019). Measured values for each level of the factor were used to describe the
273 differences among diets (see Alcaraz and García-Berthou, 2007). In addition to *P* values, the partial *eta*
274 squared (η_p^2) was used as a measure of effect size (*i.e.*, importance of factor). Similar to regression
275 coefficient (r^2), η_p^2 is the proportion of variation explained for a certain effect, and it has the advantage
276 over *eta* squared of not depending on the number of sources of variation used in the ANOVA; thus, it

277 could be compared among different designs (Tabachnick et al., 2007). In contrast to *P* value, η_p^2 has
278 the advantage that allows the proper comparison of treatments; a lower *P* value does not necessarily
279 mean that a factor has stronger effect (Alcaraz et al., 2008, 2015). For all the dietary descriptors the
280 model residuals were tested for normality by means of the Kolmogorov-Smirnov normality test; the
281 residuals of all descriptors were normally distributed ($P \geq 0.10$). The homogeneity of the variances
282 among groups was assessed using Levene tests, and verified with a mean *vs.* standard deviation plot.
283 All statistical analyses were performed with SPSS 26.0 (IBM SPSS Statistics).

284

285 **3. Results**

286 *3.1. Growth, somatic and feed performance indicators*

287 Juveniles' growth performance significantly differed among diets (ANOVA; $P < 0.05$; Supplementary
288 Tables 2 and 3). The level of dietary PLs significantly enhanced growth performance variables (BW_f,
289 BWG, SGR) in *A. stellatus* juveniles, all the above-mentioned variables related to growth performance
290 showed a quadratic response with regard to dietary PL levels (Figure 1). In particular, somatic growth
291 increased with increasing dietary PL levels, reaching maximum values in *A. stellatus* juveniles fed
292 diets ranging between 3.9 and 5.4% PLs. Regarding somatic condition indexes, although HSI did not
293 vary among diets, the level of dietary PLs had a decreasing linear effect on VSI values (Supplementary
294 Tables 2 and 3).

295 The values of FCR, PER, FI, and LER showed significant differences among experimental diets
296 (Supplementary Tables 2 and 3). The orthogonal contrast analysis revealed that for all of them the
297 quadratic component with PL levels was highly significant (Supplementary Table 3). However, FCR
298 presented a contrasting pattern when compared to PER, feed intake and LER (Figure 1). The maximum

299 values of PER, feed intake and LER were obtained in sturgeon juveniles fed diets ranging between 3.9

300 to 5.4% PLs, thus coinciding with the minimum values of FCR (Figure 1).

301

302 *3.2 Proximate body composition, and fatty acid profile of the muscle and liver*

303 Proximate body composition analyses showed that the inclusion of different levels of PLs in isolipid

304 and isoproteic diets did not modify the protein, lipid, carbohydrate, and ash content in *A. stellatus*

305 juveniles (Supplementary Tables 4 and 5). Regarding target tissues, no differences in lipid content in

306 the muscle were observed among experimental groups, whereas lipid levels in the liver were

307 significantly affected by the level of dietary PLs (ANOVA; $P < 0.05$; Supplementary Table 4). In

308 particular, there was a decreasing quadratic relationship between dietary PL levels and lipid content in

309 the liver with maximum lipid levels in fish fed 2.7% PLs (Figure 2). Experimental diets differing in the

310 PL levels modified the muscle and liver fatty acid profiles in *A. stellatus* (Supplementary Tables 6-9).

311 The relationship between selected fatty acids of both the liver and muscle with the experimental diets

312 containing graded levels of PLs are shown in Figures 3 and 4, respectively. In particular, dietary PL

313 levels had an increasing effect on the contents of total saturated fatty acids in muscle and liver,

314 including myristic (C14:0), palmitic (C16:0) and stearic (C18:0) acids. In the muscle, total

315 monounsaturated fatty acids (MUFA), including C16:1 n-7, C18:1 n-7 and oleic acid (C18:1 n-9), as

316 well as total MUFA showed an improved quadratic response to levels of PLs in experimental diets

317 (Figure 4), whereas in the liver this response was linear except for oleic acid (Figure 3). Furthermore,

318 dietary PL levels had increased the levels of total muscle and liver n-3 polyunsaturated fatty acids (n-3

319 PUFA) including alpha-linolenic (C18:3 n-3, ALA) and eicosapentanoic (C20:5 n-3, EPA); but

320 docosahexaenoic (C22:6 n-3, DHA) acid presented a contrasting pattern between the muscle

321 (increasing linear response) and the liver (decreasing linear response) samples (Figures 3 and 4). Total

322 liver and muscle n-6 PUFA, including linoleic (C18:2 n-6), eicosadienoic (C20:2 n-6) acids showed an
323 increasing quadratic response to dietary PL levels (Figures 3 and 4). Arachidonic (C20:4 n-6, ARA)
324 acid showed a contrasting pattern in muscle and liver samples (Figures 3 and 4). Diets had similar
325 importance on liver and muscle fatty acids profile differences (see η_p^2 values on Supplementary Tables
326 7 and 9),

327

328 *3.3 Levels of lipid peroxidation, activity of oxidative stress and digestive enzymes*

329 Oxidative stress levels in serum of *A. stellatus* juveniles were significantly affected by experimental
330 diets (Supplementary Tables 10 and 11). In particular, MDA content in serum showed an inverse
331 quadratic response in relation to dietary PLs (Figure 5). In contrast, CAT and SOD levels in serum
332 significantly increased linearly with dietary PL levels (Figure 5, Supplementary Table 11).

333 Regarding pancreatic and gastric digestive enzymes, dietary PL levels modified their specific
334 activities, except chymotrypsin (Supplementary Tables 12 and 13). According to the results of the
335 orthogonal contrast analysis, the activity of trypsin and α-amylase showed a quadratic response with
336 regard to dietary PL levels, reaching maximum specific activity levels in *A. stellatus* juveniles fed diets
337 containing between 3.9 and 5.4% PLs. (Figure 5). The relationship between the bile salt-activated
338 lipase and the pepsin specific activity and dietary PL levels was quadratic, in both cases, their
339 maximum specific activities were found in fish fed diets containing 2.7% PLs, while their congeners
340 fed diets containing 0.3 and 5.4% PLs showed the lowest levels in pepsin activity (Figure 6,
341 Supplementary Table 13).

342

343 *3.4 Gene expression analysis*

344 The relative quantification of *lpl* and *igf1* expression in *A. stellatus* juveniles was significantly affected
345 by diets containing graded levels of PLs (Supplementary Tables 14 and 15). Results from the
346 orthogonal contrast analysis revealed that both genes showed a positive response with regard to dietary
347 PL levels (Figure 7).

348

349 *3.5. Histology organization of the intestinal mucosa and liver*

350 The general histological organization of the intestinal mucosa and liver in juvenile stellate sturgeon fed
351 different levels of dietary PLs was normal and similar to that described for other sturgeon species
352 (Buddington and Doroshov, 1986). In particular, the hepatic parenchyma was organized in polyhedral
353 hepatocytes with central nuclei and arranged along tightly packed anastomosed laminae around veins
354 and the hepatic parenchyma was surrounded by a thin layer of connective tissue. Fat deposition within
355 hepatocytes which determines the position of the nucleus in the hepatic cells was different in response
356 to dietary PL. Juvenile sturgeon fed 0.3 and 0.9% dietary PLs showed large lipid deposits within
357 hepatocytes occupying most part of the cytoplasm of the cell and changing its polyhedral shape to
358 round (Figure 8a), whereas fish fed 1.6 to 2.7% dietary PLs showed a high degree of lipid
359 accumulation and changes in the hepatocyte shape, even though at a lower degree than fish from the
360 former groups (Figure 8b). Juveniles fed 3.9 to 5.4% PLs showed the lowest level of lipid
361 accumulation in the hepatic parenchyma with just a few and small lipid inclusions within hepatocytes
362 (Figure 8c). The histological organization of the anterior and mid-regions of the intestine in stellate
363 sturgeon juveniles was similar among experimental groups, whereas no differences in the level of lipid
364 accumulation were observed among the experimental groups (Figure 8d-f).

365

366 ## 4. Discussion

367 The importance of dietary PL levels for optimal growth and feed efficiency performance in juvenile *A.*
368 *stellatus* was clearly demonstrated in the present study. Present results revealed a positive quadratic
369 response between growth performance and dietary PLs; in this sense, somatic growth increased with
370 increasing dietary PL levels up to 3.9% when growth parameters remained stable. Growth results in
371 terms of BW, SGR and BWG were in agreement with FCR values, showing that optimal dietary PLs
372 for *A. stellatus* juveniles were 3.9% in terms of growth and feed efficiency parameters.

373 Generally, PL requirements in juvenile teleost species are comprised between 1.5 and 7 % of the
374 diet, depending on the species considered (NRC, 2011). In particular, the lowest dietary PL
375 requirements (1.5–2%) have been found in *P. dentex* and turbot (*Scophthalmus maximus*) juveniles,
376 respectively, whereas the highest requirements (5.4 and 7%) were found in amberjack (*Seriola*
377 *dumerili*) (Uyan et al., 2009) and *P. olivaceus* (NRC, 2011). Between these ranges of values, other
378 studies have reported that *P. altivelus*, *D. labrax* and *O. fasciatus* required 3% of dietary PLs, whereas
379 PL requirements in *S. salar* and rainbow trout (*Oncorhynchus mykiss*) juveniles were found to be 4%,
380 results that were in agreement with those of the current study.

