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Effects of Phospholipids in Diet on Growth of Sturgeon Fish (Huso-huso) Juveniles

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ABSTRACT

The aim of the study was to determine the influence of dietary phospholipids (PL) levels on growth and body composition of beluga (Huso-huso) juveniles. Huso-huso juveniles were fed with an isonitrogenous (45% crude protein (CP)) and isoenergetic (18.00 kJ g⁻¹) formulated diet with four levels of PL, 0, 2, 4 and 6%. The fish were then acclimated to laboratory conditions and fed with a commercial fish feed for 14 days. After acclimation, groups of 60 Huso huso fingerling (mean weight 4.5 g) were randomly stocked into the 12 circular fiberglass tanks. The results showed that during the feeding trials in (56 days), growth performance was high at 4% phospholipids groups. There were significant different (p<0.05) for Condition Factor (CF) fish fed diet D4 had a value of 0.38 followed by fish fed diet D1, D2 and D3 had CF value of 0.47, 0.43 and 0.42, respectively. Weight gain (%) and survival (%) had significant difference (p<0.05) but no significant differences were found for final weight, Feed Conversion Rate (FCR), Specific Growth Rate (SGR), Protein Efficiency Ratio (PER), Lipid Efficiency Ratio (LER), (p>0.05). Significant differences were showed in moisture and protein composition of treatments (p<0.05) but no significant effect (p>0.05) on the fat and ash composition in fish were observed.

Key words: Phospholipids, sturgeon fish, Huso-huso, juveniles, growth performance

INTRODUCTION

Fats were the main energy source for fish (Bell and Tocher, 1989) and have sensitive role in the larval growth of the fish (Rainuzzo et al., 1997). 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). Sterogeon are anadromous and potamodromous species live in the northern hemisphere. There are 28 species, six of which inhabit the Caspian basin and beluga (Huso-huso) is one of them (Bahmani et al., 2001). Unfortunately, this fish has become an endangered species because of excessive fishing, decrease of input water from the rivers due to anthropogenic and agricultural consumptions and water pollution (Asadi et al., 2006). In fact, all acipenserids are listed as threatened, vulnerable and endangered throughout their ranges (Baker et al., 2005). Thus there has been an increased demand for information on all aspects of sturgeon biology and physiology (Billard and Lecointre, 2001; Baker et al., 2005).

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It is well known that lipids constitute a major energy source for fish (Bell and Tocher, 1989) and play a critical role in larval development (Rainuzzo et al., 1997; Sargent et al., 1999). Also phospholipids (PL) have been demonstrated to significantly affect survival, growth, deformities and/or resistance to stress in several fish and crustacea (Kanazawa et al., 1985; Geurden et al., 1998; Koven et al., 1998; Cahu et al., 2003; Gisbert et al., 2005). They play a major role in maintaining the structure and function of cellular membranes (Kanazawa et al., 1985; Tocher, 2003). They have been reported to act as emulsifiers in the gut (Koven et al., 1993) and to improve intestinal absorption of long chain fatty acids (Fontagne et al., 2000). Moreover, they stimulate lipoprotein synthesis in intestinal enterocytes (Fontagne et al., 1998; Geurden et al., 1998) and play an important role in the transport of dietary lipids (Kanazawa, 1991; Teshima et al., 1986). Few studies have demonstrated their effect on the maturation of digestive structures of fish larvae (Cahu et al., 2003; Gisbert et al., 2005; Morais et al., 2007).

In fish, dietary phospholipids are required for growth and development since de novo synthesis of phospholipids is not sufficient to meet metabolic needs (Gibbs *et al.*, 2009). Generally, the levels of phospholipid requirement are around 2-4% of diet for juvenile fish and probably higher in larval fish (Tocher *et al.*, 2008). A combination of the above mentioned factors may collectively contribute to the beneficial effects of dietary phospholipids on growth but the definitive mechanism remains unclear.

In this study the complex of phospholipid was used for survey of growth, survival and biochemical and Hematological parameter of beluga sturgeon.

