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Replacement of fish meal with poultry by-product meal on body composition in practical diets for beluga sturgeon (*Huso huso*)

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Abstract. This study has been performed to evaluate the alternative effects of the poultry by-product meal (PBM) instead of the fish meal (FM) in the replacing 0 (control group), 25, 50, 75 and 100% of FM by PBM diets on the chemical compositions of the body in *Huso huso*. Five iso-nitrogenous and iso-calorical diet % of PBM were fed to three replicate groups of *H. huso* with initial weight of 1200 ± 200 g during 90 days. The results showed crude protein of body were not different among fish fed with control diet and 25% replacement. But, body protein decreased as the level of the replacement increased and fish fed with 100% replacement had showed a significant difference with the control treatment (p > 0.05). The crude lipid level showed highest value in fish fed with 100% replacement (23.19%). But did not differ significantly among control and 25% replacement groups. The ash of body in the treatment with 100% replacement showed highest value but the treatment with 50% replacement had no significant difference with the control treatment (p > 0.05). Nitrogen-Free Extract (NFE) of body showed no significant difference with the control treatment at the level replacement of 50% but it was highest in 100% replacement group.

Key Words: alternative protein, lipid, beluga sturgeon, poultry by-product.

Introduction. The sturgeons are the greatest fish in the Caspian Sea and the greatest freshwater fish throughout Northern hemisphere, Black Sea, Azov Sea and Adriatic Sea. Beluga (Huso huso) is one of the important commercial species in Iran that has been considered in the aquaculture due to its rapid and easy growth, acceptance of living in captivity and acceptance of artificial diet (Ivanov & Vlasenko 2001). With regard to the high consumption of the sturgeon meat in the world, in spite of their mass artificial breeding in Iran, culturing this kind of fish has a short history. In recent years, the culture of these fish and their diets have been regarded increasingly. Because these fish are carnivores, they need high protein amounts in their diets that fish meal (FM) is currently the major source of protein source fed to fish (Emre et al 2003). Fish meal is an imported product with high price in Iran; this is a limiting factors for its application in fish diets. Therefore, it is necessary to find a suitable protein source replacement. Poultry by product meal (PBM) is considered to be a potential animal protein alternative in aquaculture feed due to its high production volume, acceptable protein level and optimal amino acid profile (Hardy 2000). The PBM includes the feathers (Papadopoulos 1989), intestines and guts which have been used after cooking process under pressure operation, dehydration, drying and milling while Methionine and Lysine are the amino acids made to limit the PBM but their protein guality are similar to the FM (Dabrowski & Guderley 2002). Since the food is one of the major costs in aquaculture, understanding the effect of nutrition strategy on quality and Marketability of farmed fish fillet is very important (Baghaei Jezeh et al 2014).

PBM in aquatic animals diet have shown various results (Zhou et al 2006); good quality PBM can successfully replace more than half the protein from marine FM in the diet for humpback grouper *Cromileptes altivelis* (Shapawi et al 2007). The optimal level of FM replacement with PBM for cobia *Rachycentron canadum* was 30.75% on the basis of maximum protein efficiency ratio (Zhou et al 2011). PBM could replace about 50% of

FM in the diet for chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*O. mykiss*) (Alexis et al 1985; Fowler 1991; Craig et al 2006). Some authors payed attention to the nutritional quality differences of PBMs produced by different manufacturers (Dong et al 1993). Moreover research on apparent digestibility of PBM suggested that PBM is well-digested by many of fish species (Brauge et al 1994; Yang et al 2004). In 1990 decade, PBM was only able to replace FM at a level not exceeding 50%. Recent researches indicate that PBM is be able to replace FM at higher levels of up to 100% (Nengas et al 1999; Takagi et al 2000; Gaylord & Rawles 2005). There is a lack of information on nutritional quality of PBM produced in Iran for fish diets. This study has been performed to evaluate the body composition of market-size beluga sturgeon when fed practical diets containing different level of PBM.

Material and Method

Ingredient and experimental diets. This study was performed in Hantooshzadeh sturgeon (Caviar Aquatic) center in Dezful city, Iran, during a period of three months, in spring of 2014. It was conducted in 15 concrete ponds with the dimensions $2 \times 2 \times 2$ m with the density of 5 kg/m³. Ingredients used in the study were purchased from local market. According to information provided by the manufacturer, PBM used consists of viscera, heads, legs and feather, and was produced by exposing to 150-200°C under a 2.5-atm pressure for ten hours. Both FM and PBM were analyzed for proximate composition prior to the formulation of diets. Five iso-nitrogenous and iso-caloric diets were formulated to evaluate nutritional value of PBM for beluga (Table 1).

