# Dietary fat content and feed supply influence growth and body composition in juvenile beluga sturgeon (*Huso huso*)

**A. Keramat Amirkolaie · S. Mahdavi · S. A. Hosseini**

**Abstract** This study is to evaluate the impacts of two levels of fat on the fish growth, feed intake, and compositions of muscle, liver, and viscera on the juvenile *Huso huso*. There were four treatments: two experimental diets (high-fat: 30% and low-fat: 15%) and two feeding levels (100 or 60% of satiation). The treatments were assigned to 16 tanks each with 15 fish, with four replicates for each treatment. Beluga sturgeon juveniles with an initial weight of $61.3 \pm 4.4$ g were reared for 3 months. Increasing fat content of the diets resulted in an increased final weight (621.8 vs. 467.5 g for high-fat and low-fat diets, respectively, at 100% feeding level; $p < 0.01$), but had no impact on feed conversion rate (FCR; $p > 0.05$). Total feed intake was increased by the use of high-fat diet (487.8 vs. 365.0 g for high-fat and low-fat diets, respectively, at 100% feeding level; $p < 0.01$). Results also showed no interactions between fat level of the diets and feeding level in final weight and FCR. Beluga sturgeon muscle and liver fed high-fat diets contained higher fat than those fed low-fat diets (186.5 and 329.6 vs. 144.1 and 261.9 g kg$^{-1}$ for high-fat and low-fat diets, respectively, at 100% feeding level; $p < 0.01$), and feeding levels did not change protein content of the examined organs. In conclusion, beluga sturgeon shows an enhanced growth on high-fat diet, and feed intake does not reduce by the use of high-fat diets.

**Keywords** Nutrient deposition · Feed efficiency · Chemical analysis · Tissue characteristics · Dietary fat · Feed intake

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## Introduction

Beluga sturgeon has been classified as a critically endangered species in the Caspian Sea. Along with decline in the natural populations, controlled production of beluga sturgeon has grown around the world (Abdolhay and Tahori 2006; Mohseni et al. 2006). This growing trend has been attributed to excellent characters of this species such as fast growth, easy adaptation to farm environment, and, specifically, its expensive caviar (Nikcevic et al. 2003; Mohseni et al. 2006).

For successful sturgeon production, more data are needed on sturgeon nutrient requirements (Medale et al. 1995) and feeding strategy. Few studies conducted on sturgeon revealed a higher energy demand for these species. Diets containing fat from 258 to 357 g kg$^{-1}$ resulted in good growth and feed efficiency but had little effect on body composition in white sturgeon *Acipenser transmontanus* (Hung et al. 1997). Mohseni et al. (2007) reported that juvenile Persian sturgeon, *Acipenser persicus*, is capable of utilizing high levels of dietary fat (up to 250 g kg$^{-1}$). Nevertheless, information available on the impact of fat levels on feed intake and organ characteristics in sturgeon is relatively limited.

Fish are able to regulate their daily feed intake in order to meet their nutrient and energy requirements (Boujard and Medale 1994; Boujard et al. 2002). High-fat diets led to a reduced feeding level in European sea bass, *Dicentrarchus labrax* (Boujard et al. 2004), and in rainbow trout, *Oncorhynchus mykiss*, (Yamamoto et al. 2002). However, under commercial production system, rainbow trout and Arctic charr, *Salvelinus alpinus,* were not able to adjust feed intake when given a commercial diet with different levels of fat (Alanara 1994; Alanara and Kiessling 1996). This may suggest that there is still doubt about the impact of dietary energy levels on voluntary feed intake.

Feed composition and/or feeding level affect biochemical composition of the body tissue, with an obvious change in percentages of fat and moisture (Jobling 2001). Higher-fat diets will usually lead to more storage of fat in fish body part than those fed on diets with lower fat levels (Shearer 1994; Higgs et al. 1995; Bell 1998). Fat deposition is not similar among different parts of body. A large amount of fat may be stored in viscera, liver, and/or ovary leading to a reduced muscle yield during filleting (Aursand et al. 1994; Higgs et al. 1995; Jobling et al. 1998) or an impaired function of liver (Sargent et al. 1989) and, finally, low caviar yield in broodstocks (Van Eenennaam et al. 2004).

