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ORIGINAL ARTICLE

Replacement of Partial fish meal by Heat-treated Soybean meal in Juvenile Siberian sturgeon (*Acipenser baerii*) diet: effect on growth performance, body composition, hematological, Biochemical Parameters and Complement activity

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ABSTRACT
This study was carried out to investigate effect of replacing fish meal (FM) with a heat-treated soybean meal (SBM) on growth performance, body composition, hematological, biochemical parameters and complement activity of Siberian sturgeon Acipenser baerii. Experimental diets were prepared in four replacement levels of 0% (diet 1), 10% (diet 2), 20% (diet 3), and 30% (diet 4), respectively. The fish were fed to satiation twice daily for a feeding period of 60 days. The final body weight (FBW), specific growth rate (SGR), body weight increase (BWI) and condition factor (CF) of fish fed diet 3 were significantly higher than those fed diets 1, 2 and 4, and feed conversion ratio (FCR) in the group fed diet 3 was significantly lower than other groups. Whole body crude protein and ash of fish fed SBM at the level of 30% were significantly ($p<0.05$) higher than the fish fed other diets. Results suggested that some hematological parameters such as hemoglobin (Hb), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) significantly changed between treatments and with increasing of level of heat-treated soybean meal in the diet, a decreasing trend was observed whereas other hematological characteristics did not show significant difference. Among biochemical indices, cholesterol (CHO) and low- density lipoprotein (LDL) were affected by FM replacement with different levels of SBM; as with increasing dietary SBM level, these parameters decreased. There were no significant differences in plasma complement activity (C_3, C_4 and CH_{50}). According to the obtained results, the maximum levels of fish meal replacement by heat-treated soybean meal in diets of Acipenser baerii could be between 15-30% of total protein sources of diet.

Keywords: *Acipenser baerii, soybean meal, nutrition, hematology*

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INTRODUCTION

Fish meal (FM) has been used as a raw material in aquaculture industry for its unique traits including high quality of protein and well-balanced amino acid profile [9]. In recent years, world production of fish meal is approximately 6–7 million tons annually, while this level of production is expected to remain stable over the next 10 years [23]. In the last decade, the increasing demand, price and world supply fluctuations of FM has emphasized the need to look for alternative protein sources in aquafeed. Some plant protein sources such as corn gluten and SBM are widely used in fish nutrition because of their high protein content (40-60%), low cost and relative widespread availability. SBM is currently the most commonly used plant protein source in fish feeds [38]. SBM is used as a cost-effective feed ingredient and comprises up to 50% of the diet of freshwater fish species [38]. A relatively low substitute proportion of FM protein with mixture of plant protein in diets can reduce cost of feed formulation.

Partial or even total replacement of dietary fishmeal by SBM protein sources had successfully accomplished with tilapia diets [8]. Some studies have shown that considerable success in replacing FM with SBM in diets of aquatic animals. The use of SBM is limited in fish diet due to low level of methionine and the presence of anti-nutritional factors (ANFs). SBM is characterized to contain several ANFs, for example protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamins, and allergens [7]. It has shown that ANFs in a diet of raw or inadequately heated SBM adversely affect various animals including fish [24], [3], [20]. It has also been demonstrated that adequately heated SBM has the potential to improve its nutritive value [24]. Thus, heat treatments denaturing the inhibitor protein are used to make soybean suitable for feeding fish. The Siberian sturgeon *Acipenser baerii* Brandt is a migratory fish which cultured under fully controlled conditions, i.e. in ponds supplied with temperature-controlled water with artificial feed [28]. Siberian sturgeon has been the focus of much attention to date because it is a particularly interesting species in terms of rearing value. However, the feeding patterns of this species on natural food have only been studied on a small scale. No studies have yet examined the effects of heat-treated soybean meal on the Siberian sturgeon. Thus, the objective of present study was to investigate effect partial fish meal replacement with heat-treated SBM on growth, body composition, hematological, biochemical parameters and complement activity of juvenile Siberian sturgeon, *A.baerii.*