381 The improvement in feed efficiency parameters in *A. stellatus* juveniles fed graded levels of PLs
382 may be attributed to an increase in feed intake values that followed a quadratic response similarly to
383 growth performance indicators. Similar results regarding the positive effect of dietary PLs on FI have
384 been also observed in rainbow trout (Poston, 1991), *P. olivaceus* (Uyan et al., 2007) and *S. dumerili*
385 (Uyan et al., 2009). As Tocher et al. (2008) reviewed, the enhancement in FI may be attributed to the
386 phosphatidylcholine content of the dietary PL fraction that increased diet attractability and palatability.

387 Although the increase in FI might be attributed to the above-mentioned PL's properties, the
388 improvement in feed utilization may be linked to the emulsifying properties of PLs that enhanced feed
389 digestion (Tocher et al., 2008). In this sense, the current study revealed that an increase in dietary PLs
390 from 0.9 to 3.9% promoted the activity of gastric (pepsin) and pancreatic enzymes (alkaline proteases,
391 α-amylase and bile salt-activated lipase), whereas higher inclusion levels of PLs did not provide any
392 advantage in terms of *A. stellatus* digestive capacities. Plant-based lecithin promoted the secretion of
393 digestive enzymes, especially those produced by the exocrine pancreas, in *C. carpio* (Adel et al.,
394 2017). In this sense, these results may be attributed to the regulation of enzyme synthesis and secretion
395 by means of dietary PLs (SBL and chicken lecithin) through the action of cholecystokinin as it has
396 been reported in *O. mykiss* (Azarm *et al*., 2103).

397 Phosphatidylcholine and phosphatidylinositol are two important components of soybean lecithin
398 that are responsible for the enhancement of feed intake (Tocher et al., 2008; La et al., 2018). Thus, we
399 hypothesized that the increment in gastric and pancreatic enzyme activities may be a result of the
400 increase in feed intake in sturgeon juveniles that coupled with the emulsifying properties of dietary PL
401 may explain the higher performance of stellate sturgeon juveniles fed PL-supplemented diets.
402 Furthermore, values in protein and lipid efficiency ratios significantly increased with increasing dietary
403 PL levels, with no differences found between groups fed diets containing 2.7 to 5.4% PLs, indicating a
404 protein sparing effect (De Silva et al., 1991; Vergara et al., 1996). According to earlier findings, *igf1*
405 proved to correlate with protein retention and growth in different species like *S. salar* (Hevrøy et al.,
406 2007), Russian sturgeon (*A. gueldenstaedtii*) (Şener et al., 2005) and Senegalese sole (*Solea*
407 *senegalensis*) (Campos et al., 2010).

408 Regarding body condition, HIS values in *A. stellatus* juveniles were not influenced by dietary
409 PLs levels, which may be attributed to a balanced fatty acid profile of experimental diets (Reis et al.,

410 2014; Xue et al., 2006). Although no differences in HSI values were found among dietary groups,
411 some differences in lipid deposition were found as indicated by the analysis of the histological
412 organization of the liver that were in agreement to gravimetric determination of lipids in this accessory
413 digestive gland. In particular, a decreasing trend in lipid accumulation within hepatocytes of sturgeon
414 fish fed increasing dietary PL levels was observed, especially in those specimens fed diets containing
415 3.9 to 5.4% PLs, which might be attributed to the important role of PLs in the transport of triglycerides
416 from the liver to extra hepatic tissues due to the formation of very low density lipoproteins (Tocher et
417 al., 2008; Dapra et al., 2011), as well as to the hipolipidemic effect of the linolenic acid (C18:3 n-3)
418 (Caballero et al., 2004), whose levels were the highest in the livers of sturgeon fish fed these diets. The
419 above-mentioned differences in hepatic lipid accumulation were not coupled with changes in fat
420 deposits in the intestinal mucosa of sturgeon fed diets containing graded levels of PLs, since no
421 differences in the histological organization were observed among experimental groups. Such different
422 results may be associated to their different capacity of lipoprotein synthesis and secretion between the
423 intestine and the liver, as it was previously shown in *S. salar* fry (Taylor et al., 2015). Changes in lipid
424 deposition were also correlated to changes in *lpl* expression. In particular, a recent study on young
425 yellow croaker (*Larimichthys crocea*) and cobia (*Rachycentron canadum*) fed graded levels of PLs
426 showed that genes involved in the synthesis of fatty acids and their uptake, including *lpl*, were up-
427 regulated with higher levels of dietary PLs (Niu et al., 2008; Cai et al., 2016). In *R. canadum*
428 remarkable changes in the plasma lipids profile and lipoprotein metabolism the fish fed diets with low
429 levels of PLs, resulting in hypertriglyceridemia associated with lower activity of hepatic lipase and
430 LPL (Niu et al., 2008). These results were in agreement with the increasing linear response between
431 dietary PL levels and *lpl* expression in in *A. stellatus*. In this sense, LPL plays a central role in
432 incorporating plasma lipids into tissues and regulates lipid metabolism and energy balance in the

433 organism, since it hydrolyzes triglycerides from serum lipoproteins into free fatty acids and glycerol
434 (Nicoll and Lewis, 1980; Oku et al., 2006).

435 Under present experimental conditions, although different levels of dietary PLs promoted fish
436 growth, no differences in body proximate composition were detected between different experimental
437 groups. It is generally accepted that the fatty acid profiles of the fillet and liver are closely similar to
438 the fatty acid content of the diet (Vaccaro et al., 2005; Glencross et al., 2014). In this study,
439 experimental diets containing graded levels of PLs and displaying different fatty acid profiles, changed
440 the fatty acid content of the liver and muscle in *A. stellatus*. The pattern of this modification varied
441 depending on the tissue considered. For instance, increasing dietary PL levels resulted in an increasing
442 linear trend in total SFA in the muscle and liver, although the magnitude of this change was *ca.* 100
443 times in the liver with regard to the muscle. Regardless of the different mathematical relationship
444 found between SFA and MUFA, and dietary PLs, their levels in the liver of sturgeons fed diets
445 containing 03 to 1.6% PLs were similar to those contained in experimental diets, whereas the inclusion
446 of higher levels of PLs (2.7–5.4%) resulted in a larger accumulation of SFA and MUFA in this tissue.
447 These results may be attributed to the SFA and MUFA content of phosphatides in soybean lecithin
448 (Scholfied, 1981). The above-mentioned pattern of SFA and MFA deposition in the liver was also
449 found in the muscle, although the magnitude of these changes in fatty acid accumulation were lower in
450 magnitude due to the inherent differences in tissue physiology and lipid metabolism (Caballero et al.,
451 2002; Boglino et al., 2012). Total n-6 HUFA levels in the liver of *A. stellatus* juveniles were similar to
452 those in experimental diets containing 2.7–5.4% PLs, whereas fish fed lower PLs (0.3–1.6%) showed
453 lower total n-6 HUFA levels in the liver than those in feeds. Similarly, levels of alpha-linolenic (C18:3
454 n-3) in the liver were substantially lower in fish fed diets containing 0.9 to 5.3% PLs, whereas only
455 sturgeon fed the diet containing 5.4% PLs showed similar levels in C18:3 n-3 than in the diet. These

456 changes in the fatty acid profile may be attributed to the sturgeon capacity to desaturate and elongate
457 linoleic (C18:2 n-6) and alpha-linolenic (C18:3 n-3) acids to ARA (C20:4 n-6), EPA (C20:5 n-3) and
458 DHA (C22:6 n-3), as it has been shown in other sturgeon species (*Huso huso*, *A. transmontanus*, *A.*
459 *gueldenstaedtii*) (Xu et al., 1996; Şener et al., 2005; Noori et al., 2011), and data on DHA, EPA and
460 ARA levels in *A. stellatus* juveniles fed diets with lower PL levels (0.3–1.6%). Furthermore, the fatty
461 acid content, especially for the total saturated, monounsaturated, n-3 and n-6 PUFAs, of the muscular
462 tissue of *A. stellatus* juveniles fed the diet containing 5.4% PLs (SBL10 diet) was lower than expected;
463 results that may be attributed to the lower crude lipid content of this diet with regard to its theoretical
464 values (16.4 vs. 17.5%; real *vs*. theoretical crude lipid values).