Recent nutritional studies on beluga sturgeon were conducted using diets containing around 150 g kg$^{-1}$ fat (Mohseni et al. 2006, 2008; Hosseini et al. 2010). However, growing beluga sturgeon appears to require high-fat diet for meat production to supply fast-growth energy requirement as they reach to market size (5–7 kg) between 15 and 17 months after hatching (Mohseni et al. 2008).

Therefore, the main objective of the present study is to determine the effects of increasing diet energy level through incorporation of two levels of fat on the juvenile *Huso huso* growth performance and compositions of muscle, liver, and viscera. We also sought to verify whether feed intake is affected by fat content of diet in *H. huso*.

## Materials and methods

### Experimental diets

Two experimental diets were formulated with similar protein contents but different protein/ fat ratios: low-fat and high-fat diets with 15 and 30% fat contents, respectively. Nutrient

**Table 1** Feed ingredients and nutrient composition of two experimental diets in g kg$^{-1}$ or kJ g$^{-1}$ wet weight. Each value is the mean of three sub-samples

| | Diet | |
|---|---|---|
| | High fat | Low fat |
| Ingredients | | |
| Corn meal | – | 70 |
| Soybean meal | 70 | 140 |
| Wheat flour | 40 | 100 |
| Wheat gluten | 100 | 50 |
| Fish oil | 125 | 50 |
| Soybean oil | 125 | 50 |
| Fish meal | 500 | 500 |
| Mineral mix[a] | 10 | 10 |
| Vitamin mix[b] | 20 | 20 |
| Calcium phosphate | 10 | 10 |
| Nutrient composition of two experimental diets | | |
| Dry matter | 896.2 | 887.0 |
| Crude protein | 393.4 | 381.9 |
| Crude fat | 297.3 | 148.5 |
| Ash | 91.3 | 98.0 |
| Carbohydrate | 114.2 | 258.6 |
| Gross energy (kJ g$^{-1}$) | 22.9 | 19.3 |

[a] Mineral premix consisted of (g kg$^{-1}$ premix) Mn 2,600 mg, Cu 600 mg, Fe 6,000 mg, Zn 4,600 mg, Se 50 mg, I 100 mg, Co 50 mg, choline chloride 100,000 mg

[b] Vitamin premix consisted of (mg kg$^{-1}$ premix) vitamin A 1200000 IU, vitamin D3 400000 IU, vitamin E 3000 IU, vitamin C 400 mg, vitamin B1 200 mg, vitamin B2 3,360 mg B2, vitamin B3 7,200 mg, vitamin B3 9,000 mg B5, vitamin B6 2,400 mg, vitamin B9 600 mg, vitamin B12 4 mg, vitamin E 500 mg

composition was based on the current data available for feeding sturgeon (Hung and Deng 2002; Mohseni et al. 2007). The formulation and chemical composition of the two experimental diets are presented in Table 1. All ingredients were finely ground, mixed, and pelletized. A pellet size of 3 mm was used for both diets. Pellets were air-dried at 80°C and stored in a refrigerator until use.

Experimental system and animal

Beluga sturgeon juveniles with an initial weight of 61.3 ± 4.4 g were reared for 3 months. The experiment started after 2 weeks of acclimation to the system. Afterward, beluga sturgeon was randomly distributed in groups of 15 individuals to 16 rectangular tanks (2 × 2 × 0.4 m, each with a volume of around 400 L). Water quality was checked twice a week, 2 h after feeding. The measured parameters were as follows: temperature, pH, oxygen content, and $NH^{4+}$–N. A continuous flow water (15–20 L min$^{-1}$) was directed to each experimental tank throughout the experimental period. The water temperature was kept at normal range (19–24°C) during the experiment. Oxygen concentration was measured in a randomly selected tank by a digital oxygen detector and always remained above

7.5 mg $L^{-1}$. The water pH ranged between 6.7 and 7.4 during the experiment. Average ammonium concentration was $0.11 \pm 0.05$ mg $L^{-1}$ over the experimental period.

