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Serum Biochemical Change Induced by Soybean Meal in Diet on Persian Sturgeon, *Acipenser persicus*

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Abstract: Persian sturgeon, *Acipenser persicus*, is a native species of Iran. Because wild stocks are getting declined, its farming is of great importance. Achieving a cost effective diet which doesn't have negatively effects on growth is the goal of aquaculture programs. So the current experiment was conducted with the aim at evaluating hematology and plasma ion concentrations of Persian sturgeon under diets including fish meal as a control diet (C), fish meal replacing by soy bean meal along with supplementing with Phosphorus and phytase. Serum Glocose, Chlostrol, Total protein, Phosphorous, Calcium and Ferrous in different treatments of phosphorous (SP), Phytase (SpH) and phosphorous and Phytase (SPpH), phosphorous were determined. This study showed that fish meal is more sufficient for Persian sturgeon and soybean meal could be partly an alternative protein source if Phosphorous supplied for fish by incorporation with microbial phytase or Phosphorous.

Key words: Sturgeon · Serum · Biochemical · Diet

INTRODUCTION

Majority of the phosphorus in soybeans is present in the form of phytate (inositol hexaphosphate) which is not digested by fish. Furthermore, it forms complexes with other divalent ions leading to reduced availability [1, 2]. Phytate also inhibits hydrolysis of protein [3].

Phytase, an enzyme that hydrolyzes the ortho-phosphate groups from the phytate molecule, have been used as a feed additive in livestock feeds as well as aquaculture feeds to enhance the bioavailability of phytate-P and several studies have demonstrated that phytase can also successfully improves bioavailability of phytate phosphorus in diets [4-6].

Since Phosphorus (P) is an essential mineral in fish [7], one way to make phosphorous in diets replaced by soy bean meal available to fish is supplementing with phosphorous as mono or dicalciumphosphate [8]. Phytic acid chelates di cations such as magnesium at intestinal pH and reduced the availability of it [9].

Persian sturgeon, *Acipenser persicus*, is a native species of Iran. Because wild stocks are getting declined, its farming is of great importance. Therefore, preparing promising diets for good health and growth is necessary for its production. Achieving a cost effective diet which doesn't have negatively effects on growth is the goal of aquaculture programs. So an experiment was conducted with the aim at evaluating plasma ion concentration of Persian sturgeon under diets including fish meal as a control diet (C), fish meal replacing by soy bean meal along with supplementing with Phosphorus, magnesium and phytase.

MATERIALS AND METHODS

Experiment Design: Juvenile Persian sturgeon was obtained from Aquaculture Research Center of Gorgan University (average weight: 352.07±5.51g), acclimatized to the experimental condition and diets about two weeks before beginning the trial. Fish were stocked in groups in 400-L aquariums in an indoor facility. Water temperature was 20-23°C, pH = 8 and NH4-N < 0.5 mg L^{-1}. Aeration was supplied to each tank with air stone to keep dissolved oxygen throughout the trial. Fish were kept under natural photoperiodic conditions, fed twice a day at a rate of 3% of body weight. Every group was fed for 10 weeks

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with experimental diet in triplicates. Water replaced at a rate of 80% volume per day in each aquarium and uneaten feed and faeces removed by siphoning daily. Uneaten feed dried, weighted and subtracted for more exact calculation of FCR, FI and PER [8].

Blood Collecting: Every two week, fish was sampled from each trial to be weighted and calculate the required food for the following week. 24 hours before each sampling, feeding was ceased. At the end of experiment, from each trial three fish were anaesthetized. Blood (4 ml) was collected within 2 min from the caudal vasculature using a syringe. The blood divided into two aliquots, one part was transferred to a 2 ml vacationer tube containing heparin sodium, shook for 2 minutes gently and stored in refrigerator prior to hematological analysis. The other part of aliquots transferred into a 2 ml microcentrifuge tubes and centrifuged for 15 minutes at 3000g at 4°C. The plasma removed and transferred to another microtube and stored frozen at -80°C until subsequent analysis for plasma ion concentrations. After blood sampling, with a stroke to the head fish were killed. After evisceration, Skin and vertebrate removed and the carcass kept frozen at -20°C for later analysis of proximate composition.

Diets: Based on the protein requirement of Acipenser [10] and the suitable ingredients composition, one control diet with fish meal and six experimental diets in which soy bean meal replaced and supplemented with tested ingredients [phosphorous (SP), Phytase (SpH) and phosphorous and Phytase (SPpH)], were formulated (Table 1, 2).

Analysis: Moisture, crude protein and crude lipid of experimental diets and muscle were determined by standard methods [11]. Moisture determined by drying in an oven at 105°C for 24 hours; Crude protein by Kjeldahl method and crude fat by ether extraction by Soxtec System. To determine the ash and mineral contents, dried samples were placed in a muffle furnace at 550°C for 24 h. Minerals in feed and muscle determined by atomic.

Statistical Procedure: All data were subjected to one-way ANOVA in SPSS version 11.0 and presented as means ± standard deviations. If significant differences among group means identified, differences were compared using Duncan's multiple range test.

