

Research Article

Effects of fish oil replacement with poultry by-product oil in diet of Beluga sturgeon (*Huso huso*) on growth performance and carcass composition

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Keywords	Abstract
Beluga, Growth, Body composition, Poultry waste oil, Fish oil	The potential of using poultry by-product oil as a partial replacement for fish oil in the diets of young beluga sturgeon (<i>Huso huso</i>) (initial body weight, 270.6 ± 8.4 g) was studied. Three experimental diets with different levels of fish and poultry by-product oil were formulated as: 100% fish oil (FO), 100% poultry by-product oil (PO), and a blend of equal amounts of fish and poultry by-product oils (FPO). All diets were isoproteic (30% DM) and isolipidic (12% DM). After 8 weeks of feeding, the condition factor was significantly lower in the fish fed with FPO than in the fish oil group ($p < 0.05$), whereas no significant differences were observed in body weight gain, feed conversion ratio, specific growth rate, protein productive value, protein efficiency ratio, crude protein, crude fat, ash, and moisture among treatments. Furthermore, the liver and viscera fat of the fish fed with PO were significantly higher than those of the other treatments ($p < 0.05$). The results indicate that poultry by-product oil can effectively replace up to 50% of fish oil in the diet of young beluga sturgeon (as in the FPO treatment) without adversely affecting growth performance, body composition, or nutrient utilization.
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Introduction

Sturgeons are long-lived and rare species that are crucial due to their meat and caviar (Zhang *et al.*, 2021). Because of the decline in the population of these fish in their natural habitats (Pegasov, 2009), sturgeon fish culture has attracted the attention of many countries since the end of the last century. This led to ample knowledge about optimal rearing conditions, dietary requirements, formulation of diet, and so forth (Zarantonello *et al.*, 2021).

On the other hand, the production costs of aquatic animals have increased due to various factors, particularly the expenses related to their diet, which account for more than 50% of the overall costs of intensive aquatic production (Rana *et al.*, 2009). Because of the high cost of protein sources, it is more economical to use non-protein ingredients such as fats and carbohydrates as dietary energy sources. Also, there are plenty of nonprotein sources in the diet that prevent the oxidation of protein for energy production. As a result, protein can be utilized for growth and tissue building. Fats are superior sources of energy, providing approximately 8.5 kcal/g metabolizable energy, compared to carbohydrates (3.8 kcal/g ME) and proteins (4.5 kcal/g ME), (FAO, 2016). Moreover, fats are vital as essential sources of fatty acids and soluble vitamins. They also facilitate the digestion and absorption of vitamins. Fish oil is one of the main raw materials used in formulating fish diets (Reinitz and Yu, 1981). The long-chain polyunsaturated fatty acids are essential for fish to maintain optimum growth, health and development (Tocher, 2010). Although fish oil has been the primary source of essential fatty acids

for marine fish, some studies have shown that fish, specifically those containing n-3 LC-PUFA (EPA and DHA), offer significant health benefits for human nutrition, Such as prevention of cardiovascular disease and Alzheimer's disease (Perez-Velazquez *et al.*, 2019; Pourhosein Sarameh *et al.*, 2019). Therefore, there is intense global competition between the animal feed and human nutrition industries to use it (Bowyer *et al.*, 2012). Different species of fish require varying amounts and types of fatty acids for growth, which depend on their physiological characteristics and environmental conditions (Thanuthong *et al.*, 2011). Beluga or great sturgeon (*Huso huso*) is a good candidate for aquaculture due to fast growth, ease of reproduction in captivity, tolerance of variable rearing conditions (Mohseni *et al.*, 2006) and their valuable caviar and meat (Yadolahi *et al.*, 2022). Cultured beluga require essential fatty acids such as n-3, n-6, and n-9 in their diet. Among these highly unsaturated n-3 fatty acids (n-3 HUFAs), particularly EPA (20:5n-3) and DHA (22:6n-3), are crucial due to their positive effects on growth and health (Martino *et al.*, 2002).