MATERIALS AND METHODS

Fish diets were formulated by means of Lindo software (Lindo copyright, 1995, Releases 6.1) were as shown in Table 1. All diets are isonitrogenous (45% P) and isoenergetic (18 kJ g⁻¹). To

Table 1: Experimental diet formulation and proximate composition

Items	Dietary treatment				
	D1 (0% PL)	D2 (2% PL)	D3 (4% PL)	D4 (6% PL)	
Diet ingredient					
Fish meal (%)	60.00	60.00	60.00	60.00	
Wheat meal (%)	20.00	20.00	20.00	20.00	
Fish oil (%)	5.00	5.00	5.00	5.00	
Soybean oil (%)	6.00	4.00	2.00	0.00	
Phospholipids (%)	0.00	2.00	4.00	6.00	
Molasses (%)	2.30	2.30	2.30	2.30	
Vitamin mixture (%)	2.00	2.00	2.00	2.00	
Mineral mixture (%)	3.00	3.00	3.00	3.00	
Anti oxidant (%)	0.20	0.20	0.20	0.20	
Calcium phosphate (%)	1.50	1.50	1.50	1.50	
Total	100.00	100.00	100.00	100.00	
Proximate composition					
Dry mater (%)	90.30	90.10	90.90	90.90	
Protein (N x6.25) (% DM)	44.94	45.94	45.21	45.10	
Lipid (% DM)	14.70	15.10	15.30	15.50	
Ash (% DM)	13.58	13.52	13.84	13.40	
Digestible energy (kJ g ⁻¹ diet)	18.16	18.32	18.25	18.36	
Gross energy (kJ g ⁻¹ diet)	21.01	21.18	21.10	21.23	

D1 (control): 0% phospholipids, D2: 2%phospholipids, D3: 4%phospholipids, D4: 6%phospholipids

maintain the isoenergetic nature of the diets, soybean oil was added. Phospholipids complex was obtained from Bergapor Co. Germany (Berg and Schmidt) in Iran. Mineral and vitamin complements were first mixed together before added to the base diet. Mixing preliminary materials like soybeans oil and fish oil to some wheat meal was done initially for 20 min and were mixed again for 20 min after adding the required quantity of water. Then the mixture was added to the other ingredients in the electrical mixer and was passed through a die (2.5 mm) to make the required size pellets. After making the pellet, they were placed on dry trays and were moved to the drier. Diets were kept at 4°C away from light, in special plastics, after they were prepared and used to feed fish. Feed was given at 4% of the body weight in five meals at 8:00, 11:00, 13:00, 15:00 and 18:00 h. Excretion and other waste materials were siphoned off the tanks daily. At first the initial weight and length of fish was taken and after every two weeks their weights and total lengths were measured again.

This experiment was conducted in spring 2009 (period 56 days) in Shahid Margani Reproduction and Farming Center of Fisheries General Department Golestan Iran. 720 Beluga juveniles with an average weight of 4.5 g were collected from Shahid Margani Reproduction and Farming Workshop of Gorgan after categorization process.

Growth factor measurement: Mean weight gain (WG, g) per fish was calculated from the sum of weight gains at the end of the experimental period. Other growth parameters such as Correction Factor (CF), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Lipid Efficiency Ratio (LER), survival (%) and Specific Growth Rate (SGR) were calculated using the following formulas:

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CF = [BW (g)/BL^{3} (cm)] \times 100, [CF = W_{2}/(L_{2})^{3} \times 100] FCR = Dry \ feed \ consumed \ (g) \times wet \ BW \ gain \ (g) \cdot 1
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Protein efficiency ratio PER = [final BW (g)-initial BW (g)]×weight of protein consumed (g)-1

Weight gain = Final weight-initial weight (WG = W₂-W₁)

Lipid efficiency ratio LER = [final BW (g)-initial BW (g)]×weight of Lipid consumed (g)-1

 $SGR = [(Ln final BW (g) - Ln initial BW (g)) \times 100] \times (number of days) \cdot 1$

Percentage of Weight gain (WG %) = ((final weight-initial weight) × initial weight (g)-1) ×100

where, BW indicates the total body weight (g) and BL the total body length (cm) of the Huso-huso.