MATERIALS AND METHODS

Experimental diets

The study was conducted in International Sturgeon Research Institute, Rasht, Iran in 2012. White FM (production center of aquatic animals feed, Mazandaran province, Iran) and commercially heat-treated SBM (HP300**,** Hamlet**,** Denmark) were used as the main animal and plant protein ingredients, respectively. Four isonitrogenous (47% crude protein) and isolipidic (9% crude lipid) diets (Diets 1–4) were designed with different animal-plant protein values. FM protein was replaced by 0%, 10%, 20%, and 30% of SBM protein in the four experimental diets, respectively. The ingredients of four experimental diets are shown in Table 1. The ingredients of each diet were blended to a homogeneous mixture. Water was added, and the dough was extruded to form strands and then pelleted to suitable size through a 2.5-mm die. The pellets were dried in a ventilated oven at 45°C for 12 h and then placed at room temperature before sealing in plastic bags and stored at -20°C until feeding.

Fish and feeding

A total of 120 juveniles of *A.baerii* weighing 198.2 ± 17.9 g were provided from International Sturgeon Research Institute, Rasht, Iran. Fish were randomly stocked in 12 batches of 10 fish each, in 400-l cylindroconical fiberglass tanks. Three replicate tanks per dietary treatment were used. Water was supplied by recirculated freshwater. During experimentation, continuous aeration was provided, and water temperature was 21.36±3.68°C. In addition, the tanks were siphoned daily, before the first feeding, to remove fecal material, and they were thoroughly scrubbed and completely flushed fortnightly, when fish were removed for weighing. Fish were acclimatized to experimental conditions for 14 days prior to the feeding trial during which they received a control diet. At the beginning of the experimental feeding trial, each diet was assigned randomly to three tanks. At the start of the feeding trial, the fish were fasted for 24 h. The fish were hand fed to apparent satiation (i.e., until the first feed item was refused) two times daily. Daily feed consumption was obtained for each tank by weighing the feed at the start and end of each day.

Sample collection

At the end of experiment, five fish were randomly sampled from each replicate and then pooled in plastic bags and stored at -20ºC for whole body composition analysis. The proximate body composition (Moisture, crude protein, crude lipid, and ash) was determined using the standard methods of the Association of Official Analytical Chemists [2]. Protein by estimating the Kjeldahl nitrogen (×6.25), moisture by heating at 105ºC to constant weight, ash by incinerating in a crucible at 600ºC for 18 h and crude lipid was determined by using Soxhlet apparatus. Another five fish from each tank were randomly sampled, anesthetized with clove oil (100 mg/l). Blood sample was taken gently from the caudal vein by 5-mL plastic syringe, for hematological and biochemical tests. Two different of blood samples were used for different analyses. The first sample was transferred to an eppendorf tube coated with heparin as anticoagulant and was used for hematological indices. Red blood cell (RBC) and white blood cell (WBC) were determined with a Neubauer using Rees diluting solution. To determination differential count of leukocyte, that is, measure of lymphocyte, neutrophil, eosinophil and monocyte, the obtained smears were first air dried, fixed in 96% ethanol for 30 min, stained by Giemsa (Merck, Germany) staining for 30 min and were examined for leukocyte differential count under light microscope [17]. Hemoglobin

concentration (Hb) was determined with Drabkin's reagent and read at absorbance at 540 nm [15]. According to the procedure of Rehulka [27], haematocrit (Hct) was measured in microhaematocrit heparinised capillaries, using a microhematocrit centrifuge at 13,000 rpm for 3 min (Hettrich, Germany). Hematological indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Haney et al [10]. Another blood samples were collected in centrifuge tubes without anticoagulant for serum. Blood was centrifuged at 3000 rpm for 15 min in cooling centrifuge (Heraeus sepatech, Germany) for separation of plasma which was stored at -80ºC until used for biochemical analysis. Blood glucose, cholesterol, triglyceride, total protein, albumin, high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL) in serum were determined with their corresponding kits (Pars Azmoon Company, Tehran, Iran). All these parameters were determined using a spectrophotometer (S200- UV/VIS England). Plasma complement activities (C_3, C_4 and CH_{50}) were assayed according to Yano [39] with some modification according to Takahaschi et al [34].