465 Dietary fatty acid profiles and lipid levels, and their form of inclusion in diets (neutral *vs.* polar
466 lipids) have a direct impact on body condition and tissue health. In this sense, SOD and CAT activities
467 are generally used to measure the antioxidant defense of fish in response to diet. In addition, MDA is
468 produced during lipid, and especially polyunsaturated fatty acids, peroxidation and it is measured to
469 evaluate the oxidative stress damage in tissues (Solé et al., 2004; Fontagné-Dicharry et al., 2104).
470 Different studies have shown an inverse relationship between dietary PL and oxidative stress (MDA
471 levels) in different tissues (Kumar et al., 2014; Chen et al., 2015; Cai et al., 2016). Under current
472 experimental conditions, an inverse quadratic response was found between dietary PLs and serum
473 MDA levels; thus, increasing dietary PLs resulted in a decreasing trend in serum MDA content with
474 the exception of juveniles fed 5.4% PLs, which showed MDA levels similar to those from fish fed 0.3
475 and 0.9% PLs. Unexpectedly, MDA levels in serum from fish fed the diet containing 5.4% PLs (SBL
476 10) were higher than those found in the fish fed the 5.3% PL diet (SBL 8), regardless of their similar
477 content in dietary PLs. This higher level of MDA in the serum of *A. stellatus* juveniles might be
478 associated to a higher rate of polyunsaturated fatty acid peroxidation in these animals as data on the

479 content of total n-6 PUFA in the liver and muscle in this experimental group indicated (Li et al., 2015).
480 However, the reason for such high lipid peroxidation values in this experimental group deserves further
481 attention, since data presented in this study is not conclusive regarding this issue. Data on SOD and
482 CAT activities may explain the trend observed between MDA levels and dietary PLs, since we found a
483 linear increasing response between the activities of the above-mentioned antioxidative stress enzymes
484 and the levels of PL in diets (Gao et al., 2014).

485

486 **5. Conclusions**

487 According to the results from the current study, the inclusion of PLs at 3.9% in compound diets for *A.*
488 *stellatus* maximized growth performance and improved feed efficiency variables, as well as enhanced
489 feed intake. Furthermore, increasing the levels of dietary PLs reduced the accumulation of fat stores in
490 the liver as well as up-regulated the expression of *lpl* confirming the important role of this enzyme in
491 incorporating plasma lipids into tissues, whereas the activities of CAT and SOD showed an increasing
492 linear increase with dietary PL levels. Increasing dietary PLs from 0.9 to 3.9% promoted the activity of
493 gastric and pancreatic enzymes, whereas higher inclusion levels of PLs did not provide any advantage
494 in terms of *A. stellatus* digestive capacities.

495

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501

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741

742 **Figure captions**

743

744 **Figure 1.** Relationship between dietary phospholipids and growth performance final (BW, BWG,
745 SGR) and feed efficiency (FCR, FI, PER, LER) variables in stellate sturgeon (*A. stellatus*) juveniles.
746 Significant ($P < 0.05$) linear or quadratic components are also shown considering the results presented
747 in Supplementary Table 3; the dotted line is the confidence interval at 95%). *Abbreviations*: BW, body
748 weight; BWG, body weight gain; SGR, specific growth rate in BW; FCR, feed conversion ratio; FI,
749 feed intake; PER, protein efficiency ratio; LER, lipid efficiency ratio.

750

751 **Figure 2.** Relationship between dietary phospholipids and hepatic lipid content in stellate sturgeon (*A.*
752 *stellatus*) juveniles.

753

754 **Figure 3.** Relationship between dietary phospholipids in the liver and selected fatty acid profile in the
755 liver of stellate sturgeon (*A. stellatus*) juveniles. Significant ($P < 0.05$) linear or quadratic components
756 are also shown considering the results presented in Supplementary Table 9; the dotted line is the
757 confidence interval at 95%. *Abbreviations*: ∑SFA, total saturated fatty acids; ∑MUFA, total
758 monounsaturated fatty acids; ∑n–3 PUFA, total polyunsaturated fatty acids from the n-3 series; ∑n–3
759 HUFA total highly unsaturated fatty acids from the n-3 series; ∑n–6 PUFA, total polyunsaturated fatty
760 acids from the n-6 series.

761

762 **Figure 4.** Relationship between dietary phospholipids and selected fatty acid profile in the muscle of
763 stellate sturgeon (*A. stellatus*) juveniles. Significant ($P < 0.05$) linear or quadratic components are also
764 shown considering the results presented in Supplementary Table 7; the dotted line is the confidence
765 interval at 95%. *Abbreviations*: ∑SFA, total saturated fatty acids; ∑MUFA, total monounsaturated
766 fatty acids; ∑n–3 PUFA, total polyunsaturated fatty acids from the n-3 series; ∑n–3 HUFA total highly
767 unsaturated fatty acids from the n-3 series; ∑n–6 PUFA, total polyunsaturated fatty acids from the n-6
768 series.

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770

771 **Figure 5.** Relationship between dietary phospholipids and serum lipid peroxidation levels (MDA),
772 activity of catalase (CAT) and superoxide dismutase (SOD) enzymes in stellate sturgeon (*A. stellatus*)
773 juveniles. Significant ($P < 0.05$) linear or quadratic components are also shown considering the results
774 presented in Supplementary Table 11; the dotted line is the confidence interval at 95%.
775
776 **Figure 6.** Relationship between dietary phospholipids and gastric (pepsin) and pancreatic (trypsin, α-
777 amylase and bile salt-activated lipase) digestive enzymes in stellate sturgeon (*A. stellatus*) juveniles.
778 Significant ($P < 0.05$) linear or quadratic components are also shown, considering the results presented
779 in Supplementary Table 13; the dotted line is the confidence interval at 95%. As chymotrypsin was not
780 affected by dietary phospholipid levels, data for this pancreatic enzyme is not shown in this figure, but
781 values may be found in Supplementary Table 14.
782
783 **Figure 7.** Relationship between dietary phospholipids and insulin growth factor 1 (*igf1*) and
784 lipoprotein lipase (*lpl*) expression from the liver of stellate sturgeon (*A. stellatus*) juveniles. Significant
785 ($P < 0.05$) linear or quadratic components are also shown considering the results presented in
786 Supplementary Table 15; the dotted line is the confidence interval of the mean at 95%.
787
788
789 **Figure 8.** Histological images of the liver and intestine of stellate sturgeon (*Acipenser stellatus*)
790 juveniles fed graded levels of dietary phospholipids (PLs). (a) Detail of the hepatic parenchyma of a
791 sturgeon fed 0.3 and 0.9% PLs. Note the large size of lipid inclusions within hepatocytes. (b) Detail of
792 the hepatic parenchyma of a specimen fed 1.6 and 2.7% PLs. Note the reduction in size of lipid
793 inclusions within hepatocytes with regard to fish fed 0.3–0.9 % PLs. (c) Detail of the hepatic
794 parenchyma from a sturgeon fed 3.9–5.4% PLs. Note the reduced presence of very small lipid
795 inclusion within hepatocytes in comparison to the other dietary groups. Detail of a villi from the
796 anterior-mid intestine of sturgeons fed diets containing 0.3–0.9% PLs (d), 1.6–2.7% PLs (e) and 3.9–
797 5.4% PLs (f). No major differences were found regarding the level of lipid inclusion within
798 enterocytes. Staining: hematoxylin-eosin.

Table 1. Formulation, lipid class and proximate composition of experimental diets.

Ingredient	Experimental diet (% SBL)						
	SBL 0	**SBL 1**	**SBL 2**	**SBL 4**	**SBL 6**	**SBL 8**	**SBL 10**
Kilka fishmeal[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[b]	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[c] and mineral[d] mix	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 (%)	43.6	44.0	44.0	44.0	44.0	44.0	43.5
Crude lipid (%)	17.6	17.3	17.6	17.1	17.0	17.7	16.4
Neutral lipids (% of total lipid)	98.6	94.9	91.3	84.3	77.3	69.8	66.9
Phospholipids (% of total lipid)	1.4	5.1	8.7	15.7	22.7	30.2	33.1
Phospholipids (%)	0.25	0.88	1.55	2.68	3.86	5.35	5.43
Ash (%)	10.0	10.7	9.7	10.2	10.5	11.9	11.7
Gross energy (J kg^{-1})	2,130.1	2,112.9	2,130.7	2,110.7	2,098.5	2,092.6	2,062.5

[a] Saba Company, Tehran, Iran; [b] Ettehad khazar shomal company, Babolsar, Mazandaran, Iran; [c] Composition of vitamin premix (IU or g kg^{-1}): Vitamin A, 800,000 IU; Vitamin D3, 300,000, IU; Vitamin E, 2,500 mg; Vitamin K, 1,000 mg; Vitamin B1, 1,200 mg; Vitamin B2, 1,200 mg; Vitamin B3, 2,400 mg; Vitamin B5, 3,500 mg; Vitamin B6, 1,300 mg; Vitamin B7, 600 mg; Vitamin B9, 600 mg; Vitamin B12, 750 µg; Vitamin C, 35,000 mg (ATA Company, Tabriz, Iran); [d] Mineral premix (g kg^{-1} premix): Magnesium, 6,400 mg; Copper, 2,000 mg; Iron, 11,000 mg; Zinc, 7,000 mg; Selenium, 100 mg; Iodine, 300 mg; Cobalt, 50 mg; Natrium, 5,000 mg (ATA Company, Tabriz, Iran). The composition of the SBL used in this study was 74.4% of PLs (32.5% phosphatidylcholine, 16.9% phosphatidylserine + phosphatidylinositol, 16.7% phosphatydilethanolamine, 1.4% lysophosphatidylcholine and 6.9% unknown lipid class) and 25.6% of neutral lipids (11.0% phosphatidylglycerol + sulfoquinovosyl diacylglycerols, 5.2% free fatty acids, cholesterol, 2.5% triacylglycerides).