RESULTS

Analyses data of formulation and proximate composition of reference and the experimental diets are in Table 1. However, P and phytase content of experimental diets are separately included in Table 2. No obvious difference in the diet acceptance between the control and experimental diets was noticed during the feeding trial. The mortality of experimental fish during the feeding trial was lower than 5% in all the dietary groups.

The proximate composition of Carcass is different treatments also shown in Table 3. some blood factors (Glucose, Cholesterol, Total protein, Phosphorous, Calcium, Ferrous) are presented in Table 4 as well.

Table 1: Formulation and proximate composition of reference and the experimental diets

	Content (g Kg^{-1} diet)	
Ingredients	Reference diet	Experimental diet
Fish meal	550	218
Soybean meal	0	500
milk powder	150	150
wheat flour	170	0
fish oil	30	40
Soybean oil	50	40
Lysine	15	15
methionin	12.5	15
vitamin mineral premix	20	20
Mold inhibitor	2.5	2
	1,000	1,000
Proximate chemical composition		
Dry Matter (DM)	891.81	904.49
In DM		
Crude protein	426.1	433.3
Crude fat	84.58	84.26
Ash	77.92	71.74

Table 2: P[a] and phytase[a] content in experimental diets

	Supplement (g Kg^{-1})			Analyzed (g Kg^{-1} dry diet)		
Diet	Mga	Pa	Phytasea	Mg	P	Ca
C	0	0	0.0	1.1	10.47	12.6
SP		6		1.2	4.19	2.2
SpH			0.2	1.5	10.54	4.4
SPpH		6	0.2	1.3	9.64	5.1

[a]Ca as DCP, Mg as MgO, Phytase as microbial

Table 3: The proximate composition of Carcass in the Persian sturgeon

	Diets			
	C	SP	SpH	SPpH
Composition of carcass (%)				
Moisture	76.59±7.68[a]	78.44±0.01[ab]	79.96±0.01[ab]	77.73±0.45[ab]
Protein	25.91±8.40[ab]	21.38±1.19[ab]	26.36±0.6[a]	25.04±3.84[ab]
Lipid	14.65±0.57	14.46±0.22	14.34±0.53	14.58±0.13
Ash	7.5±0.81[ab]	6.26±0.63[cd]	6.41±0.5[bcd]	7.99±1.26[a]
Calcium	0.05±0.02[a]	0.05±0.02[a]	0.05±0.01[a]	0.05±0.02[a]
Phosphorous	1.02±0.04[b]	1.06±0.1[a]	0.92±0.04[d]	0.91±0.03[b]

Table 4: Blood indices of Persian sturgeon exposed to different diets

	Diets			
	C	SP	SpH	SppH
Blood factors				
Glucose	38.04±1.3	34.52±2.45	49.04±19.12	35.41±9.53
Cholesterol	210.26±170.73[bc]	187.1±26.33[bc]	337.73±84.73[ab]	390.48±10.2[a]
Total protein	5.29±0.56	4.61±0.44	4.41±0.37	4.41±0.19
Phosphorous	2.26±0.91	2.18±0.92	1.96±0.55	1.38±0.14
Calcium	10.56±0.69	9.60±0.56	9.97±1.2	10.29±0.92
Ferrous	55.95±0.27	26.79±0.9	38.93±0.6	26.79±0.4

DISCUSSION

Hematocrit concentration decreased as soybean included in diet and in fishmeal diet was higher. The few data that have been published on the effect of the dietary soybean concentration on hematocrit are not always consistent. What's more, there are no clear relationships between the replacement level of fishmeal with alternative proteins in the diet and the deterioration of hematological parameters of the cultured fish. However, it seems that significant reduction of the hematological parameters accompany the growth reduction [12]. Our results agree with those of Kim and Lee [13] for parrot fish, *Oplegnathus fasciatus* and used gossypol by Barros *et al.* [14] for channel catfish and El-Said and Gaber [15] for Nile tilapia.

It could be explained by adverse effects of gossypol on iron absorption in the intestine, the gossypol-iron complex in liver, or increased erythrocyte fragility [8]. However, none of these mechanisms has been confirmed in fish because of some contradictory [16] and complicated [14] results on the haematological values.

Total protein, Glucose, magnesium, phosphorous, calcium and ferrous in blood did not show any differences among treatments. Cholesterol was significantly higher in group SpH, SpHMg and SPpH. Reasons for the discrepancy are not clear from the present data.

The finndings in this study suggested that dietary supplementation of phosphorus; Magnesium and phytase could not increase the inclusion of SBM for FM replacement in diets for Persian sturgeon species.

In this study, supplementing minerals did not apparently resulted in better growth performances with comparison to C group. It showed that fish meal is more sufficient for Persian sturgeon and soybean meal could be partly an alternative protein source if phosphorous supplied for fish by incorporation with microbial phytase or phosphorous. It also might be attributed to the deficiency of a digestible essential amino acids in soybean meal compare to fish meal which would lead to poor utilization of the soybean protein [17]. The whole body protein and moisture content responded to dietary P and phytase did not exhibit any dependant manner. In the case of whole body moisture, similar results have been reported for other fish species [13]. Whole body lipid was not also correlated to dietary [18].

In conclusion, no significant difference were observed among almost serum biochemical indices with supplemented P and phytase in diet among fed groups compared to the C fed group.

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