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Full Length Research Paper

Effects of phospholipids in the diet on biochemical factors of sturgeon fish (*Huso-huso*) juveniles

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A study was carried out to determine the influence of dietary phospholipids biochemical factors parameters of beluga sturgeon (*Huso huso*) juveniles. Juveniles were fed formulated diet with four varying dietary levels of PL, that is, 0 (D1), 2 (D2), 4 (D3) and 6% (D4). At the end of the experimental period (56 days), there were no significant differences (P > 0.05) found for hepatic enzymes among treatments. The enzyme alanine aminotransferase (ALT) was highest in fish fed diet D2, while the lowest was found in fish fed diet D1 with values of 46.07 and 24.70 (IU/L), respectively. The enzyme aspartate aminotransferase (AST) was found highest in fish fed diet D3 and lowest was found in fish fed diet D2 with values 16.43 and 12.47 (IU/L), respectively. The enzyme lipase was found to be highest in fish fed diet D4, while the lowest was shown in fish fed diet D3 with values of 32.47 and 29.03 (IU/L), respectively. Among the biochemical factors, there was a significant difference (P < 0.05) observed in blood sugar, in which case, the highest reading was found in fish fed diet D3 with amount of 83 mg/dl and the lowest amount was found in fish fed diet D2 with a value of 46 mg/dl. However, the other biochemical parameters such as cholesterol and triglyceride did not showed significant differences (P > 0.05) among treatments. The triglyceride values ranged from the highest and lowest values were found in fish fed diets D4 and D2 with values of 349.33 and 263.00 (mg/dl), respectively. The cholesterol values which ranged from the highest to lowest were found in fish fed diets D4 and D2 with values of 121.67and 104.67 (mg/dl), respectively.

Key words: Phospholipids, sturgeon fish, *Huso huso,* biochemical factors.

INTRODUCTION

Phospholipids have a great effect on the growth, deformity and resistance against stress in some fish and shellfish species (Cahu et al., 2003; Koven et al., 1998). Phospholipids contain choline (phosphatidylcholine and sphingomyelin) accumulate in the external part of cell wall and phosphatidyl ethanolamine and phosphatidylserine and little amount of phosphatidylinositol in the internal part of cell wall. Lipoproteins formed with phospholipids, cholesterol and proteins cause lipids like triglyceride and steryl esters to be able to transfer to the serum (Tocher, 1995). In fishes, phosphatidylcholine is the main phospholipids in liver. However, sphingomyelin can be dominant phospholipids in the species with little amount of phospholipids and phospholipids bilious salt (Moschetta et al., 2005). Phospholipids have a very big role in protecting the structure and function of cell membrane (Tocher, 2003; Kanazawa, 1985).

Presently, much research has not been conducted on

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Table 1. Experimental diet formulation and proximate composition.

Diet Ingredient (%)	Dietary treatment			
	D1 (0% PL)	D2 (2% PL)	D3 (4% PL)	D4 (6% PL)
Fish meal	60	60	60	60
Wheat meal	20	20	20	20
Fish oil	5	5	5	5
Soybean oil	6	4	2	0
Phospholipids	0	2	4	6
Molasses	2.3	2.3	2.3	2.3
Vitamin mixture	2	2	2	2
Mineral mixture	3	3	3	3
Anti oxidant	0.2	0.2	0.2	0.2
Calcium phosphate	1.5	1.5	1.5	1.5
Total	100	100	100	100
Determined composition				
Dry matter (%)	90.3	90.1	90.9	90.9
Protein (N x 6.25)(% DM)	44.94	45.94	45.21	45.1
Lipid(% DM)	14.7	15.1	15.3	15.5
Ash (% DM)	13.58	13.52	13.84	13.4
Gross energy (kJ g^{-1} diet)	21.01	21.18	21.10	21.23

the nutritional importance of *Huso huso* diets formulation. These have resulted to non commercial feed diets available for *Huso huso,* instead of the nutritionally substandard feeds formulated for other species that are commonly used for feeding *Huso huso*. In comparison with other sturgeon fishes, there is much less information currently available on *Huso huso* dietary requirements, especially for lipid utilization and fatty acid composition (Hosseini et al., 2010).

Beluga like other species of sturgeon fishes in the Caspian Sea, population is reducing due to the excessive fishing and destruction of their habitat and unfortunately, they have limited artificial reproduction. Beluga species are currently endangered with extinction. Due to the increasing world population, importance and demand for the sturgeon product, that is, caviar and its meat, the price of sturgeon became very expensive and as a result, there is difficulty in buying them in most of the 3rd world countries. Very few populations can afford buying them. It seems that large part of this demand can be provided in future by aquaculture (Hafezie and Hasani, 2007).