In addition, the consumption of fish meal supplies and increases fish oil prices make this ingredient environmentally and economically unsustainable (Monteiro *et al.*, 2018; Campos *et al.*, 2019). Due to the decline in the population of wild fish used for making fish oil, marine capture fishery does not seem to be an appropriate solution for meeting the demands of aquaculture in the future (Pauly *et al.*, 2005; Shapawi *et al.*, 2007; Regan *et al.*, 2010). Therefore, finding a suitable alternative for fish oil in

the diet of fish has become a main principle in the aquaculture industry. Fish metabolism and health, final tissue fatty acid composition, growth performance, and final product quality are all adjusted and influenced by replacing dietary fish oil (Campos *et al.*, 2019). Because poultry by-product meal and oil contain high levels of protein, many essential amino acids and fatty acids (Yaghobfar, 2004), they are reasonable protein and oil sources for marine fish. Therefore, they have been examined in a vast range of fish species (Subhadra *et al.*, 2006).

Fish oil is a commodity used not only for aquaculture and the terrestrial animal feed industry, but there is also competition for its use in human food. The global supply of FO production has reached a maximum of about 1.3million tones (FAO, 2010; Jackson, 2010) and competition between industries, as well as natural environmental impacts such as El Nino events, affects the price of FO (Turchini *et al.*, 2009). The future expansion and long-term sustainability of the aquaculture industry is highly dependent on the identification of suitable alternative lipid compounds for FO. In recent years, efforts have been made to replace dietary FO with lipids from terrestrial plant and animal sources.

This study aimed to evaluate the effects of partially (50%) and fully (100%) replacing dietary fish oil with poultry by-product oil on growth performance, feed efficiency, nutrient retention, and body composition in young beluga sturgeon.

Materials and methods

Fish and culture system

The fish were obtained from the Shahid Marjani sturgeon reproduction and breeding Center (Gorgan, Iran), and transported to the aquaculture research facility of Isfahan University of Technology. To adapt to the experimental conditions, fish were placed in quarantine tanks for two weeks and fed to apparent satiation with a commercial diet (crude protein, 40%; crude fat, 16%; and moisture, 10%). Then 135 healthy juvenile belugas were transferred to the experimental semi-closed culture system, and randomly distributed in nine polyethylene tanks (with 60 cm depth and 350 L volume) with three treatments and three replicates (containing 15 fish with an average weight of 270.6 ± 8.4 g). The water flow rate of about 10 L/min and an average temperature of $20.3 \pm 3.9^\circ\text{C}$ (mean \pm standard error), 8.8 \pm 0.2 mg/L dissolved oxygen, and 0.44 \pm 0.15 mg/L ammonia during rearing fish. Tap water was stored in a 10 m³ tank at the same time as aeration for dichlorination, and it was used to compensate the water of the semi-closed system. Each tank was aerated with a separate air stone by means of a central air pump (Vortex Gas Pump of Type HG-400SB, CHINA). A 12 h light: 12 h dark photoperiod was established throughout the trial. In addition, the remaining excreta in the tanks were flushed twice daily in order to maintain a better water quality. The fish of each tank were randomly assigned to one of the replicates of each treatment.

Diet preparation and feeding

The ingredients were purchased from Roshd Daneh Company (Sharekord, Iran) and transported to the fish-rearing establishment of the Department of Natural

Resources of Isfahan University of Technology. The poultry by-product oil and fish oil were purchased from Riz Daneh Company (Isfahan, Iran). The diets were adjusted in three levels of 0, 50, and 100%, based on the amount of poultry by-product oil that replaced fish oil.

Each treatment was made with a part of oil equal to 12% of their respective weights. The 0% replacement level (i.e., fish oil only) was considered as the control treatment. All ingredients were milled into

fine powder and blended with 100 mL of water per 1 kg of diet, then passed through a meat grinder equipped with a 4 mm screen (Sepahkar, Isfahan, Iran) to obtain uniform pellets. The pelleted diets were air-dried at room temperature for 48 h (moisture content of about 7.5%) and then packed in plastic bags at -2°C until use. The Formulation and Proximate composition of the experimental diets is presented in Table 1.

Table 1: Ingredient and proximate composition of experimental diets (%).