Growth performance

At the end of the feeding trial, fish were fasted for 24 h and then body weight increase (BWI), feed conversion ratio (FCR), specific growth rate (SGR) and condition factor (CF) were calculated according to Huang et al [13].

FCR = dry feed intake (g)/wet weight gain (g)

$BWI = (W_f - W_i) \times 100 / W_i$

$SGR = (\ln W_f - \ln W_i) \times 100/t$

$CF = \text{Weight (g)} \times 100/\text{TL (cm)}^3$

Where W_f and W_i were final and initial fish weights (g), respectively; TL was total length and t is the experimental duration in day.

Statistical analysis

All data were subjected to one-way ANOVA test to determine whether significant differences occurred among dietary treatments after confirmation of normality and homogeneity of variance, where differences were identified, multiple comparisons among means were performed with Duncan's multiple range test using SPSS software (Version 20, SPSS Inc. Chicago, Illinois, USA). Significance was declared at $p<0.05$. Experimental results were expressed as mean ± SD.

RESULTS AND DISCUSSION

All experimental diets were well ingested by the Siberian sturgeon during the experimental period. The survival rate of fish in each dietary treatment was 100%. The growth performance of the experimental fish is shown in Table 2. With the increase of dietary SBM levels, growth factors including FBW, BWI, SGR and CF increased significantly in diet 3, while FCR decreased significantly in diet 3 ($p<0.05$). There were significant differences in moisture, protein, fat and ash of body among all dietary treatments (Table 3). As shown in Table 4, Hb, MCH and MCHC significantly changed between all dietary treatments. With the increase of dietary SBM levels, Hb, MCH and MCHC decreased significantly ($p<0.05$). Other hematological indices such as WBC, RBC, Hct, eosinophil and neutrophil among all dietary treatments did not have considerable differences. Some biochemical indices such as cholesterol, HDL and LDL concentrations were significantly ($p<0.05$) influenced by the dietary treatments (Table 5). In diet 3, total protein was higher than in comparison with others. Cholesterol concentration in the fish fed diet 1 was significantly lower than that in fish fed other diets while HDL and LDL concentrations were significantly ($p<0.05$) lower than that of fish fed other experimental diets. Data on plasma complement activity (C_3, C_4 and CH_{50}) are shown in Table 6. Different dietary soybean meal inclusion did not affect on complement activity ($p>0.05$).

Table 1 Ingredients of the experimental diets (as fed basis)

Ingredients (%)	Level of soybean meal			
	Diet 1 (0%)	Diet 2 (10%)	Diet 3 (20%)	Diet 4 (30%)
Fish meal	68	61.3	55	46
Soybean meal	0	10	20	30
Wheat flour	18	13.7	9	7
Fish oil	3.5	4	4.5	5
Sunflower oil	3.5	4	4.5	5
Vitamin premix*	2	2	2	2
Mineral premix**	1.5	1.5	1.5	1.5
Choline chloride	1.5	1.5	1.5	1.5
Methionine	1	1	1	1
Lysine	1	1	1	1

* Vitamin mixtures (IU or mg/kg diet): DL-alpha tocopherol acetate. 60 IU; DL-cholecalciferol, 3000 IU; thiamin, 15 mg; ribofelavin, 30 mg; pyridoxine, 15 mg; B_{12}, 0.05 mg; nicotinic acid, 175 mg; folic acid, 5 mg; ascorbic acid, 500 mg; inositol, 1000 mg; biotin, 2.5 mg; calcium panthoteate, 50 mg; choline chloride, 2000 mg.
** Mineral mixture (g or mg/kg diet): calcium carbonate (40% Ca), 2.15 g; magnesium oxide (60% Mg), 1.24 g; ferric citrate, 0.2 g; potassium iodide (75% I), 0.4 mg; zinc sulphate (36% Zn), 0.4 g; copper sulphate (25% Cu), 0.3 g; manganese sulphate (33% Min), 0.3 g; dibasic calcium phosphate (20% Ca, 18% p), 5 g; cobalt sulphate, 2 mg; sodium selenite (30% Se), 3 mg; KCl,0.9 g; NaCl, 0.4 g.