Table 2. Fatty acid profile of experimental diets (g kg^{-1} dry weight). *n.d.* = not detected.

Fatty acid	Experimental diet (% SBL & % PL)						
	SBL 0 PL 0.25	**SBL 1 PL 0.88**	**SBL 2 PL 1.55**	**SBL 4 PL 2.68**	**SBL 6 PL 3.86**	**SBL 8 PL 5.35**	**SBL 10 PL 5.43**
C14:0	0.5	0.6	0.7	0.7	0.7	0.9	0.8
C16:0	15.3	17.0	19.0	20.1	19.4	25.2	20.3
C18:0	2.9	3.0	3.3	3.6	3.5	4.5	3.7
C20:0	0.2	0.0	0.0	0.4	0.3	0.4	0.3
C22:0	0.0	0.0	0.0	0.2	0.2	0.3	0.2
∑SFA	19.5	20.7	23.2	25.2	24.4	31.6	25.5
C14:1 n–5	0.06	0.02	0.02	0.06	0.08	0.12	0.09
C16:1 n–7	1.0	1.1	1.4	1.3	1.3	1.7	1.3
C18:1 n–7	0.9	1.0	0.9	1.0	1.2	1.5	1.2
C18:1 n–9	31.3	33.4	35.5	34.4	30.1	33.9	24.5
C20:1 n–9	0.40	0.44	0.47	0.03	0.02	0.05	0.04
C22:1 n–9	0.13	0.16	0.14	0.04	*n.d.*	*n.d.*	*n.d.*
∑MUFA	33.9	36.2	38.5	37.1	32.8	37.4	27.3
C18:3 n–3	0.42	1.3	1.6	1.9	2.3	2. 9	2.6
C20:3 n–3	0.09	0.17	0.10	0.04	0.03	0.04	0.04
C20:5 n–3	0.9	0.9	1.2	1.2	1.2	1.6	1.3
C22:6 n–3	3.1	3.5	3.9	4. 1	4.0	4.8	4.0
∑n–3 PUFA	4.4	5.4	5.7	6.0	6.4	6.7	6.9
∑n–3 HUFA	4.6	6.0	6.9	7.4	7.7	9.4	8.1
C18:2 n–6	50.7	55.6	59.5	57.9	51.1	58.7	53.2
C20:2 n–6	0.34	0.35	0.36	0.27	0.08	0.13	0.10
C20:4 n–6	0.22	0.11	0.06	0.27	0.39	0.45	0.28
∑n–6 PUFA	51.3	56.1	59.9	58.4	51.6	59.3	53.6

Abbreviations: ∑SFA, total saturated fatty acids; ∑MUFA, total monounsaturated fatty acids; ∑n–3 PUFA, total polyunsaturated fatty acids from the n-3 series; ∑n–3 HUFA total highly unsaturated fatty acids from the n-3 series; ∑n–6 PUFA, total polyunsaturated fatty acids from the n-6 series.