	Experimental diets		
	FO	PO	FPO
Ingredients			
Fish meal	30	30	30
Soybean meal	12	12	12
Meat meal	8	8	8
Wheat gluten meal	9	9	9
Wheat meal	16	16	16
Corn flour	10	10	10
Fish oil	6	0	3
Poultry oil	0	6	3
Vitamin premix ^a	1.3	1.3	1.3
mineral premix ^b	1.2	1.2	1.2
Lysine	1	1	1
Methionine	0.5	0.5	0.5
Rice bran	2	2	2
Molas	2	2	2
Salt	1	1	1
Proximate composition (%DM)			
Crude protein	30.24	29.87	30.54
Crude lipid	12.09	12.22	12.89
Ash	16.51	15.84	16.52
Moisture	7.30	8.18	7.66

Diet abbreviations, FO: 100% fish oil; PO 100% poultry by-product oil and FPO 1:1 blends of fish and poultry by-product oil.

a-Unit kg⁻¹ of vitamin mixture: retinol acetate (A), 1 200 000 IU; DL-a-tocopheryl acetate (E), 30 IU; cholecalciferol (D₃), 400 000 IU; menadione sodium bisulphite (K₃), 1200 mg; L-ascorbic acid (C), 5400 mg; D-biotin (H₂), 200 mg; thiamin mononitrate (B₁), 200 mg; riboflavin (B₂), 3600 mg; calcium D-pantothenate (B₃), 7200 mg; niacinamide (B₅), 9000 mg; pyridoxine hydrochloride (B₆), 2400 mg; folic acid (B₉), 600 mg; cyanocobalamin (B₁₂), 4 mg; antioxidant 500 mg; Carrier up to 1 kg.

b- Unit kg⁻¹ of mineral mixture: Fe, 4500 mg; Cu, 500 mg; Se, 50 mg; Zn, 6000 mg; Mn, 5000 mg; I, 150 mg; choline chloride, 150 000 mg; carrier up to 1 kg.

The fish were fed with the experimental diets (2% of the body weight per day) for eight weeks. Feeding was done by hand 3

times daily at 8:00 am, 13:00 pm, and 19:00 pm in the rearing tanks.

Growth performance measurements

The total length and body weight of some fish were randomly measured at the beginning and end of the experiment. During the experiment period, the average biomass of each tank was measured every two weeks, and the results were used to determine the amount of feed needed for the next two weeks.

Water quality analysis

During the experiment, water quality parameters were measured from the outflow of the experimental tanks. Hardness and pH were measured by a portable pH-meter (AZ 8651, AZ Instrument Corp, Taichung, Taiwan). Dissolved oxygen and temperature were assessed daily by an oxygen meter (WTW 3205, Xylem Analytics, Weilheim, Germany). The inorganic nitrogenous compounds including TAN, NO₂, and NO₃ were determined weekly using spectrophotometry analysis according to APHA (2005) standard method. The mean dissolved oxygen, hardness, pH, and ammonium (NH₄) concentration in all treatments were 8.0 ± 0.7 mg/L, 430 ± 30 μ z, 7.00 ± 0.08 , and 0.44 ± 0.15 mg/L, respectively. The tanks had no significant differences in ammonia (NH₃) concentration.

Experiment design This study was conducted with a completely randomized design. It lasted eight weeks from

November 2012 to January 2013. The fish were reared with three different diets in terms of the composition of fat sources includes: treatment 1 containing 100% fish oil (FO); treatment 2 containing 100% poultry waste oil (PO); and treatment 3 containing equal parts (50-50%) of fish and poultry waste oil (FPO).

Methods used in the experimental analyses

Before the experiment, 10 fish were randomly selected from the fish population for carcass analysis. At the end of the experimental period, 2 fish were randomly taken from each replicate for conducting the experiments. For experimental analysis, the selected fish were treated with MS222 anesthetic at a concentration of 1000 ppm until death (Soivio *et al.*, 1977).