Table 2 Effect of dietary soybean meal inclusion on growth performance of juvenile *Acipenser baerii*

Parameters	Level of soybean meal			
	Diet 1 (0%)	Diet 2 (10%)	Diet 3 (20%)	Diet 4 (30%)
BWI (%)	126.54 ± 26.11^{ab}	100.12 ± 12.53^{b}	164.7 ± 1.36^{a}	158.11 ± 25.07^{a}
FBW (g)	420.4 ± 44.37^{ab}	374.03 ± 23.34^{b}	488.25 ± 5.35^{a}	485.66 ± 51.05^{a}
FCR	2.03 ± 0.038^{ab}	2.55 ± 0.032^{b}	1.59 ± 0.021^{a}	1.65 ± 0.03^{a}
SGR (%/day)	0.90 ± 0.012^{ab}	0.76 ± 0.068^{b}	1.07 ± 0.057^{a}	1.05 ± 0.011^{a}
Condition factor (%)	0.31 ± 0.0028^{a}	0.26 ± 0.0031^{a}	0.35 ± 0.0081^{a}	0.32 ± 0.0042^{a}

Table 3 Effect of dietary soybean meal inclusion on whole body composition of juvenile *Acipenser baerii*

Parameters (%)	Level of soybean meal			
	Diet 1 (0%)	Diet 2 (10%)	Diet 3 (20%)	Diet 4 (30%)
Moisture	63.25 ± 0.25^{c}	66.36 ± 0.5^{ab}	67.18 ± 0.22^{a}	65.47 ± 0.24^{b}
Crude protein	39.58 ± 1.01^{b}	46.94 ± 0.3^{a}	48.04 ± 3.07^{a}	45.87 ± 0.4^{a}
Crude lipid	33.25 ± 0.35^{c}	35.92 ± 0.31^{b}	39 ± 0.7^{a}	30.75 ± 0.35^{d}
Ash	2 ± 0.1^{b}	3.5 ± 0.7^{b}	4.7 ± 0.35^{a}	3.5 ± 0.7^{ab}

Table 4 Hematological profiles of juvenile *Acipenser baerii* in response to dietary soybean meal inclusion

Parameters	Level of soybean meal			
	Diet 1 (0%)	Diet 2 (10%)	Diet 3 (20%)	Diet 4 (30%)
RBC (×10^{6}mm)	0.11 ± 0.02	0.12 ± 0.03	0.14 ± 0.05	0.1 ± 0.04
WBC (×10^{3}mm)	22.65 ± 7.74	23.91± 5.21	15.93 ± 5.99	15.63 ± 6.33
Hematocrit (%)	27.46 ± 2.79	27.10 ± 4.35	26.56 ± 3.84	25.56 ± 0.84
Hemoglobin (g/dl)	6.87 ± 0.84^{a}	5.54 ± 0.40^{ab}	4.09 ± 0.81^{b}	4.30 ± 1.08^{b}
MCV (fl)	262.3 ± 87^{b}	228 ± 64^{ab}	204.3 ± 73^{a}	283.1 ± 131^{b}
MCH (pg)	64.03 ± 14.13^{a}	47.02 ± 15.91^{ab}	30.19 ± 6.42^{b}	48.27 ± 23.63^{ab}
MCHC (g/dl)	25.39 ± 5.64^{a}	20.45 ± 1.48^{ab}	15.47 ± 2.98^{b}	16.85 ± 4.08^{b}
Lymphocyte (%)	87.2 ± 2.87^{a}	80.13 ± 3.19^{b}	81.66 ± 2.14^{b}	85.38 ± 2.92^{ab}
Monocyte (%)	1.23 ± 0.75^{b}	3.8 ± 1.67^{a}	2.43 ± 0.51^{ab}	1.88 ± 0.4^{b}
Neutrophil (%)	9.53 ± 1.5	13.27 ± 1.66	10.1 ± 2.91	9.82 ± 1.19
Eosinophil (%)	2.6 ± 1.44	3.6 ± 1.77	5.63 ± 3.26	2.88 ± 1.54