RESULTS

The results of growth parameter of *Huso-huso* fed phospholipids after 56 days were shown in (Table 2). There were no significant differences in the initial weights of the fish at the beginning of the trial. The weight gain was significantly different (p<0.05) in fish fed the experimental diets (Table 2). Fish fed diet D3 had the highest weight gain with a value of 70.3 g followed by fish fed diets D1, D2 and D4 with values of 63.05, 56.42 and 47.94 g, respectively as showed in Fig. 1. Similarly, the percentage weight gain had a significant different (p<0.05). With fish fed diet D3 had a value of 1389 followed by fish fed D1, D2 and D4 with values of 1264, 1136 and 993%

Table 2: Growth parameter of Huso-huso fed phospholipids in 8 weeks¹

Performance parameters	Dietary treatment				
	D1 (0% PL)	D2 (2% PL)	D3 (4% PL)	D4 (6% PL)	
Initial weight (g)	4.99±0.01 ^a	4.97±0.03ª	5.06±0.07ª	4.92±0.03ª	
Final weight (g)	68.08 ± 3.58^{ab}	61.39 ± 6.4^{ab}	75.36±3.11ª	52.76±9.45 ^b	
Weight gain (g)	63.05±3.59 ^{ab}	56.42 ± 6.42^{ab}	70.30±3.05ª	47.94 ± 9.43^{b}	
Food consumption (g)	6036.67±297ª	5376.67 ± 454^{ab}	6420.00±599a	4663.333±665 ^b	
Weight gain (%)	$1264.47{\pm}75.32^{ab}$	1136.16±135.68 ^{ab}	1389.07 ± 44.18^a	993.44±46.35 ^b	
FCR	2.52 ± 0.33^{a}	2.80 ± 0.22^{a}	1.98 ± 0.10^a	2.36 ± 0.52^{a}	
SGR	4.66 ± 0.10^{a}	4.47 ± 0.19^{a}	4.82±0.05ª	4.22 ± 0.32^{a}	
CF	0.47 ± 0.01^a	0.43 ± 0.01^{b}	0.42 ± 0.00^{b}	$0.38\pm0.01^{\circ}$	
PER	0.92 ± 0.13^{a}	0.79 ± 0.06^{a}	1.12±0.06ª	1.03 ± 0.21^{a}	
LER	2.80 ± 0.38^a	2.39 ± 0.18^{a}	3.32±0.17ª	2.99 ± 0.60^a	
Survival (%)	65.00 ± 5.10^{ab}	57.80±4.85b	76.70±2.89ª	73.33±3.84ª	

¹D1 (control): 0% phospholipids, D2: 2% phospholipids, D3: 4% phospholipids, D4: 6% phospholipids, Values in each row with the same superscripts are not significantly different (Duncan significance level is defined as p>0.05)

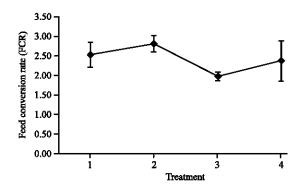


Fig. 1: Relationship between feed conversion rate (FCR with deferent level of phospholipids, No significant differences were found for FCR, the best value (1.98±0.01) of FCR was observed for fish fed diet D3 and the poorest was (2.80±0.22) again for fish fed diet D2