There were two experimental diets and two feeding levels, totally four treatments according to a $2 \times 2$ factorial design. These treatments were the following: LF100, low-fat diet/apparent satiation level; LF60, low-fat diet/60% satiation level; HF100, high-fat diet/apparent satiation feeding; and HF60, high-fat diet/60% satiation feeding. This design was to assess the interaction between fat levels of the diets and feeding levels in *H. huso*. The intended design would enable determining the impacts of feeding levels on the growth and nutrient deposition when high-fat and/or low-fat diets were used.

Experimental procedure

The experimental treatments were randomly assigned to each of the 16 tanks, having four replicates (tanks) for each treatment. Experimental tanks were fed at 100 or 60% of satiation feeding level. The fish were fed by hand four times a day at 06:00, 12:00, 18:00, and 24:00 h.

At 100% satiation feeding level, feed was provided to the fish, until the fish stopped eating and 3–4 pellets were remained on the tank bottom uneaten for 6 min. The feed intake at 100% satiation was recorded for all the four feedings during the experiment. The daily feed ration at 60% satiation (DF 60%) was calculated according to the following formula

$$\mathrm{DF\,60\%} = \text{Average feed consumed by four tanks of 100\% satiation} \times 0.6.$$

On day 90, all fish were weighed individually. Afterward, three fish were randomly selected from each tank and killed using overdosed clove essence solution for the analysis of body part composition. The whole viscera were removed, and livers were separated from the viscera. Viscera weight was measured after removal of the liver. The muscle was selected from skin–free dorsal muscle between operculum and third bony dermal plate. Muscle, liver, and viscera from three fish in each tank were pooled before chemical analysis.

On day 90, blood sample for plasma triglyceride, glucose, and protein assessments was also taken. The last feeding was made on day before at 24.00 h. From each tank, three fish were randomly selected and immediately put in anesthesia (clove essence solution). Around 1 mL of blood was collected from the caudal blood vessels in all the selected fish by hypodermic syringe (containing 3 mg Na2EDTA). The collected samples were placed in cooled, 1.5-mL plastic tubes; mixed; and centrifuged at $6{,}000 \times g$ for 5 min at 4°C. After centrifugation, plasma was collected and stored at $-20$°C for further analysis.

Chemical analysis

Feed samples were collected and pooled at regular intervals during the experimental period and ground using a 1-mm screen before analyses. Feed and body part were analyzed for dry matter through drying samples for 24 h at 103°C until constant weight (ISO 1983). Ash content was determined by incineration in a muffle furnace for 4 h at 550°C (ISO 1978). Crude protein (N × 6.25) was measured by the Kjeldahl method after acid digestion according to ISO (1979). Fat was extracted by petroleum ether extraction in a Soxhlet apparatus. Energy content was measured by direct combustion in an adiabatic bomb calorimeter (IKA-C-7000, Fa. IKA- Analysentechnik, Weitersheim, Germany). Carbohydrate fraction was determined as dry matter minus fat, protein, and ash in the feed.

Plasma protein, triglycerides, and glucose were assayed by using automatic chemistry system (Vitros DT60 II, Vitros DTE II, DTSC II Chemistry System, Johnson and Johnson Clinical Diagnostics Inc., New York, USA).

Fish performance

Weight gain was determined by the difference between total initial and final body weights. Feed conversion ratio (FCR) was calculated per tank from total feed intake and total weight gain for the period of 3 months between the initial and the final weightings. Protein efficiency ratio (PER) was calculated per tank by dividing total weight gain to total protein consumed during 3 months. The calculations were based on the wet weight of the diet.