All chemical analyses followed the AOAC (2000) methods and were performed on four levels: dorsal muscular, visceral, liver, and whole fish carcasses. The moisture, ash, crude protein, and fat in the tissues of the fish were analyzed for dry matter (105°C until a constant weight). The dried samples were placed in a furnace for 12 hours at an inside temperature of 600°C to measure the amount of ash. The crude protein was determined ($N \times 6.25$), crud fat by petroleum ether extraction, using the Kjeldahl method and Soxtec method, respectively (AOAC, 2000). The following formulas were used to calculate the growth parameters (Halver, 2013):

$$\text{Weight Gain (WG, \%)} = [(BW_f - BW_i) / BW_i] \times 100$$

$$\text{Specific Growth Rate (SGR, \% day}^{-1}\text{)} = [(\ln BW_f - \ln BW_i) / T] \times 100$$

$$\text{Condition factor (FC, \%)} = [BW_f (\text{g}) / (\text{body length (cm)})^3] \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \text{Feed intake (g)} / \text{WG (g)}$$

Protein Efficiency Ratio (PER) = weight gain (g) / protein intake (g)

protein productive value (PPV) = protein retention (g)/protein intake (g).

Survival = (number of fish remaining in each group on day 56 / initial number of fish) \times 100

Where BW_i and BW_f are initial and final body weight of fish (g) and T is equal to 56 days.

Statistical Analysis

Statistics were performed using the SPSS software, version 15.0 for Windows. The normality of data and homogeneity of variances were tested using the Kolmogorov-Smirnov and Levine's F tests, respectively. The possible differences in the variables among the treatments were tested using one-way ANOVA. Post hoc comparisons between means were performed using Tukey's test. Data were expressed as mean \pm standard error (SE), and differences were considered significant at the $p<0.05$ level.

Results

The results of the growth performance of the experiments are portrayed in Table 2. At the end of the experiment, the amounts of

the weight gain between all experimental groups were more than 1.3 times. The final body weight between 360 and 368 grams was observed among different treatments. Similar FCR among dietary treatments has shown that all diets were well accepted by the fish. The CF of the FPO treatment was significantly lower than the FO treatment ($p<0.05$), while the PO treatment showed no significant differences compared with the FO treatment ($p>0.05$). The other parameters reflecting growth performance, including the final weight, weight gain percentage, feed conversion ratio, specific growth rate protein efficiency ratio and, protein productive value, showed no significant differences between the different treatments (Table 2).

Table 2: Comparison of the growth performance of beluga fed diets containing fish oil (FO), poultry oil (PO) and a blend of equal amounts of fish and poultry oil (FPO).

Parameters	Experimental diets		
	FO	PO	FPO
Initial weight (g)	269.92 \pm 15.78	269.10 \pm 20.92	270.17 \pm 11.71
Final weight (g)	368.56 \pm 23.43	360.11 \pm 17.54	364.54 \pm 9.51
Weight gain (%)	36.60 \pm 4.75	33.82 \pm 7.43	34.93 \pm 8.10
Feed conversion ratio	2.18 \pm 0.19	2.09 \pm 0.20	2.23 \pm 0.32
Condition factor	0.44 \pm 0.01 ^b	0.40 \pm 0.00 ^a	0.42 \pm 0.00 ^{ab}
Specific growth rate (%/day)	0.55 \pm 0.04	0.51 \pm 0.09	0.53 \pm 0.10
Protein efficiency ratio	1.48 \pm 0.15	1.43 \pm 0.27	1.38 \pm 0.30
Protein productive value	33.21 \pm 3.70	34.96 \pm 2.60	24.75 \pm 7.12
Survival (%)	86.66 \pm 0.00	93.33 \pm 0.00	100 \pm 0.00

Different superscripts in the same row denote significant differences between the experimental groups.

The results of using different levels of poultry waste oil and their effects on the chemical composition of the carcasses are presented in Table 3. The amount of ash in fish fed PO was significantly lower than

that in the control ($p<0.05$), while none of the examined parameters for the fish fed FPO showed any significant differences compared with the control. The other chemical compositions of the carcasses,

including crude protein, crude fat, and moisture, did not show any significant differences between the treatments ($p>0.05$). Based on the results from the chemical analysis of the liver and viscera at the end of the experiment, the liver moisture indicated a significant difference in fish fed PO compared to the FPO

($p<0.05$), while none of them showed any statistical differences compared with the FO. It was also observed a significant difference in the viscera so that the amount of visceral fat of the fish fed PO was significantly higher than that of the fish fed FO ($p<0.05$).