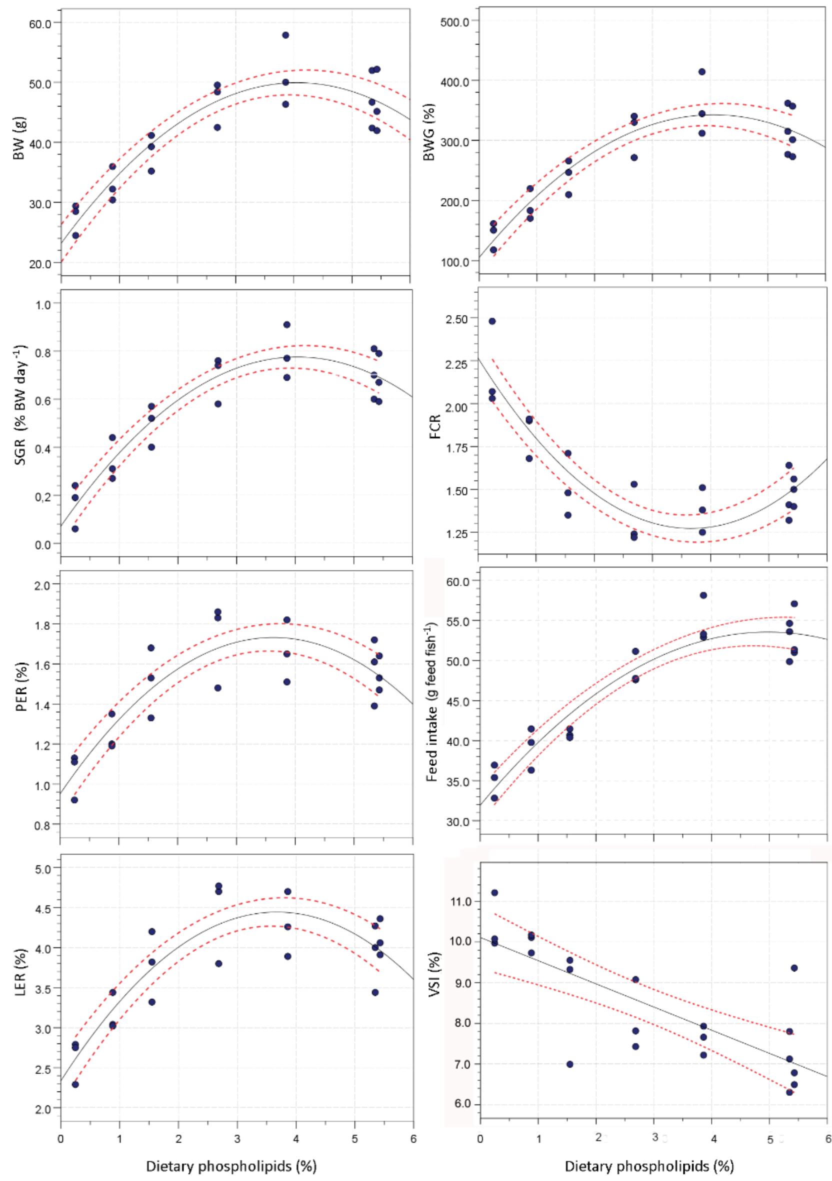


Figure 1

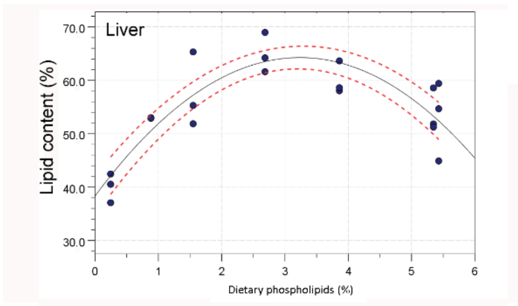


Figure 2

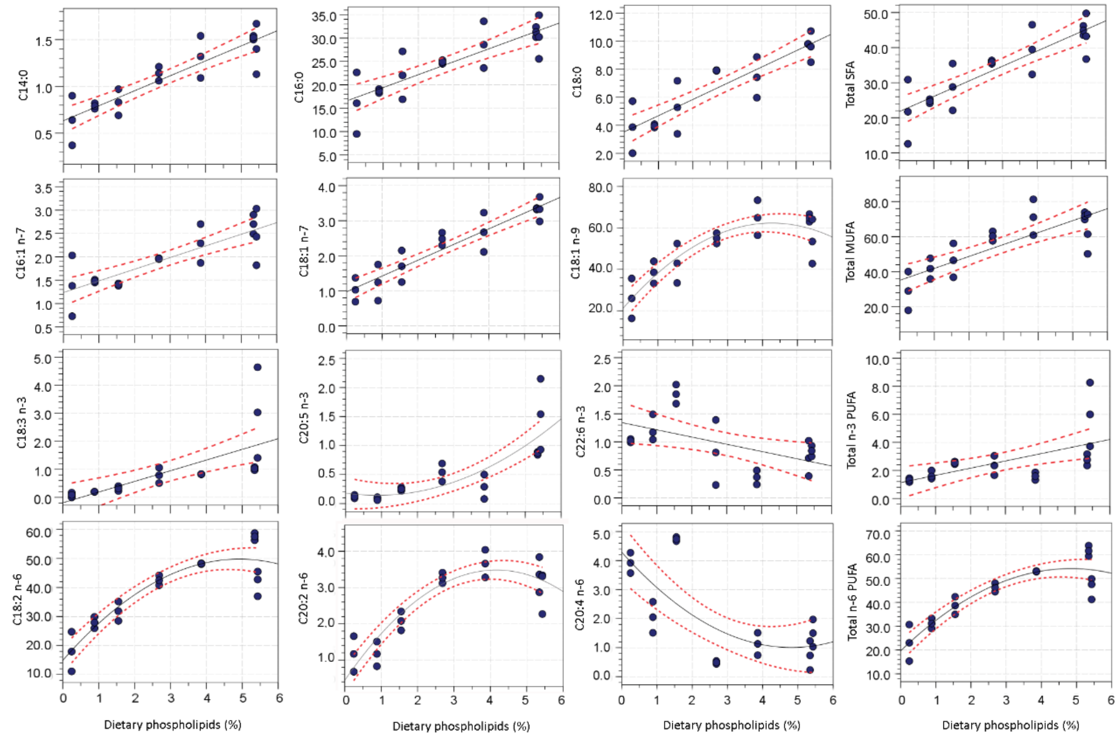
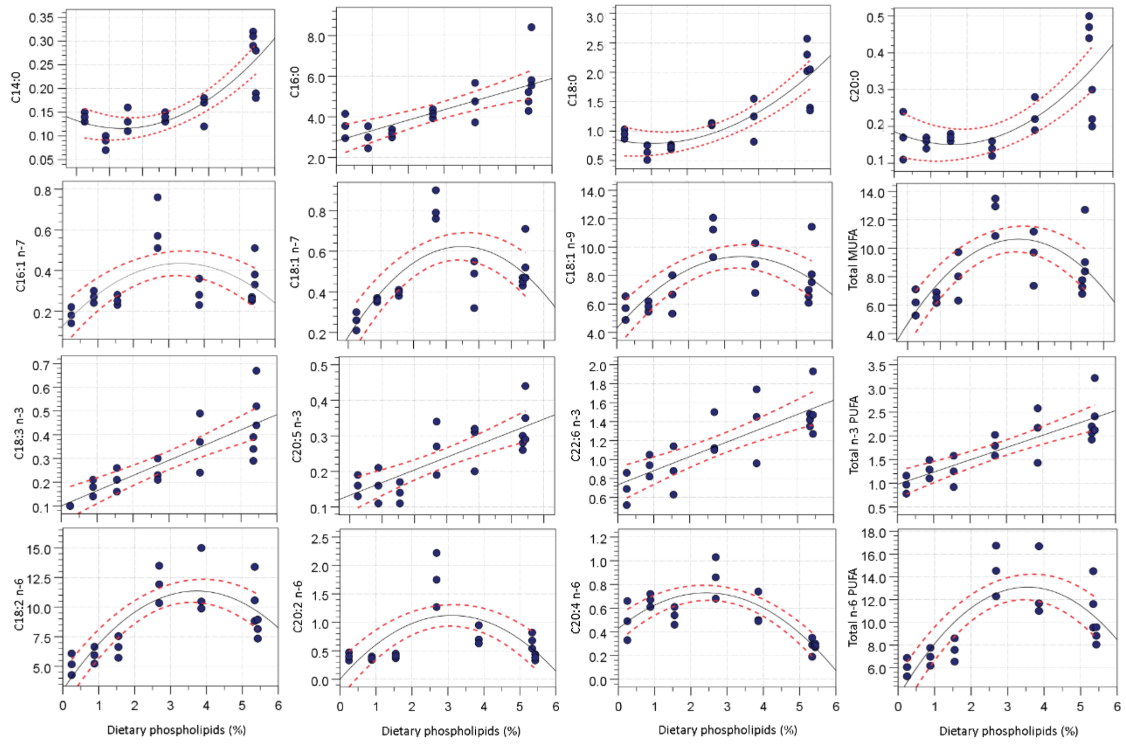


Figure 3



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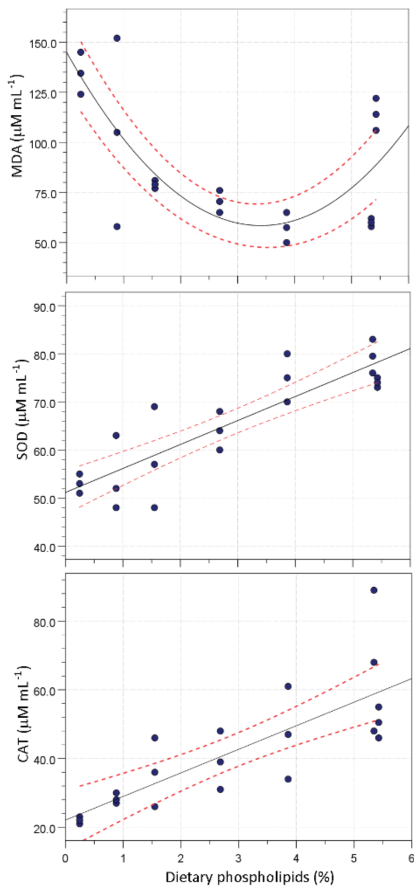

Figure 5

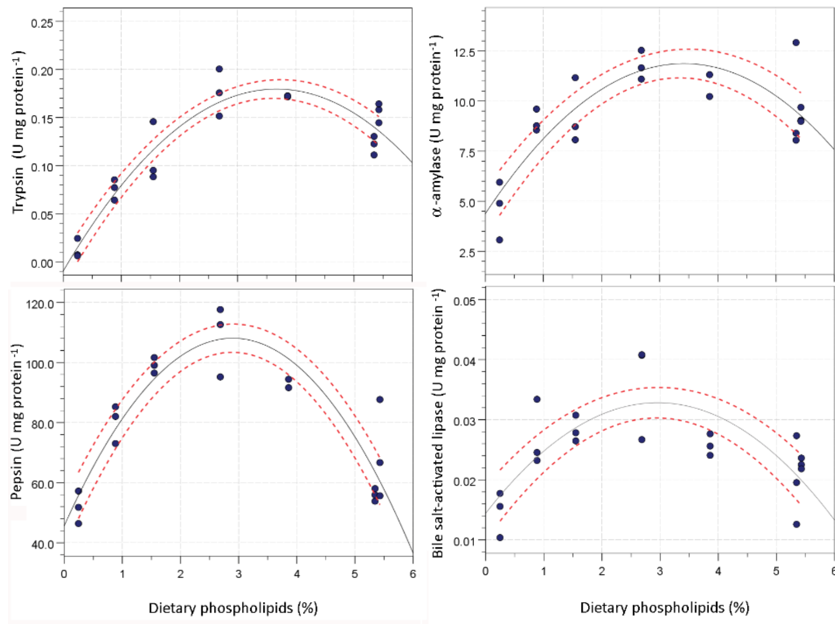

Figure 6

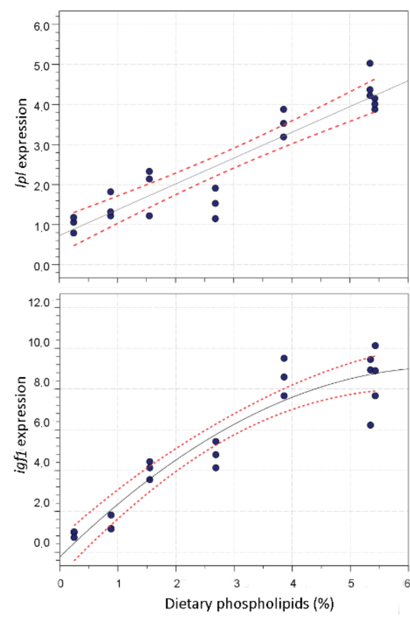

Figure 7

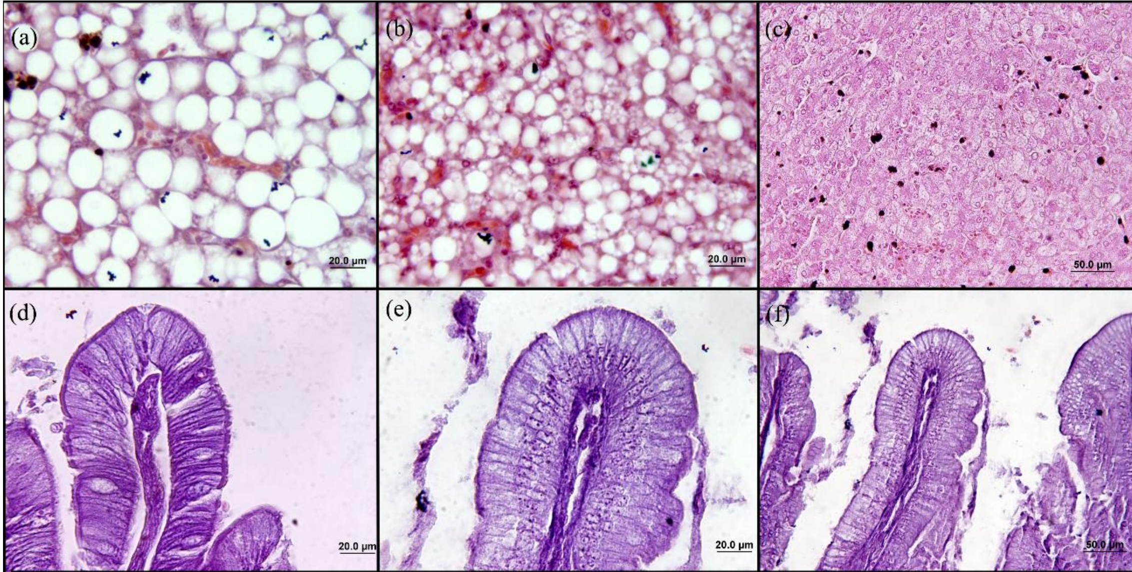

Figure 8

Electronic supplementary material to **“Phospholipids improve the performance, physiological, antioxidative responses and lpl, igf gene expression in juvenile stellate sturgeon (*Acipenser stellatus*)”**

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Supplementary Table 1. Nucleotide sequences of the primers used to assay gene expression by real-time PCR.