MATERIALS AND METHODS

Location of study

The experiment was conducted in May 24th to July 18th 2009 in Shahid Margani Reproduction and Culture Center for sturgeon fish, Gorgan, Iran. A sample of 720 Beluga juveniles were collected with an average weight of 4.5±0.1 g and were acclimatized for three weeks at Shahid Margani Reproduction and Culture Center for sturgeon fish.

Preparation of tanks

Twelve 200 L fiberglass tanks (four treatments and three replications) supplied with water, were randomly arranged and numbered. Tanks were completely washed with water and disinfected with materials like sodium hypochlorite.

Fish diet was formulated (Table 1) using fish meal as the main source of protein with 45% of protein, lipid 15% containing 21.00 kJ/g of gross energy by means of Lindo software (Lindo copyright, 1995, Releases 6.1). Purified soybean phospholipids (Bergapor Co. Germany were added at four dietary levels (0, 20, 40 and 60 g kg^{-1}) by reducing soybean oil. The formulations and proximate compositions of the experimental diets are shown in Table 1.

Biochemical factors measurement

Blood biochemical parameters such as triglyceride, total cholesterol, glucose and liver enzymes (Aspartic amino transferase, alanine amino transferase) were also measured. About 2 ml of blood for these biochemical factors measurement was left to clot and centrifuged to at least 1 cc obtained serum. The serum samples were separated from clotted blood by centrifuging for 10 min at 3000 rpm (350 g). The collected serum were transferred to tubes and were then quickly stored at -20°C prior to biochemical parameters (Velisek et al., 2005).

Table 2. Biochemical factors of *Huso huso* fed with four dietary levels of phospholipids in 8 weeks[1].

Serum index/treatment	Dietary treatment			
	D1(0%PL)	D2(2%PL)	D3(4%PL)	D4 (6%PL)
Triglyceride (mg/dl)	344.67±20.92[a]	263.00±39.59[a]	310.00±42.25[a]	349.33±33.79[a]
Cholesterol (mg/dl)	119.67±12.47[a]	104.67±21.62[a]	119.00±5.03[a]	121.67±22.92[a]
Blood sugar (mg/dl)	48±7.37[b]	46±6.03[b]	83±7.00[a]	70±10.44[ab]

[1]D1 (control); 0% phospholipids; D2: 2% phospholipids; D3: 4% phospholipids; D4: 6% phospholipids. Values in each row with the same superscripts are not significantly different at P ≥ 0.05.

Determination of blood, total cholesterol, triglyceride and glucose concentration

Serum samples were analyzed for total cholesterol, triglyceride and glucose concentration. The enzyme calorimetric method was used to determine the quantity of total cholesterol, triglyceride and glucose in the serum. To measure triglyceride level, glycerol was first separated from fatty acid by lipoprotein lipase enzyme and then the released oxygen from glycerol forms chinonimin with -4 amino antipirin and phenol beside peroxidize enzyme. The formed chinonimin level which is measurable photometrically has a direct relation with triglyceride quantity (Cole et al., 1997). In the experiment of total cholesterol (Rifai et al., 1999) and glucose (Barham and Trinder, 1972) measurement, the released oxygen from cholesterol beside cholesterol and glucose oxidize respectively forms chinonimin with -4 amino antipirin beside oxidize cholesterol and phenol besides peroxidize enzyme. The formed chinonimin quantity which is measurable photometrically has a direct relation with cholesterol and glucose quantities. The determination of total cholesterol, triglyceride and serum glucose quantities was done at 546 nm wave length using kits from Pars Azmoon Company. All biochemical parameters were measured by photo 100 lab system machine. The total cholesterol, triglyceride and glucose quantities were calculated as mg/dl.

Liver enzymes measurement

The auto analyzer (DANA, model 1700) was used to measure liver enzymes including aspartic amino transferase (AST), alanine amino transferase (ALT) and lipase anzyme. The serum was done using kits (Pars Azmoon Chemical Co. Tehran, IRAN). The spectrophosphorimetric method was used to measure lipase enzyme through photometrical method (Rehulka, 2000; Lott et al., 1986).

Statistical data analysis

All data used were analyzed with one-way ANOVA method. LSD and Duncan statistical tests were used to compare means at 5% reliability level. SPSS was used for statistical analysis.

RESULTS

Biochemical parameters

Based on Table 2, a significant difference ($P < 0.05$) was observed in blood sugar of fish, but in other parameters including triglyceride and cholesterol, there was no significant effect with different dietary levels of phospholipids ($P > 0.05$).

The highest blood sugar was found in fish fed diet D3 with a value of 83 mg/dl followed by fish fed diets D4, D1 and D2 with values of 70, 48 and 46 mg/dl, respectively. Figures 1 to 3 show the relationship between cholesterol, triglyceride and blood sugar of fish treated with four dietary levels of phospholipids.

Hepatic enzymes

The results showed that there was no significant effect observed in ALT, AST and lipase (Table 3) in fish fed with different dietary levels of phospholipids ($P > 0.05$).