Table 5 Biochemical indices of juvenile *Acipenser baerii* in response to dietary soybean meal inclusion

Parameters	Level of soybean meal			
	Diet 1 (0%)	Diet 2 (10%)	Diet 3 (20%)	Diet 4 (30%)
Total protein (g/l)	3.84 ± 0.21	3.44 ± 0.55	3.98 ± 0.75	3.85 ± 1.73
Glucose (mmol/l)	25.8 ± 5.07	62.25 ± 13.46	55.4 ± 32.38	54.58 ± 13.7
Cholesterol (mmol/l)	95.84 ± 14.42^{c}	149.47 ± 7.49^{a}	125.9 ± 8.88^{ab}	134.99 ± 14.13^{a}
Triglyceride (mmol/l)	963.26 ± 97.42	587.4 ± 29.83	816.67 ± 294.48	868.56 ± 142.09
Albumin (mg/dl)	1.81 ± 0.88	1.13 ± 0.25	1.64 ± 0.25	1.8 ± 0.31
HDL (mmol/l)	29.6 ± 2.81^{a}	25 ± 3.14^{b}	24.2 ± 5.33^{bc}	21.6 ± 8.45^{d}
LDL (mmol/l)	43.3 ± 3.14^{a}	38.3 ± 2.97^{b}	32 ± 2.06^{c}	29.6 ± 3.30^{cd}

Table 6 *Complement activity* of juvenile *Acipenser baerii* in response to dietary soybean meal inclusion

Parameters	Level of soybean meal			
	Diet 1 (0%)	Diet 2 (10%)	Diet 3 (20%)	Diet 4 (30%)
C_3 (mg/dl)	15.33 ± 1.53	16 ± 1	14.33 ± 4.16	19.33 ± 4.93
C_4 (mg/dl)	13.33 ± 1.53	13.66 ± 1.15	12 ± 4	12.33 ± 2.08
CH_{50} (U/ml)	17.66 ± 1.53	20 ± 6	15.33 ± 1.53	16 ± 1

There are many studies on the replacement of fishmeal with soybean products in sturgeon feeds. Substitution 40% of fish meal in diet with soy protein concentrate in a diet for juvenile white sturgeon led to poorer growth compared with a purified test diet [33]. Ustaoglu and Rennert [35] reported that