Gene	Forward (5'–3')	Reverse (5'–3')	Amplicon length	Efficiency (%)
Igf1	GCGTGTTCTGTGCCTGACT	AGAAGCCTCTCTCCCCACAC	104	98
lpl	CATTGCCGGCAGTCTCACA	AAGTTAGCATCGTCCGGGGA	117	102
ef1a	GGACTCCACTGAGCCACCT	GGGTTGTAGCCGATCTTCTTG	90	96

Supplementary Table 2. Growth performance (μ ± Standard Deviation) of juvenile stellate sturgeon (*A. stellatus*) in experimental diets with different levels of phospholipids (PLs). Phospholipids were added into experimental diets as soybean lecithin (SBL). See Supplementary Table 5 for detailed statistical analysis.

Variable	**Experimental diet (% SBL & % PL)**						
	SBL 0 PL 0.25	**SBL 1 PL 0.88**	**SBL 2 PL 1.55**	**SBL 4 PL 2.68**	**SBL 6 PL 3.86**	**SBL 8 PL 5.35**	**SBL 10 PL 5.43**
BW_i (g)	11.29 ± 0.07	11.29 ± 0.07	11.32 ± 0.06	11.31 ± 0.11	11.25 ± 0.00	11.25 ± 0.00	11.31 ± 0.09
BW_f (g)	27.46 ± 2.61	32.86 ± 2.83	38.55 ± 3.04	46.80 ± 3.78	51.40 ± 5.88	47.01 ± 4.80	46.42 ± 5.22
BWG (%)	143.2 ± 22.72	191.1 ± 25.65	240.8 ± 28.57	314.0 ± 37.20	356.9 ± 52.29	317.9 ± 42.67	310.4 ± 42.76
SGR (% day^{-1})	0.16 ± 0.09	0.34 ± 0.08	0.49 ± 0.08	0.69 ± 0.09	0.79 ± 0.11	0.70 ± 0.10	0.68 ± 0.10
FCR	2.19 ± 0.24	1.83 ± 0.13	1.51 ± 0.18	1.33 ± 0.17	1.38 ± 0.13	1.45 ± 0.16	1.48 ± 0.08
PER	1.05 ± 0.11	1.24 ± 0.09	1.51± 0.17	1.72 ± 0.21	1.66 ± 0.15	1.57 ± 0.16	1.54 ± 0.08
LER	2.61 ± 0.27	3.16 ± 0.23	3.78 ± 0.44	4.42 ± 0.54	4.28 ± 0.40	3.90 ± 0.42	4.11 ± 0.22
Feed Intake	35.05 ± 2.08	39.18 ± 2.62	40.84 ± 0.56	48.84 ± 2.00	54.79 ± 2.91	52.71 ± 2.51	53.15 ± 3.41
HSI (%)	2.51 ± 0.21	2.65 ± 0.70	2.68 ± 0.59	2.74 ± 0.44	2.72 ± 0.49	2.33 ± 0.20	2.56 ± 0.30
VSI (%)	10.42 ± 0.68	10.00 ± 0.23	8.62 ± 1.41	8.10 ± 0.86	7.60 ± 0.35	7.07 ± 0.74	7.54 ± 1.57

Supplementary Table 3. Analysis of variance (ANOVAs) of the growth performance of stellate sturgeon (*A. stellatus*) with diet as factor. The variation among diets was decomposed into a linear component and a residual term (deviation) with polynomial contrasts. See Supplementary Table 4 for variables summary and Figure 1 for polynomial contrast representation.

Variable	**Explained variation (Adj. R^2)**	**Among diets**			**Phospholipids content (%)**		
					Linear contrast	**Deviation test**	**Quadratic contrast**
		$F_{6, 14}$	P	η_p^2	P	P	P
BW_i (g)	-0.19	0.45	0.831	0.16			
BW_f (g)	0.78	13.07	<0.001	0.84	<0.001	0.012	<0.001
BWG (%)	0.78	13.10	<0.001	0.84	<0.001	0.012	<0.001
SGR (% day^{-1})	0.82	16.38	<0.001	0.87	<0.001	0.005	<0.001
FCR	0.73	10.28	<0.001	0.81	<0.001	0.003	<0.001
PER	0.66	7.64	0.001	0.76	<0.001	0.009	<0.001
LER	0.69	8.71	<0.001	0.78	<0.001	0.007	<0.001
Feed Intake (g/day)	0.90	31.09	<0.001	0.93	<0.001	0.008	<0.001
HSI (%)	-0.27	0.30	0.931	0.11			
VSI (%)	0.56	5.34	0.005	0.69	<0.001	0.530	

Supplementary Table 4. Proximate body composition (μ ± Standard Deviation) of juvenile stellate sturgeon (*A. stellatus*) in experimental diets with different levels of phospholipids (PLs). Phospholipids were added into experimental diets as soybean lecithin (SBL). See Supplementary Table 7 for detailed statistical analysis.

Variable	Experimental diet (% SBL & % PL)						
	SBL 0 PL 0.25	SBL 1 PL 0.88	SBL 2 PL 1.55	SBL 4 PL 2.68	SBL 6 PL 3.86	SBL 8 PL 5.35	SBL 10 PL 5.43
Crude protein (%)	53.00 ± 0.45	53.18 ± 0.55	54.50 ± 0.44	55.42 ± 1.80	55.08 ± 0.82	54.56 ± 1.57	54.50 ± 0.45
Crude lipids (%)	25.71 ± 2.60	24.64 ± 1.15	20.50 ± 4.17	23.34 ± 4.49	26.18 ± 0.87	23.12 ± 6.26	24.47 ± 1.86
Carbohydrates (%)	7.67± 3.78	9.08 ± 1.08	13.81 ± 6.98	11.13 ± 2.98	9.45 ± 2.55	11.20 ± 5.94	9.87 ± 1.98
Ash (%)	13.62 ± 1.33	13.10 ± 0.48	11.19 ± 2.36	10.11 ± 2.50	9.29 ± 0.99	11.12 ± 2.34	11.25 ± 1.12

Supplementary Table 5. Analysis of variance (ANOVAs) of the proximate body composition of stellate sturgeon (*A. stellatus*) with diet as factor. See Supplementary Table 6 for variables summary.

Variable	**Explained variation (Adj. R^2)**	**Among diets**		
		$F_{6,14}$	P	η_p^2
Crude protein (%)	0.29	2.37	0.086	0.50
Crude lipids (%)	-0.04	0.85	0.551	0.26
Carbohydrates (%)	0.10	0.68	0.665	0.22
Ash (%)	0.27	2.27	0.096	0.49

Supplementary Table 6. Fatty acid profile (mg g^{-1} lipid) of muscle (μ ± Standard Deviation) of juvenile stellate sturgeon (*A. stellatus*) in experimental diets with different levels of phospholipids (PLs). Phospholipids were added into experimental diets as soybean lecithin (SBL). See

Variable	Experimental diet (% SBL & % PL)						
	SBL 0 PL 0.25	**SBL 1 PL 0.88**	**SBL 2 PL 1.55**	**SBL 4 PL 2.68**	**SBL 6 PL 3.86**	**SBL 8 PL 5.35**	**SBL 10 PL 5.43**
C14:0	0.14 ± 0.01	0.08 ± 0.01	0.13 ± 0.02	0.14 ± 0.01	0.15 ± 0.03	0.30 ± 0.01	0.21 ± 0.05
C16:0	3.55 ± 0.60	2.99 ± 0.54	3.18 ± 0.19	4.15 ± 0.22	4.72 ± 0.97	4.75 ± 0.46	6.58 ± 1.58
C18:0	0.95 ± 0.08	0.63 ± 0.12	0.73 ± 0.04	1.12 ± 0.02	1.20 ± 0.36	2.29 ± 0.27	1.60 ± 0.39
C20:0	0.17 ± 0.06	0.15 ± 0.01	0.17 ± 0.01	0.14 ± 0.02	0.23 ± 0.04	0.47 ± 0.03	0.24 ± 0.05
C22:0	0.04 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.02	0.02 ± 0.00
∑SFA	4.85 ± 0.76	3.88 ± 0.70	4.23 ± 0.27	5.56 ± 0.26	6.32 ± 1.30	7.86 ± 0.23	8.67 ± 2.08
C14:1 n–5	0.01 ± 0.00	0.04 ± 0.02	0.65 ± 0.31	0.06 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
C16:1 n–7	0.18 ± 0.04	0.27 ± 0.03	0.25 ± 0.02	0.61 ± 0.13	0.29 ± 0.06	0.26 ± 0.01	0.40 ± 0.09
C18:1 n–7	5.71 ± 0.83	5.83 ± 0.37	6.67± 1.35	10.87 ± 1.42	8.62 ± 1.75	6.53 ± 0.45	9.01 ± 2.10
C18:1 n–9	0.25 ± 0.04	0.36 ± 0.01	0.39 ± 0.01	0.81 ± 0.07	0.45 ± 0.11	0.45 ± 0.02	0.56 ± 0.12
C20:1 n–9	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.00	0.07 ± 0.03	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
∑MUFA	6.19 ± 0.92	6.52 ± 0.37	8.02 ± 1.70	12.43 ± 1.38	9.41 ± 1.92	7.27 ± 0.48	10.03 ± 2.33
C18:3 n–3	0.10 ± 0.00	0.17 ± 0.03	0.21 ± 0.05	0.24 ± 0.04	0.36 ± 0.12	0.34 ± 0.05	0.54 ± 0.11
C20:3 n–3	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	0.11 ± 0.05
C20:5 n–3	0.16 ± 0.03	0.16 ± 0.05	0.14 ± 0.03	0.26 ± 0.07	0.27 ± 0.06	0.28 ± 0.02	0.36± 0.07
C22:6 n–3	0.69 ± 0.17	0.93 ± 0.11	0.88 ± 0.25	1.24 ± 0.22	1.38 ± 0.39	1.41 ± 0.06	1.55 ± 0.33
∑n–3 PUFA	0.97± 0.19	1.29 ± 0.19	1.25 ± 0.33	1.79 ± 0.22	2.06 ± 0.58	2.06 ± 0.14	2.58 ± 0.57
C18:2 n–6	5.17 ± 0.90	5.94 ± 0.71	6.63 ± 0.91	11.92 ± 1.57	11.78 ± 2.79	10.93 ± 2.30	8.14 ± 0.80
C20:2 n–6	0.40 ± 0.07	0.37 ± 0.03	0.41 ± 0.04	1.74± 0.47	0.76 ± 0.16	0.68 ± 0.14	0.38 ± 0.05
C20:4 n–6	0.49 ± 0.16	0.66 ± 0.05	0.53 ± 0.07	0.85 ± 0.17	0.57± 0.14	0.27 ± 0.08	0.28 ± 0.01
∑n–6 PUFA	6.07± 0.81	6.97 ± 0.78	7.57 ± 1.02	14.52 ± 2.22	13.12 ± 3.11	11.89 ± 2.48	8.81 ± 0.76