Table 3: Comparison of body composition of bluga fed diets containing fish oil (FO), poultry oil (PO) and a blend of equal amounts of fish and poultry oil (FPO).

Parameters	Experimental diets		
	FO	PO	FPO
Carcass			
Protein (%)	67.29±1.57	66.62±3.22	64.11±0.95
Lipid (%)	18.58±2.24	23.22±1.52	22.68±1.35
Moisture (%)	78.62±0.63	76.65±1.05	78.60±1.17
Ash (%)	10.53±0.06	9.89±0.74	9.95±1.07
Viscera			
Lipid (%)	26.71±1.22 ^a	34.22±2.32 ^b	30.11±1.15 ^{ab}
Moisture (%)	86.29±2.31	82.27±1.13	83.70±1.89
Liver			
Lipid (%)	24.62±3.28	36.14±5.44	31.93±4.10
Moisture (%)	71.40±3.56 ^{ab}	59.81±3.70 ^a	74.21±3.33 ^b

Different superscripts in the same row denote significant differences between the experimental groups.

Discussion

Due to the high cost of ingredients used in the aquaculture industry, especially fish oil and fish meal, many studies have been conducted to find suitable replacements for these ingredients (Regan *et al.*, 2010; Bowyer *et al.*, 2012; Monteiro *et al.*, 2018; Pourhosein Sarameh *et al.*, 2019). One of the reliable sources that can be used in fish diets is poultry waste oil (Gallagher and Degni, 1988). Poltry by-product oil has great potential as an alternative to fish oil in fish diets. Similar to other sources of fat used in fish diets, the effect of this product on growth indices, health, and carcass composition depends on the replacement amount and water temperature (Perez-Velazquez *et al.*, 2009). The results of present study showed that diets were well

consumed by beluga, and the replacement of fish oil by PO had no effect on the growth performance and carcass composition. Although, a high survival rate (86.66-100%) along with no significant differences in the growth parameters and carcass composition, indicate that fish oil replacement by PO did not impair the dietary utilization of lipids, nitrogen and energy, which are in agreement with the results presented for; yellowtail kingfish (*Seriola lalandi*), European seabass (*Dicentrarchus labrax*), barramundi (*Lates calcarifer*), and largemouth bass (*Micropterus salmoides*) (Bowyer *et al.*, 2012; Monteiro *et al.*, 2018; Campos *et al.*, 2019; Ahmad *et al.*, 2013; Yun *et al.*, 2013).

The suitable growth rate of beluga and no significant differences in survival rate,

final weight, WGP, FCR, SGR, PER, and PPV in the present study indicate that poultry oil used with the suggested ratios had no negative effects on the growth and health of the juvenile beluga. The results of a study conducted by Monteiro *et al.* (2018) showed no significant decrease in the feed efficiency of seabass when they replaced fish oil with land animal fats. Studies on European seabass juveniles (Campos *et al.*, 2019), yellowtail kingfish (*Seriola lalandi*) (Bowyer *et al.*, 2012), largemouth bass (*Micropterus salmoides*) (Subhadra *et al.*, 2006), and rainbow trout (*Oncorhynchus mykiss*) (Liu *et al.*, 2004) have shown similar results with the present study findings. Contrarily, the total replacement of fish oil by a blend of poultry fat and mammal fat reduced growth in European seabass (Monteiro *et al.*, 2018). Some results confirmed that 100% poultry oil and 50% canola oil can replace fish oil in the diets of yellowtail kingfish without reducing growth, but 100% canola oil resulted in poor fish growth compared with the control (Bowyer *et al.*, 2012). These findings demonstrate that poultry oil can be a more suitable oil source for fish diets.