total replacement of fishmeal with soy protein reduced the growth rate in sterlet, although the soy protein diet was better digested (93.6%) than the fishmeal diet (91.2%). The present study showed that different SBM levels significantly affected the growth, body composition and feed utilization of Siberian sturgeon. The positive effects of FBW, FCR, SGR, CF, protein and ash of carcass in response to dietary SBM increase, indicating that dietary SBM level below 30% was suitable for Siberian sturgeon. Pham et al [25] reported that 20% FM protein replacement by SBM (8.7%) had no adverse effect on the growth of Japanese flounder, *Paralichthys olivaceus*. Dietary SBM inclusion of up to 20 or 32% did not affect the fish growth in feeding experiments [30], [4]. On the contrary, growth did not influence on gilthead sea bream, *Sparus aurata* [31], Sterlet, *Acipenser ruthenus* [35] and Japanese flounder, *P.olivaceus* [40]. This discrepancy observed in this experiment with others might be attributed to difference response to SBM levels in diet and also experimental conditions. Another explanation is due to methionine and phosphorus deficiencies in the soy protein diet. Although soy protein has a well-balanced amino acid profile for fish, it is low in methionine [32] and contains phytic phosphorus which is of limited availability for fish [19]. Alyakrinskyaya and Dolgova [1] demonstrated hematological studies of sturgeons are important in understanding their evolution and also controlling the physiological condition of fish in rearing conditions. However, few investigation have been assessed the hematological parameters of fish, particularly sturgeon, in response to partial replacement of dietary fish meal with SBM. In the present experiment, replacing fish meal with heat-treated SBM resulted in a significant ($p<0.05$) different in the Hb, MCH, MCHC, CHO, HDL and LDL while significant differences were not observed in WBC, RBC, haematocrit, neutrophil, eosinophil, triglyceride, total protein, albumin and glucose plasma ($p>0.05$). Similar results have been reported on beluga (*Huso huso*) juveniles fed different replacement of FM with SBM in diet [12]. Food quality strongly influences blood indices and the qualitative and quantitative properties of Hb in sturgeon juveniles [6]. However, there are no clear relationship between the replacement level of FM with plant proteins in the diet and deterioration of hematological parameters of the cultured fish [22]. Hct concentration decreased parallel to inclusion plant protein in diet of great sturgeon; however, in fish meal diet, it was higher [14]. They reported Juvenile sturgeons had a higher Hb concentration in contrast to other fish species [37]. In agreement to our results, a reduction of Hct occurred in juvenile sturgeons given a diet of oligochaeta [1]. Our present results showed decreased serum CHO, HDL and LDL levels with the increase in dietary SBM, several studies showed serum CHO levels decreased with the elevation of dietary SBM in Atlantic cod [11], rainbow trout [29], European Sea bass, *Dicentrarchus labrax* [5] and turbot [26]. On the contrary, increased dietary SBM level elevated CHO level in cobia, *Rachycentron canadum* [41], European Sea bass [16] and Japanese flounder, *P. olivaceus* [40]. Kritchevsky [18] demonstrated increase in CHO, LDL and HDL levels imply that the fish may have had some disorder in lipid metabolism, leading to hyperlipidemia. In conclusion, according to the results obtained in the present experiment, maximum proposed levels of fish meal replacement by SBM in diets for Siberian sturgeon, *A. baerii* can be recognized at 15–30 % of total protein.

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REFERENCES

1. Alyakrinskyaya, I.O. & Dolgova, S.N. (1984). Hematological features of young sturgeons. Vopr. Ikhtiol., 4:135–139.
2. AOAC, (1995). Official methods of analysis, 16th edition Association of Official Analytical Chemists, Arlington, VA, 1298pp.
3. Barrows, F.T., Stone, D.A.J. & Hardy, R.W. (2007). The effects of extrusion conditions on the nutritional value of soybean meal for rainbow trout (Oncorhynchus mykiss). Aquaculture, 265: 244-252.
4. Choi, S.M., Wang, X., Park, G.J., Lim, S.R., Kim, K.W., Bai, S.C. & Shin, I.S. (2004). Dietary dehulled soybean meal as a replacement for fish meal in fingerling and growing olive flounder Paralichthys olivaceus. Aquacult. Res., 35(4): 410–418.
5. Dias, J., Alvarez, M.J., Arzel, J., Corraze, G., Diez, A., Bautista, J.M. & Kaushik, S.J. (2005). Dietary Protein Source Affects Lipid Metabolism In The European Sea Bass (Dicentrarchus Labrax). Com. Biochem. Physiol., 142: 19-31.
6. Domezain, A.E., Martínez-Álvarez, R.M., Hidalgo, M.C, Morales, A., García Gallego, M. & Sanz, A. (2002). Physiological changes of sturgeon Acipenser naccarii caused by increasing environmental salinity. J. Exp. Biol., 205: 3699-3706.
7. Francis, G., Makkar, H.P.S. & Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture, 199: 197-227.
8. Gaber, M.M. (2006). The Effects of Plant-protein-based Diets Supplemented with Yucca on Growth, Digestibility,