respectively. There were significant different (p<0.05) for CF (condition factor) fish fed diet D4 had a value of 0.38 followed by fish fed diet D1, D2 and D3 had CF value of 0.47, 0.43 and 0.42, respectively as showed in Fig. 2. Fish fed diet D3 also had the highest final weight with a value of 75.36 g followed by fish fed diet D1, D2 and D4 with value of 68.08, 61.39 and 52.76 g, respectively. No significant differences were found for FCR, SGR, PER, LER (Table 2, p>0.05). The best value (1.98±0.01) of FCR was observed for fish fed diet D3 and the poorest was (2.80±0.22) again for fish fed diet D2 as showed in Fig. 3. The highest and lowest Specific Growth Rate (SGR) was observed for fish fed diet D3 (4.82) and diet D4 (4.22) respectively as showed in Fig. 4. The highest and lowest protein efficiency ratio (PER g g⁻¹) was observed for fish fed diet D3 (1.12±0.06) and diet D2 (0.79±0.06), respectively as showed in Fig. 5. Lipid efficiency ratio (LER g g⁻¹) did not differ for any treatment but the highest and lowest was observed in fish fed diet D3 (3.32±0.17) and fish fed diet (2.39±0.18), respectively as showed in Fig. 6. There was significant difference in survival that highest (76.70) and lowest (57.80) was showed in fish fed diet D3 and fish fed diet D2, respectively.

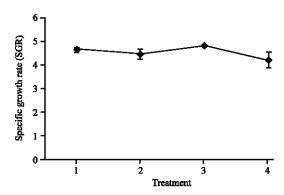


Fig. 2: Relationship between Specific Growth Rate (SGR), with deferent level of phospholipids, Specific growth rate (SGR, % day⁻¹) did not differ for any treatment but the highest and lowest was observed in fish fed diet D3 (4.82±0.05) and fish fed diet D4 (4.22±0.32), respectively

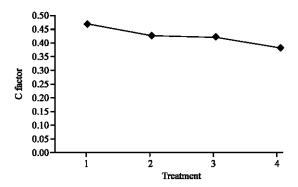


Fig. 3: Relationship between Condition Factors (CF) with deferent level of phospholipids, There were significant different (p<0.05) for CF (condition factor) fish fed diet D4 had a value of 0.38 followed by fish fed diet D1, D2 and D3 had CF value of 0.47, 0.43 and 0.42, respectively

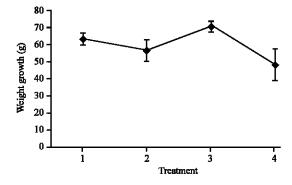


Fig. 4: Relationship between Weight Growths (WG) with deferent level of phospholipids, The weight gain was significantly different (p<0.05) in fish fed the experimental diets. Fish fed diet D3 had the highest weight gain with a value of 70.3 g followed by fish fed diets D1, D2 and D4 with values of 63.05, 56.42 and 47.94 g, respectively

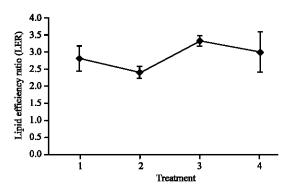


Fig. 5: Relationship between Lipid Efficiency Ratio (LER) with deferent level of phospholipids, Lipid efficiency ratio (LER g g⁻¹) did not differ for any treatment but the highest and lowest was observed in fish fed diet D3 (3.32±0.17) and fish fed diet (2.39±0.18), respectively

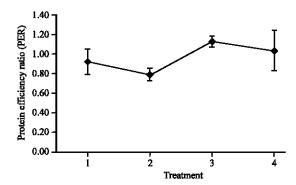


Fig. 6: Relationship between Protein Efficiency Ratio (PER) with deferent level of phospholipids, No significant differences were found for Protein Efficiency Ratio (PER). The highest and lowest protein efficiency ratio (PER g g⁻¹) was observed for fish fed diet D3 (1.12±0.06) and diet D2 (0.79±0.06), respectively

Table 3: Mean concentrations (% dry weight basis except for % moisture±SE) of the proximate constituents in the sturgeon fish (Huso-huso), reared on different levels of phospholipids¹

Proximate constituent	Dietary treatment				
	D1 (0% PL)	D2 (2% PL)	D3 (4% PL)	D4 (6% PL)	
Moisture	75.82±0.81bc	78.08±0.33ª	74.77±0.32°	77.69±0.82ªb	
Protein	68.38±0.58ª	67.75±1.82a	62.39±0.52b	70.04±1.20 ^a	
lipid	19.96±0.36a	21.36 ± 0.99^{a}	21.80 ± 1.28^{a}	20.37 ± 0.55^{a}	
Ash	8.81 ± 0.65^{a}	8.86±0.47a	8.48±0.33ª	8.52±0.35ª	