Supplementary Table 9 for detailed statistical analysis.

Supplementary Table 7. Analysis of variance (ANOVAs) of the muscle fatty acid profile (mg g^{-1} lipid) of stellate sturgeon (*A. stellatus*) with diet as factor. See Supplementary Table 8 for variables summary and Figure 2 for polynomial contrast representation.

Variable	Explained variation (Adj. R^2)	Among diets			Phospholipids content (%)		
					Linear contrast	Deviation test	Quadratic contrast
		$F_{6, 14}$	P	η_p^2	P	P	P
C14:0	0.85	20.29	<0.001	0.90	<0.001	0.001	0.002
C16:0	0.65	7.2	0.001	0.76	<0.001	0.120	
C18:0	0.83	17.80	<0.001	0.89	<0.001	0.005	0.008
C20:0	0.88	25.31	<0.001	0.92	<0.001	<0.001	0.001
C22:0	0.36	2.89	0.048	0.55	0.914	0.030	0.008
∑SFA	0.72	9.43	<0.001	0.80	<0.001	0.305	
C14:1 n–5	0.77	11.91	<0.001	0.84	0.028	<0.001	0.018
C16:1 n–7	0.78	13.12	<0.001	0.85	0.018	<0.001	<0.001
C18:1 n–7	0.83	17.58	<0.001	0.88	<0.001	<0.001	<0.001
C18:1 n–9	0.61	6.33	0.002	0.73	0.014	0.004	0.002
C20:1 n–9	0.64	6.97	0.001	0.75	0.014	0.002	0.001
∑MUFA	0.64	6.82	0.002	0.74	0.018	0.002	0.001
C18:3 n–3	0.77	12.01	<0.001	0.84	<0.001	0.092	
C20:3 n–3	0.71	9.00	<0.001	0.800	<0.001	0.003	0.148
C20:5 n–3	0.64	6.95	0.001	0.75	<0.001	0.260	
C22:6 n–3	0.55	5.08	0.006	0.69	<0.001	0.794	
∑n–3 PUFA	0.66	7.44	0.001	0.76	<0.001	0.545	
C18:2 n–6	0.72	9.40	<0.001	0.80	<0.001	0.003	0.001
C20:2 n–6	0.84	18.24	<0.001	0.89	0.185	<0.001	<0.001
C20:4 n–6	0.72	9.52	<0.001	0.81	0.001	0.001	<0.001
∑n–6 PUFA	0.72	9.66	<0.001	0.80	0.001	0.001	<0.001

Supplementary Table 8. Fatty acid profile (mg g^{-1} lipid) of liver (μ ± Standard Deviation) of juvenile stellate sturgeon (*A. stellatus*) in experimental diets with different levels of phospholipids (PLs). Phospholipids were added into experimental diets as soybean lecithin (SBL). See Supplementary Table 11 for detailed statistical analysis.

Variable	Experimental diet (% SBL & % PL)						
	SBL 0 PL 0.25	**SBL 1 PL 0.88**	**SBL 2 PL 1.55**	**SBL 4 PL 2.68**	**SBL 6 PL 3.86**	**SBL 8 PL 5.35**	**SBL 10 PL 5.43**
C14:0	0.63 ± 0.26	0.79 ± 0.03	0.83 ± 0.14	1.13 ± 0.07	1.31 ± 0.22	1.52 ± 0.02	1.40 ± 0.27
C16:0	16.07 ± 6.57	18.67 ± 0.42	22.03 ± 5.12	24.89 ± 0.38	28.59 ± 5.00	31.28 ± 1.08	30.23 ± 4.67
C18:0	3.86 ± 1.85	3.96± 0.12	5.28± 1.89	7.90 ± 0.02	7.42 ± 1.45	9.79 ± 0.00	9.61 ± 1.11
C20:0	1.08 ± 0.44	1.25± 0.04	0.57 ± 0.50	1.90 ± 0.00	2.00 ± 0.35	2.13 ± 0.26	1.83± 0.52
C22:0	0.07 ± 0.02	0.040 ± 0.00	0.06 ± 0.02	0.057 ± 0.00	0.127 ± 0.06	0.160 ± 0.00	0.18 ± 0.04
∑SFA	21.71 ± 9.16	24.71 ± 0.60	28.78 ± 6.67	35.87 ± 0.48	39.45 ± 7.08	44.89 ± 1.33	43.25 ± 6.52
C14:1 n–5	0.16 ± 0.13	0.09 ± 0.06	0.20 ± 0.15	0.71 ± 0.05	0.97 ± 0.72	0.78 ± 0.05	1.82 ± 0.24
C16:1 n–7	1.38 ± 0.65	1.48 ± 0.03	1.40 ± 0.02	1.96 ± 0.01	2.28 ± 0.41	2.69 ± 0.20	2.427 ± 0.60
C18:1 n–7	1.03 ± 0.34	1.23 ± 0.51	1.70 ± 0.45	2.480 ± 0.18	2.670 ± 0.56	3.34 ± 0.02	3.33 ± 0.35
C18:1 n–9	25.98 ± 9.63	38.56 ± 5.32	42.95 ± 9.48	54.98 ± 2.50	64.91 ± 8.46	64.81 ± 1.84	53.46 ± 10.70
C20:1 n–9	0.47 ± 0.28	0.39± 0.05	0.23 ± 0.10	0.18± 0.03	0.25 ± 0.06	0.22 ± 0.06	0.40 ± 0.14
∑MUFA	29.02 ± 11.04	41.76 ± 5.88	46.49 ± 9.65	60.31 ± 2.72	71.09 ± 10.21	71.86 ± 2.07	61.45 ± 11.27
C18:3 n–3	0.08 ± 0.08	0.20 ± 0.00	0.31± 0.08	0.78 ± 0.27	0.82 ± 0.00	1.02 ± 0.05	3.02 ± 1.61
C20:3 n–3	0.08 ± 0.05	0.14 ± 0.05	0.13 ± 0.03	0.22 ± 0.00	0.11 ± 0.06	0.18 ± 0.04	0.94 ± 0.21
C20:5 n–3	0.12 ± 0.03	0.08 ± 0.02	0.25 ± 0.03	0.53 ± 0.15	0.29 ± 0.21	0.86 ± 0.02	1.54 ± 0.61
C22:6 n–3	1.02 ± 0.03	1.23 ± 0.23	1.85 ± 0.17	0.81 ± 0.58	0.36 ± 0.12	0.70 ± 0.31	0.83 ± 0.09
∑n–3 PUFA	1.30 ± 0.13	1.66 ± 0.29	2.54 ± 0.09	2.35 ± 0.69	1.59 ± 0.27	2.75 ± 0.40	5.99 ± 2.27
C18:2 n–6	17.92 ± 6.82	27.92 ± 1.95	31.84 ± 3.35	42.49 ± 1.70	48.20 ± 0.14	57.53 ± 1.22	41.73 ± 4.33
C20:2 n–6	1.17 ± 0.49	1.17 ± 0.34	2.08 ± 0.26	3.26 ± 0.14	3.66 ± 0.38	3.35 ± 0.48	2.97 ± 0.60
C20:4 n–6	3.92 ± 0.35	2.04 ± 0.53	4.74 ± 0.07	0.49 ± 0.05	1.12 ± 0.38	0.73 ± 0.50	1.50 ± 0.47
∑n–6 PUFA	23.01 ± 7.67	31.13 ± 2.14	38.65 ± 3.69	46.25 ± 1.79	52.98 ± 0.15	61.61 ± 2.20	46.20 ± 4.46

Supplementary Table 9. Analysis of variance (ANOVAs) of the liver fatty acid profile (mg g^{-1} lipid) of stellate sturgeon (*A. stellatus*) with diet as factor. See Supplementary Table 10 for variables summary and Figure 3 for polynomial contrast representation.