and Chemical Composition of Nile Tilapia (Oreochromis niloticus, L) Fingerlings. J. World Aquac. Soc., 37: 74-81.
9. Gatlin, D.M. III., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R. & Wurtele, E. (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquacult. Res., 38: 551-579.
10. Haney, D.C., Hursh, D.A., Mix, M.C. & Winton, J.R. (1992). Physiological and hematological changes in chum salmon artificially infected with erythrocytic necrosis virus. J. Aquat. Anim. Health, 4: 48-57.
11. Hansen, A.C., Rosenlund, G., Karlsen, O., Koppe, W. & Hemre, G.I. (2007). Total replacement of fish meal with plant proteins in diets for Atlantic cod (Gadus morhua L.) I-Effects on growth and protein retention. Aquaculture, 272: 599-611.
12. Hosseini, S.A. & Khajepour, F. (2012). Effect of partial replacement of dietary fish meal with soybean meal on some hematological and serum biochemical parameters of juvenile Beluga, Huso huso. Iran J. Fish Sci., 12: 348-356.
13. Huang, C.H., Chang, R.J., Huang, S.L., & Chen, W. (2003). Dietary vitamin E supplementation affects tissue lipid peroxidation of hybrid tilapia, Oreochromis niloticus × O. aureus. Com. Biochem. Physiol. 134: 265-70.
14. Jahanbakhshi, A., Imanpoor, M.R., Taghizadeh, V. & Shabani, A. (2013). Hematological and serum biochemical indices changes induced by replacing fish meal with plant protein (sesame oil cake and corn gluten) in the Great sturgeon (Huso huso). Comp. Clin. Path., 22: 1087-1092.
15. Jain, N.C. (1993). Essentials of Veterinary Hematology. Wiley-Blackwell publishing. Lea and Febiger, Philadelphia. USA. pp. 417.
16. Kaushik, S.J., Cove`s, D., Dutto, G. & Blanc, D. (2004). Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European Sea bass, Dicentrarchus labrax. Aquaculture, 230: 391-404.
17. Klontz, G.W. (1994). Fish hematology. Techniques in fish immunology. (Eds. Stolen, J.S., Fletcher, T.C., Rowley, A.F., Kelikoff, T.C., Kaatari, S.L. and Smith, S.A). Vol. 3. SOS Publications, Fair Haven, New Jersey, USA. p.121-132.
18. Kritchevsky, D. (1995). Dietary protein, cholesterol and atherosclerosis: a review of the early history. J. Nutr., 125: 589-593.
19. Lall, S.P. (1991). Digestibility, metabolism and excretion of dietary phosphorus in fish. (Eds. Cowey, C.B. and Cho, C.Y.), Nutritional Strategies and Aquaculture Waste. University of Guelph, Ontario, Canada. p. 21-36.
20. Machado, F.P.P., Queiro, J.H., Oliveira, M.G.A., Piovesan, N.D., Peluzio, M.C.G., Costa, N.M.B., & Moriera, M.A. (2008). Effects of heating on protein quality of soybean flour devoid of Kunitz inhibitor and lectin. Food Chem., 107: 649-655.
21. Martínez-Llorens, S., Tomás, A., Jauralde, I., Pla, M., & Jover, M. (2009). Optimum dietary soybean meal level for maximizing growth and nutrient utilization of on-growing gilthead sea bream (Sparus aurata). Aquacult. Nutr., 15: 320-328.
22. Moreau, R., Dabrowski, K., Czesny, S. & Chila, F. (1999). Vitamin C- vitamin E interaction in juvenile lake sturgeon (Acipenser fulvescens), a fish able to synthesize ascorbic acid. J. Appl. Ichthyol., 15: 250–257.
23. New, M.B. & Wijkstrom, U.N. (2002). Use of Fishmeal and Fish oil in Aquafeeds: Further Thoughts on the Fishmeal Trap, FAO Fish Circ, Rome, Italy. No.975 FIPP/C975.
24. Peres, H., Lim, C. & Klesus, P.H. (2003). Nutritional value of heat-treated soybean meal for channel catfish (Ictalurus punctatus). Aquaculture, 225: 67-82.
25. Pham, M.A., Lee, K.J., Lim, S.J., & Park, K.H. (2007). Evaluation of cottonseed and soybean meal as partial replacement for fishmeal in diets for juvenile Japanese flounder Paralichthys olivaceus. Fish Sci., 73: 760–769.
26. Regost, C., Arzel, J. & Kaushik, S.J. (1999). Partial or replacement of fish meal by corn gluten meal in diet for turbot (Psetta maxima). Aquaculture, 180: 99-177.
27. Rehulka, J. (2000). Influence of astaxanthin on growth rate condition and some blood indices of rainbow trout Oncorhynchus mykiss. Aquaculture, 190: 27-47.
28. Reichle, G., Bercsenyi, M., & Belger, H. (1991). Store im Bruthans und in der Teichwirtschaft – Fisch Teich., 42: 339-341.
29. Romarheim, O.H., Skrede, A., Gao, Y., Krogdahl, A., Denstadli, V., Lilleeng, E. & Storebakken, T., (2006). Comparison of white flakes and toasted soybean meal partly replacing fish meal as protein source in extruded feed for rainbow trout (Oncorhynchus mykiss). Aquaculture, 256: 354–364.
30. Saitoh, S., Koshio, S., Harada, H., Watanabe, K., Yoshida, T., Teshima, S.I., & Ishikawa, M. (2003). Utilization of extruded soybean meal for Japanese flounder *Paralichthys olivaceus* juveniles. Fish Sci., 69: 1075–1077.
31. Sitjà-Bobadillaa, A., Peña-Llopisa, S., Gómez-Requenia, P., Médaleb, F., Kaushikb, S. & Pérez-Sáncheza, J. (2005). Effect of fish meal replacement by plant protein sources on non-specific defence mechanisms and oxidative stress in gilthead sea bream (Sparus aurata). Aquaculture, 249: 387-400.
32. Storebakken, T., Refstie, S. & Ruyter, B. (2000). Soy products as fat and protein sources in fish feed for intensive aquaculture. (Eds. Drackley, J.K.), Soy in Animal Nutrition. Federation of Animal Science Societies. Savoy, Illinois, USA. p. 127-170.
33. Stuart, S.J. & Hung, S.O. (1989). Growth of juvenile white sturgeon (*Acipenser transmontanus*) fed different proteins. Aquaculture, 76: 303-316.
34. Takahaschi, M., Takahaschi, S., Brade, U. & Nussenzweig, V. (1978). Requirement for the solubilization of immuno-aggregates by complement. The role of the classical pathway. J. Clini. Invest., 62: 349-54.
35. Ustaoglu, S. & Rennert, B. (2002). The apparent nutrient digestibility of diets containing fishmeal or isolated soy protein in sterlet (Acipenser ruthenus). II. Rearing techniques of sturgeon. Int. Revue Hydrobiol., 87: 577-584.