¹D1 (control): 0% phospholipids, D2: 2% phospholipids, D3: 4% phospholipids, D4: 6% phospholipids, Values in each row with the same superscripts are not significantly different (Duncan significance level is defined as p>0.05)

Fish proximate: The proximate compositions of fish fed different diets are shown in Table 3. Slight change in the composition of the fish fed was observed for all measured components. Significant differences were shown in moisture and protein composition of treatments (p<0.05) but the phospholipids had no significant effect on the fat and ash composition (p>0.05). The highest

moisture content was found in fish given diet D2 with value of 78.08% followed by fish fed diets D4, D1 and D3 with values of 77.69, 75.82 and 74.77%, respectively. There were significant deferent in protein content (p<0.05). Fish fed diet D4 had the highest percentage of protein with a value of 70.04% followed by fish fed diet D1, D2 and D3 with values of 68.38, 67.75 and 62.39%, respectively. There were no significant deferent lipid content (p>0.05). The highest lipid content was found in fish diet D3 with value 21.8% and the lowest was found in fish fed diet D1 with a value 19.96%. There were no significant deferent in ash content (p>0.05). Fish fed diet D2 had highest ash content with value of 8.86% and the lowest ash content was found in fish fed diet D3 with a value of 8.48%. The proximate composition of *Huso-huso* reared in all feeding trials was in accordance with the proximate composition data of aquatic animals (NRC, 1993).

DISCUSSION

In this study the 4% level of phospholipids improve the growth of beluga juveniles especially in early days with no significant effect (p>0.05). SGR and WG% in 4% level was better. Other researchers had presented seminar results (Fontagne et al., 1998; Cyprinus carpio; Gisbert et al., 2005; Dicentrachus labrax; Izquierdo et al., 2001, gilthead sea bream).

Complement of diet with phospholipid is improving the growth of fish with reducing energy consumption that require for synthesis of phospholipid (Craig and Gatlin, 1997). Although it discovered that biosynthesis of phospholipid in early days of fish a limited is done (Tocher *et al.*, 2008) but the rate of this synthesis is very low (Kanazawa *et al.*, 1985).

Generally, the levels of phospholipid requirement are around 2-4% of diet for juvenile fish and probably higher in larval fish (Tocher $et\ al.$, 2008). Also positive impact of this kind of lipid may be because of emulsifier property that causes increase of digestion of neutral lipids (Kasper and Brown, 2003). On the other hand phospholipids have important role on transport of lipid particularly fatty acid (Fontagne $et\ al.$, 1998). The effect of dietary PL appears to diminish with age and is generally not essential in adults (Sufang $et\ al.$, 2008). Our results were showed that great sturgeon in juvenile stage requires less phospholipid complement.

In the present investigation, the moisture and protein composition were affected by diet treatment but the fat and ash were not affected by diet treatment. Although slight differences between the fat and ash were observed, these differences were not significant.

Xu et al. (1993) and Deng et al. (1998) on white sturgeon, McKenzie et al. (1999) on Adriatic sturgeon and Sener and Savas (2005) on Russian sturgeon found that except moisture, the proximate composition were not affected by diet treatment.

Present results showed that there is a relationship between lipid and protein composition of muscle in *Huso huso*. This relationship between muscle protein and lipid content has been observed in previous study with Russian sturgeon (Sener and Savas, 2005) and other fish, i.e., salmonids (Bell *et al.*, 2001). It seems that in high level of phospholipid the retention of protein increased but the lipid consumed.

CONCLUSIONS

Fat (phospholipids) in sturgeon fish (*Huso-huso*) juvenile's diet can increase growth and improve nutritional indices. Adding phosphatidylcholine to (*Huso-huso*) juvenile's diet up to 4% leads to improvement in food digestion and absorption.

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