Table 1

	Treatments						
Dietary compounds	PBM 0	PBM 25	PBM 50	PBM 75	PBM 100		
	(%)	(%)	(%)	(%)	(%)		
Poultry-by product meal	-	12	24	36	48		
Fish meal	48	36	24	12	-		
Soy bean meal	16	16	16	17	17		
Wheat flour	12	12	13	13	12		
Rice bran	3	3	2	2	2		
Molasses	3	3	3	2	3		
Fish oil	5	5	5	5	5		
Canola oil	5	5	5	5	5		
Probiotic	2	2	2	2	2		
Binder	2	2	2	2	2		
Betafyn	1	1	1	1	1		
Vitamin premix (1)	1.5	1.5	1.5	1.5	1.5		
Mineral premix (2)	1.5	1.5	1.5	1.5	1.5		
Protein (%)	40/80	40/18	39/55	39/37	38/81		
Lipid (%)	14/65	15/05	15/40	15/81	16/24		
Digestible energy (kcal kg ⁻¹)	3478/06	3503/5	3534/2	3584/4	3610/72		

Formulation and proximate composition of the experimental diets that these diets were fed to *H. huso*. Nutrients levels determined by analysis

(1) - Per kg premix: 4,000,000 IU vitamin A, 480,000 IU vitamin D3, 40,000 mg vitamin E, 2,400 mg vitamin K3, 4,000 mg vitamin B1, 6,000 mg vitamin B2, 40,000 mg niacin, 10,000 mg Ca-panthothenate, 4,000 mg vitamin B6, 10 mg vitamin B12, 100 mg D-biotin, 1,200 mg folic acid, 40,000 mg vitamin C and 60,000 mg inositol; (2) - Per kg premix: 23,750 mg Mn, 75,000 mg Zn, 5,000 mg Zn, 2,000 mg Co, 2,750 mg I, 100 mg Se, 200,000 mg Mg.

In Table 1 the control diet contained 48% of FM and 16% of soy bean meal as main protein sources. PBM was tested at five inclusion levels (0, 25, 50, 75 and 100% replacement of fish meal) by reducing FM levels. The diets were prepared by mixing the dry ingredients and oil, followed by the addition of water until stiff dough was obtained. The moist diet was extruded through a mincer with a 2 mm die. The resulting pellets

were then dried on the shelves at the room temperature. The diets were stored in the plastic bags under ambient conditions over the experimental period. The feeding trial was conducted in outdoor concrete ponds with holding capacity of 4000 L. Each pond was supplied with a water flow of 0.5-2 cm³ s⁻¹. Over the experimental period, water temperature (°C), dissolved oxygen (mg L⁻¹) and pH changed between, 23.1-24.5, 8.0-8.7 and 7.3-7.8, respectively. Fish of one year (initial mean weight 1200 ± 200 g), were randomly allocated at a stocking rate of 16 fish per pond with three replicate tanks for each experimental diet. All fish were fed three times daily at a fixed, feeding rate of 2% body weight per day for 90 days. Total biomass of the fish from each pond was weighed at twice a week intervals and feeding rates adjusted accordingly.

Experiment of the chemical composition of body. The protein content of the diets and the whole body was determined by Kjeldahl method, fat by solvent extraction, ash by placing the samples in a muffle furnace (550°C) for 12 h, fiber by placing the samples remaining in a muffle furnace (550°C) for 6 h after acid and alkali hydrolysis and moisture by drying (105°C) until constant weight has been attained. Nitrogen free extract was calculated by subtracting the protein, fat, fiber and ash from the dry weight (AOAC 1990). Results were analyzed by a one-way analysis of variance and the treatment means compared by Duncan's multiple range tests. Significance was tested at the p < 0.05 level.

Statistical analysis method. There was used one way Anova test to compare the treatments in the final results statistically and there was used Duncan Separator Test according to multiple range test to compare the averages between treatments. The presence or the absence of the significant difference was compared at the level 95% by using SPSS Software (version XVI) and then there were drawn related diagrams by Excel Software.

Results and Discussion. The results of this study were gained on the Table 2. The results showed that the diet with 25% replacement from the PBM had no significant difference with the control treatment - 73.5%, and PBM25 - 72.5%. But by increasing the alternative level of the PBM, crude protein of body decreased and fish fed with 100% replacement had 56.9% crude protein that it showed significant difference with the control (p < 0.05) (Figure 1). There was an inverse relationship between the body protein level and dietary inclusion level of PBM in fish diet (Reinitz 1983). It was reported that the replacement of 80% PBM reduced the protein level in rainbow trout (Nengas et al 1999). Emre et al (2003) obtained similar results for the carp (Cyprinus carpio). The findings of the present study are in agreement with the study of (Shapawi et al 2007). Steffens (1994) reported that PBM could be used as the sole animal protein source in diets for rainbow trout with amino acid supplementation. PBM can be used to replace 75% of the FM in diets without amino acid supplementation for gilthead sea bream -Sparus auratus (Nengas et al 1999), up to 100% for red sea bream - Pagrus major (Takagi et al 2000) and for humpback grouper - Cromileptes altivelis (Shapawi et al 2007). The results for African catfish (Clarias gariepinus) demonstrate that PBM can replace up to 40% of a high-quality FM without adverse effects on body protein (Abdel-Warith et al 2001). Rawles et al (2006) founded that PBM was able to replace 35% of protein from FM in diets for hybrid striped bass, without adverse effects on body protein. The major reasons for these variable findings were probably due to the different fish species and the varying quality of the tested PBM, which are significantly influenced by their origin and processing methods used (Dong et al 1993; Shapawi et al 2007).