DISCUSSION

Liver is the most important organ metabolizing drugs and other substances in fish (Souba and Wolmore, 1983). Physiological changes which occurred in the liver were mainly caused by metabolical processes in a living thing. Generally, liver damages linked to liver cell membrane permeability resulted in leaking some enzymes into plasma, so their activities increase. An increase of enzymes such as AST, ALT, LDH and ALP in the serum is a criterion of liver damage. Cowey (1976) suggested that dietary needs for cultural fish should be based on biochemical evaluations including specific enzyme

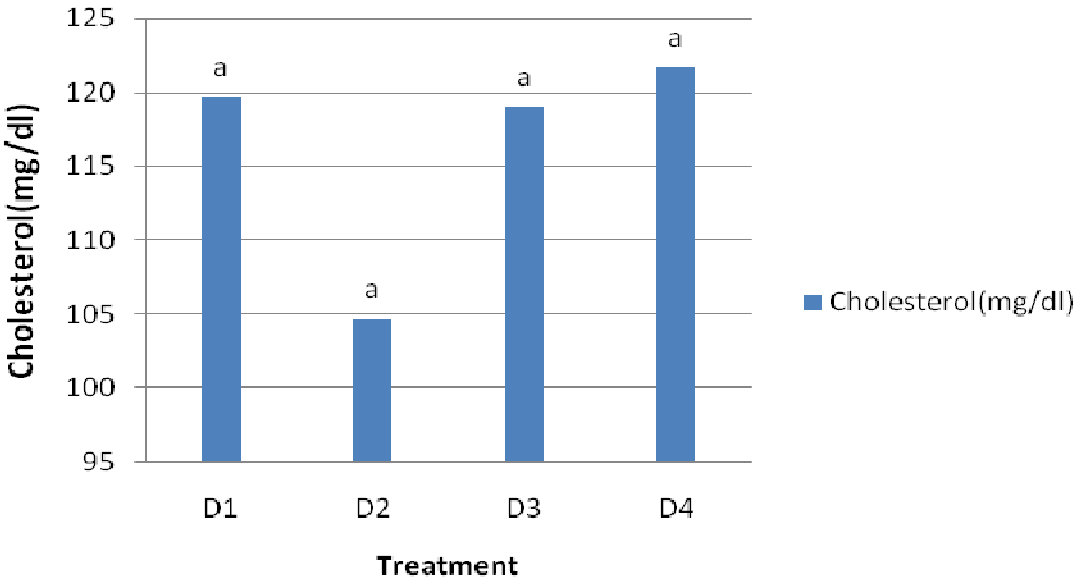

Figure 1. Relationship of cholesterol in fish treated with four dietary levels of phospholipids.

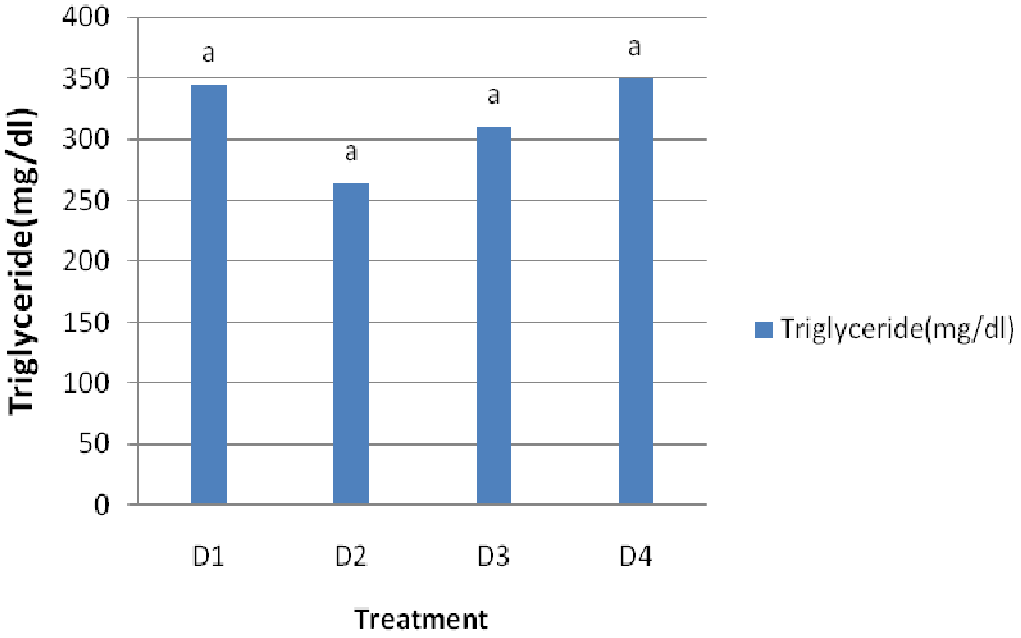

Figure 2. Relationship between triglyceride with four dietary levels of phospholipids.