36. Ustaoglu, S. & Rennert, B. (2006). Effects of Partial Replacement of Fishmeal with Isolated Soy Protein on Digestibility and Growth Performance in Sterlet (Acipenser ruthenus). Isr. J. Aquacult. – Bamidgeh, 58: 170-177.
37. Wedemeyer, G.A., Barton, B.A. & McLeay, D.J. (1990). Stress and acclimation. (Eds. Schreck, C.B. and Moyle, P.B.), Methods for fish biology. American Fisheries Society, Bethesda, MD, USA. p. 451–489.
38. Yan, W., Kong, L.J., Cui, L. & Dominique, P.B. (2006). Effect of replacing fish meal with soybean meal on growth, feed utilization and carcass composition of cuneate drum (Nibea miichthioides). Aquaculture, 261: 1307–1313.
39. Yano, T. (1992). Assays of hemolytic complement activity. (Eds. Stolen, J.S., Fletcher, T.C. and Rowley, A.F.), Techniques in fish immunology. SOS Publications, Poland. p. 131-141.
40. Ye, J., Liu, X., Wang, Z. & Wang, K. (2011). Effect of partial fish meal replacement by soybean meal on the growth performance and biochemical indices of juvenile Japanese flounder Paralichthys olivaceus. Aquacult. Int., 19: 143-153.
41. Zhou, Q.C., Mai, K.S., Tan, B.P. & Liu, Y.J. (2005). Partial replacement of fishmeal by soybean meal in diets for juvenile cobia (Rachycentron canadum). Aquacult. Nutr., 11: 175–182.