Variable	Explained variation (Adj. R^2)	Among diets			Phospholipids content (%)		
					Linear contrast	Deviation test	Quadratic contrast
		$F_{6, 14}$	*P*	η_p^2	*P*	*P*	*P*
C14:0	0.75	11.08	<0.001	0.82	<0.001	0.784	
C16:0	0.60	6.14	0.002	0.72	<0.001	0.927	
C18:0	0.77	12.65	<0.001	0.84	<0.001	0.480	
C20:0	0.66	7.61	0.001	0.76	<0.001	0.027	0.491
C22:0	0.72	9.76	<0.001	0.80	<0.001	0.135	
∑SFA	0.67	7.86	0.001	0.77	<0.001	0.933	
C14:1 n–5	0.77	12.42	<0.001	0.84	<0.001	0.020	0.702
C16:1 n–7	0.60	6.06	0.003	0.72	<0.001	0.807	
C18:1 n–7	0.83	17.96	<0.001	0.88	<0.001	0.783	
C18:1 n–9	0.74	10.61	<0.001	0.82	<0.001	0.033	0.003
C20:1 n–9	0.26	2.200	0.105	0.48			
∑MUFA	0.74	10.90	<0.001	0.82	<0.001	0.066	
C18:3 n–3	0.67	7.83	0.001	0.77	<0.001	0.030	0.187
C20:3 n–3	0.91	34.51	<0.001	0.93	<0.001	<0.001	0.001
C20:5 n–3	0.78	12.90	<0.001	0.84	<0.001	0.011	0.032
C22:6 n–3	0.69	8.51	0.001	0.78	0.001	0.002	0.752
∑n–3 PUFA	0.70	8.87	<0.001	0.79	<0.001	0.004	0.083
C18:2 n–6	0.93	43.80	<0.001	0.95	<0.001	<0.001	<0.001
C20:2 n–6	0.84	19.33	<0.001	0.89	<0.001	0.002	<0.001
C20:4 n–6	0.94	54.29	<0.001	0.96	<0.001	<0.001	0.001
∑n–6 PUFA	0.90	34.17	<0.001	0.93	<0.001	0.001	<0.001

Supplementary Table 10. Oxidative stress levels and activities of antioxidant enzymes (μ ± Standard Deviation) of juvenile stellate sturgeon (*A. stellatus*) in experimental diets with different levels of phospholipids (PLs). Phospholipids were added into experimental diets as soybean lecithin (SBL). See Supplementary Table 13 for detailed statistical analysis.

Variable	**Experimental diet (% SBL & % PL)**						
	SBL 0 PL 0.25	**SBL 1 PL 0.88**	**SBL 2 PL 1.55**	**SBL 4 PL 2.68**	**SBL 6 PL 3.86**	**SBL 8 PL 5.35**	**SBL 10 PL 5.43**
MDA (μM mL^{-1})	134.50 ± 10.50	105.00 ± 47.00	79.00 ± 2.00	70.50 ± 5.50	57.50 ± 7.50	60.00 ± 2.00	114.0 ± 8.00
CAT (μM mL^{-1})	22.00 ± 1.00	28.33 ± 1.52	36.00 ± 10.00	39.33 ± 8.50	47.33 ± 13.50	68.33 ± 20.50	50.50 ± 4.50
SOD (μM mL^{-1})	53.03 ± 2.00	54.33 ± 7.76	58.00 ± 10.54	64.00 ± 4.00	75.00 ± 5.00	79.50 ± 3.50	74.00 ± 1.00

Supplementary Table 11. Analysis of variance (ANOVAs) of the oxidative stress levels and activities of antioxidant enzymes of stellate sturgeon (*A. stellatus*) with diet as factor. The variation among diets was decomposed into a linear component and a residual term (deviation) with polynomial contrasts. See Supplementary Table 12 for variables summary and Figure 4 for polynomial contrast representation.

Variable	**Explained variation (Adj. R^2)**	**Among diets**			**Phospholipids content (%)**		
					Linear contrast	**Deviation test**	**Quadratic contrast**
		$F_{6, 14}$	P	η_p^2	P	P	P
MDA (μM mL^{-1})	0.65	7.38	0.001	0.76	0.008	0.002	<0.001
CAT (μM mL^{-1})	0.61	6.23	0.002	0.72	<0.001	0.463	
SOD (μM mL^{-1})	0.74	10.64	<0.001	0.82	<0.001	0.556	

Supplementary Table 12. Pancreatic and gastric enzymes specific activity (μ ± Standard Deviation) of juvenile stellate sturgeon (*A. stellatus*) in experimental diets with different levels of phospholipids (PLs). Phospholipids were added into experimental diets as soybean lecithin (SBL). See Supplementary Table 15 for detailed statistical analysis.

Variable	**Experimental diet (% SBL & % PL)**						
	SBL 0 **PL 0.25**	**SBL 1** **PL 0.88**	**SBL 2** **PL 1.55**	**SBL 4** **PL 2.68**	**SBL 6** **PL 3.86**	**SBL 8** **PL 5.35**	**SBL 10** **PL 5.43**
Trypsin (U mg protein^{-1})	0.01 ± 0.01	0.07 ± 0.01	0.11 ± 0.03	0.17 ± 0.02	0.17 ± 0.00	0.12 ± 0.01	0.15 ± 0.01
α-Amylase (U mg protein^{-1})	4.63 ± 1.45	8.97 ± 0.55	9.31 ± 1.63	11.76 ± 0.72	10.77 ± 0.76	9.78± 2.71	9.23 ± 0.39
Chymotrypsin (U mg protein^{-1})	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.02	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.02	0.03 ± 0.01
Lipase (U mg protein^{-1})	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Pepsin (U mg protein^{-1})	51.82 ± 5.41	80.12 ± 6.33	99.07 ± 2.57	108.5 ± 11.77	93.03 ± 1.98	55.95 ± 2.09	70.01 ± 16.28

Supplementary Table 13. Analysis of variance (ANOVAs) of the pancreatic and gastric enzymes specific activity of stellate sturgeon (*A. stellatus*) with diet as factor. The variation among diets was decomposed into a linear component and a residual term (deviation) with polynomial contrasts. See Supplementary Table 14 for variables summary and Figure 5 for polynomial contrast representation.

Variable	Explained variation (Adj. R^2)	Among diets			Phospholipids content (%)		
					Linear contrast	Deviation test	Quadratic contrast
		$F_{6, 14}$	P	η_p^2	P	P	P
Trypsin (U mg protein^{-1})	0.91	36.34	<0.001	0.94	<0.001	<0.001	<0.001
α-Amylase (U mg protein^{-1})	0.66	7.11	0.002	0.76	0.004	0.004	<0.001
Chymotrypsin (U mg protein^{-1})	0.25	2.13	0.114	0.47			
Lipase (U mg protein^{-1})	0.58	5.62	0.004	0.70	0.935	0.002	<0.001
Pepsin (U mg protein^{-1})	0.85	18.43	<0.001	0.90	0.281	<0.001	<0.001

Supplementary Table 14. Liver lipoprotein lipase (*lpl*) and insulin growth factor 1 (*igf1*) expression (μ ± Standard Deviation) of juvenile stellate sturgeon (*A. stellatus*) in experimental diets with different levels of phospholipids (PLs). Phospholipids were added into experimental diets as soybean lecithin (SBL). See Supplementary Table 17 for detailed statistical analysis.

Variable	**Experimental diet (% SBL & % PL)**						
	SBL 0 PL 0.25	**SBL 1 PL 0.88**	**SBL 2 PL 1.55**	**SBL 4 PL 2.68**	**SBL 6 PL 3.86**	**SBL 8 PL 5.35**	**SBL 10 PL 5.43**
lpl expression	1.01 ± 0.20	1.45 ± 0.32	1.89 ± 0.59	1.53 ± 0.38	3.53 ± 0.34	4.54 ± 0.43	4.01 ± 0.13
igf1 expression	0.89 ± 0.15	1.37 ± 0.38	4.04 ± 0.44	4.78 ± 0.64	8.59 ± 0.92	8.20 ± 1.73	8.90 ± 1.23

Supplementary Table 15. Analysis of variance (ANOVAs) of the liver lipoprotein lipase (*lpl*) and insulin growth factor 1 (*igf1*) expression of stellate sturgeon (*A. stellatus*) with diet as factor. The variation among diets was decomposed into a linear component and a residual term (deviation) with polynomial contrasts. See Supplementary Table 16 for variables summary and Figure 6 for polynomial contrast representation.

Variable	**Explained variation (Adj. R^2)**	**Among diets**			**Phospholipids content (%)**		
					Linear contrast	**Deviation test**	**Quadratic contrast**
		$F_{6, 14}$	P	η_p^2	P	P	P
lpl expression	0.92	44.00	<0.001	0.95	<0.001	0.006	0.069
igf1 expression	0.920	39.22	<0.001	0.94	<0.001	0.022	0.017