Based on the results of this study, the only factor influenced by diet fat changes is the condition factor (CF) when fish were fed a diet containing poultry oil. Thus, increasing poultry oil consumption caused lower growth in our experimental fish. This conclusion is supported by the lower percentage of body weight gain in fish fed PO ($33.82 \pm 7.43\%$) compared to other treatments. However, the weight gain decrease was not significant among the others treatments. Perez-Velazquez *et al.* (2019) found that hybrid striped bass

(*Morone chrysops* ♀ \times *M. saxatilis* ♂) fed the diet with 30% replacement of fish oil with algal meals grew better than fish fed the control diet, but the growth was not significantly different from fish fed the 10, 20, 40, or 50% replacement diets, suggesting that there is a suitable level of replacing fish oil with other fat sources for each species. Therefore, it seems that cultured beluga responds well to the replacement treatments and can be used well on poultry oil as a source of fat.

The final body composition of fish indicated no significant difference in the amount of moisture, protein, and lipid among experimental diets. This suggests the suitability of poltry by-product oil application as a good source of fat in the diet of cultured beluga in the context of this research. The results of this study are in line with the previous reports on European eels (*Anguilla anguilla*) (Gallagher and Degani 1988), rainbow trout (Liu *et al.*, 2004), yellowtail kingfish (Bowyer *et al.*, 2012), and European seabass (*Dicentrarchus labrax*) (Regan *et al.*, 2010; Monteiro *et al.*, 2018). Likewise, Pourhosein *et al.* (2019) did not observe any changes in lipoprotein levels, blood lipid indices or hematological parameters in broodstock of sterlet sturgeon (*Acipenser ruthenus*). They stated that the deferent oil sources did not have any adverse effects on the health of the fish during their feeding trial. Therefore, under our experimental conditions, it is possible to replace fish oil with poltry by-product oil in the diet of beluga without any negative effects on the growth performance and body composition.

Guo *et al.* (2011), have suggested that the required amount of fat in the diet of

juvenile sturgeon is above 11.5%. In the present study, the amount of fat in the diets containing fish oil, poultry oil, and a mixture of both with equal parts was estimated 12.09%, 12.22%, and 12.89% respectively, which were all in a suitable range ensuring a suitable growth for the fish. Experiments on the Japanese sea bass (*Lateolabrax japonicus*) indicate that when sufficient essential fatty acids are provided in the feed diets to meet the fish demands, the consumption of suggested fats will not have a negative effect on the growth parameters (Xue *et al.*, 2006). Based on the existing evidence, this conclusion agrees with the findings of the present study.

The maximum visceral fat was observed in fish fed PO, whereas, the lowest fat amount was observed in fish fed FO. Regarding liver fat, despite no significant difference, the highest and lowest fat amounts were observed in fish fed PO and FO, respectively. The result showed that when fish oil was completely replaced by poultry oil, lipid deposition in carcass, viscera and liver compared to FO fed fish. It seems that this was caused by the lower metabolism capacity and energy value of poultry oil compared with fish oil. Also, it may have been due to the high dietary levels of fatty acids that direct absorption and esterification in the muscle and also a high mobilization from liver to muscle and viscera (Henderson, 1996). Therefore, these sources of fat will be less used for energy production and accumulate in body tissues, viscera, and the liver. The difference in the energy metabolism capacity can be attributed to the poultry oil compound produced from slaughterhouse waste, its saturated and unsaturated fatty

acid ratios, and its low digestibility and absorption. Studies have estimated that the energy that can be produced from the metabolism of poultry fat and fish oil are 5.37 ± 0.08 and 8.85 ± 0.07 kcal per gram, respectively, which can be recommended for diet formulation (Yaghobfar, 2004).

Conclusion

Using poultry by-product oil as a fat source in the diet has no significant negative effects on the survival, growth, and body composition of juvenile beluga. Due to its availability and cost, it is recommended to use poultry by-product oil appropriately. Based on the results, a 50% replacement of fish oil with poultry by-product oil in the diet of bluga sturgeon is recommended. It is important to continue monitoring the long-term effects of using poultry by-product oil in the diet of bluga sturgeon to ensure that there are no unforeseen consequences. Furthermore, future research could explore the potential advantages of incorporating poultry by-product oil into the diets of additional aquatic species. It is also advisable to study the prolonged impact of using poultry by-product oil on bluga sturgeon and other fish species. Overall, the findings of this study suggest that poultry by-product oil can be a viable alternative to fish oil in the diets of beluga, offering potential economic and environmental benefits.

Conflicts of interest

The authors declare no conflict of interest.

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