Table 2 Proximate composition in whole body of *Huso huso* fed on test diets (Mean +SD)

Treatment index	PBM 0	PBM 25%	PBM 50%	PBM 75%	PBM 100%
Moister Crude protein (dry weight %)	69 73.5±2.6ª	73 72. 56±2.3 ª	74 67.2±2.1 ^b	79 62.9±2.6 ^b	84 56.9±2.6 ^c
Crude lipid (dry weight %)	13.9 ± 2.6^{a}	13.9 ± 2.2^{a}	18.29±2.6 ^b	18.63±2.6 ^b	23.94 ± 2.6^{c}
Ash (dry weight %) NFE (dry weight %)	5.2±2.6 ^a 7.4±0.02 ^a	5.8±3.6 ^{ab} 7.74±0.06 ^a	5.9 ± 3.6^{ab} 8.61 ± 0.08^{b}	6.3±2.6 ^b 12.17±0.06 ^c	6.6±2.6 ^b 12.56±0.3 ^c

Values in the same column with different superscript letters are significantly different (p < 0.05).

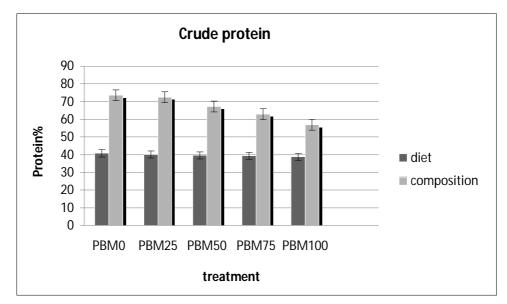


Figure 1. Diet protein and body composition protein in *Huso huso* fed different level of PBM.

In such process, some part of the protein in the by product may be damaged due to high temperature of the process. The degradation and vulnerability levels are subject to some factors such as temperature, time, humidity and physic-chemical conditions of the production of the PBM (Kalogeropoulos et al 1992). Another reason to limit the use of the PBM in the diet of the sturgeons is the low levels of Lysine, Methionine and Arginine, because these substances are sensitive to temperature due to the processing conditions and the production of the diet (Nengas et al 1999; Allan & Booth 2010). In current study, the highest level of body lipid was related to 100% replacement of the PBM (23.19) and there was no significant difference in the control treatment and the treatment with 25% replacement (13.9% and 13.7%) (p > 0.05) but body lipid increased with increased dietary replacement of FM with PBM in diets (Figure 2). Also, the primary analysis on the PBM showed the lipid content (fat% = 8) was more than the lipid in the FM (fat% = 1.93). This is actually due to the full fat ingredients such as poultry by product, skin and viscera in the poultries (Kalogeropoulos et al 1992).

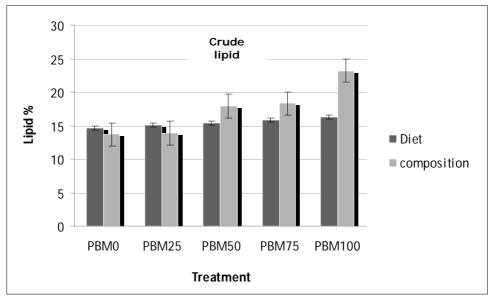


Figure 2. Diet lipid and body composition lipid in *Huso huso* fed different level of PBM.

This observation was in agreement with Fowler (1990), Gouveia (1992), Higgs et al (1979), Reinitz (1983), Cruz-Suárez et al (2007). Also, Steffens (1994) reported that the lipid level of body in rainbow trout fed by 50% replacement diet and 100% the PBM was higher than the control treatment. Javaheri Baboli et al (2013) had similar results with Steffens (1994), Cruz-Suarez et al (2007) obtained same results in *Litopenaus vannamei*. Abdel-Warith et al (2001) investigated Channa striata fish and they did not observe any significant difference in the chemical compositions of the body to the 40% level replacement. Such replacement needs to investigate vastly the PBM and the permitted amounts for the replacement in the diets of the sturgeons. So it was necessary to perform the current study. The results of the current study showed that there was not observed any significant difference in the experiments of the chemical compositions body. The ash level in the treatment with 100% replacement showed highest value but the treatment with 50% replacement had no significant difference with the control treatment (p > 0.05). Moisture content of the body of fish fed different test diets was different among diets and with body moisture increased with increased dietary replacement of FM with PBM in diets.

Conclusions. The results showed that the replacement of the PBM up to 25% had no effect on the protein content of the body but by increasing the levels of the replacement, the protein percentage of the body was reduced. Also, there was not observed any significant difference in the body lipid up to 25% replacement but by increasing the replacement levels, the lipid percentage of the body was increased. In this study, there was not observed any significant difference in the body ash up to 50% replacement but by increasing the replacement levels, the ash percentage of the body was increased. Overall, chemical composition of the fish body affected by PBM replacement at the end of experimental period.

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