activities. The amount of liver enzymes of *Huso huso* fed (D3) phospholipids in their diet, had a higher amount of AST at the end of experimental period. However, the lipase enzyme was higher in fish fed diet with 6% phospholipids when compared with fish fed with other diets. These results implied that diets have an impact on with 2% (D2) was highest in ALT, while fish fed with 4% enzymes. Hamza et al. (2008), studying on larval pikeperch (*Sander lucioperca*), found that the brush border membrane enzyme activates aminopeptidase N (AN) and alkaline phosphatase (AP) which would enhance an increased in phospholipids level.

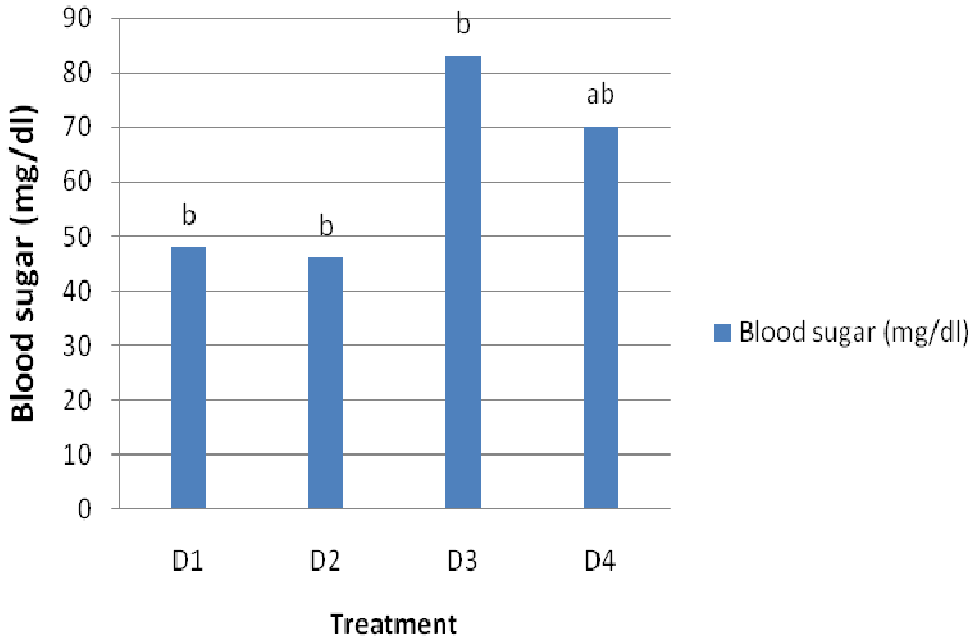

Figure 3. Relationship between blood sugar with four dietary levels of phospholipids.

Table 3. Effect on hepatic enzymes of *Huso huso* fed with four dietary levels of phospholipids after 8 weeks[1].

Proximate constituent (U/L)	Dietary treatment			
	D1(0%PL)	D2(2%PL)	D3(4%PL)	D4 (6%PL)
Alanine aminotransferase (ALT)	24.70±2.32[a]	46.07±9.34[a]	34.93±8.01[a]	39.45±22.95[a]
Aspartate aminotransferase (AST)	15.60±4.61[a]	12.47±3.28[a]	16.43±7.59[a]	12.83±5.7[a]
Lipase	30.50±0.76[a]	30.77±2.3[a]	29.03±1.53[a]	32.47±4.72[a]

[1]D1(control); 0% phospholipids; D2: 2% phospholipids; D3: 4% phospholipids; D4: 6% phospholipids. Values in each row with the same superscripts are not significantly different at P ≥ 0.05.

Researchers showed that the release of these enzymes is affected by dietary elements, as well as pesticides and chemical substances. As an example, a study conducted by Soltani and Rostami (2002) showed the effect of diazinon pesticides on biochemical factors in *Acipenser gueldenstaedtis*' blood. It was shown that ALT, AST, ALP and LDH levels were higher in control treatment than test groups. They concluded that because these enzymes are produced in liver, any decrease of them could be resulted from diazinon accumulation in liver parenchyma tissue and dysfunction of the earlier-mentioned enzyme synthesis.

Conclusions

However, there is no information about dietary phospholipids effect on the enzyme synthesis in sturgeon fish. In this study, the effect of phospholipids on some mentioned enzymes was presented, but the way in which they metabolized is still under question.

Similarly, other parameters, including triglyceride, cholesterol, alanine aminotransferase, aspartate aminotransferase and lipase remained the same as in other treatments with the exception of blood sugar level. According to mentioned results, it is be useful to consider 4% phospholipids (D3) levels as suitable biochemical factors of *Huso huso* juveniles.

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