Hepatosomatic and visceral somatic indices were calculated according to the following formulas.

$$\text{Hepatosomatic index (HSI\% )} = 100 \times (\text{liver weight/live weight})$$

$$\text{Visceral somatic index (VSI\% )} = 100 \times (\text{visceral weight/live weight}).$$

Statistical analysis

Data are presented as means of each treatment with standard deviation. All data were verified for normality after transformation (ASIN). Two-way ANOVA was used to determine the effects of both fat (15 vs. 30%) and feeding levels (low vs. high) and also the interactions between fat and feeding levels (SAS 1989). Tukey's test was used to compare differences between the means. For all statistical analyses, each tank was considered as the experimental unit. Differences among treatment means were considered significant when $p < 0.05$.

# Results

Data on the growth performance and tissue characteristics of beluga sturgeon are presented in Table 2. There were no interactions between fat levels of the diets and feeding levels on final weight and FCR ($p > 0.05$). An increase in fat level improved growth ($p < 0.01$), but no effects were found on FCR and PER. Total feed intake increased by the use of high-fat diet (487.8 vs. 365.0 g for high-fat and low-fat diets, respectively, at 100% feeding level; $p < 0.01$). Fat level of the diet increased HSI (3.0 vs. 2.0% for high-fat and low-fat diets, respectively, at 100% feeding level; $p < 0.01$), but feeding level had no significant effects on HSI. A tendency toward lower VSI was observed in fish fed the high-fat diet and those fed to satiation (Table 2).

Fat level of the diets influenced muscle moisture and fat content ($p \leq 0.01$), but feeding levels did not affect those parameters. Liver fat content elevated with increasing both fat level and feeding level of the diets ($p < 0.01$). High-fat diets led to a larger content of protein in the liver, while a lower protein observed in the liver of 100% satiation feeding level. There were significant interaction effects between fat and feeding levels for protein and moisture contents of the liver (Table 3). Fat only accounted for 5–6% of visceral mass, and diet composition did not have any impact on chemical composition of the viscera ($p > 0.05$; Table 3).

Glucose and triglyceride were higher ($p < 0.05$) with the high-fat than with the low-fat diets. However, increasing feeding level did not cause any changes in these parameters for

**Table 2** Growth-related parameters and tissue characteristics in juvenile beluga fed with two dietary fat contents (high and low) and two feeding levels (60 and 100% satiation) over a 90-day experimental period

| Fat level: | High fat | High fat | Low fat | Low fat | $p$ values of the factors | | |
| Feeding level: | 100% | 60% | 100% | 60% | Fat | Feed level | Fat*level |
|---|---|---|---|---|---|---|---|
| Initial weight(g) | $61.4 \pm 1.4$ | $60.6 \pm 0.4$ | $61.8 \pm 1.5$ | $61.1 \pm 0.7$ | | | |
| Final weight(g) | $621.2 \pm 29.1^a$ | $481.9 \pm 77.9^{ab}$ | $467.5 \pm 70.7^b$ | $399.3 \pm 77.3^b$ | 0.004 | 0.009 | 0.308 |
| FCR | $0.9 \pm 0.0^a$ | $0.7 \pm 0.0^b$ | $0.9 \pm 0.0^a$ | $0.7 \pm 0.1^b$ | 0.452 | 0.001 | 0.355 |
| PER | $2.92 \pm 0.04^b$ | $3.56 \pm 0.30^a$ | $2.90 \pm 0.15^b$ | $3.73 \pm 0.33^a$ | 0.549 | 0.001 | 0.447 |
| Total feed intake(g) | $487.8 \pm 23.5^a$ | $305.1 \pm 76.5^b$ | $365.0 \pm 49.3^{ab}$ | $242.8 \pm 73.3^b$ | 0.001 | 0.001 | 0.329 |
| HSI (%) | $3.0 \pm 0.3^a$ | $2.8 \pm 0.4^a$ | $2.0 \pm 0.4^b$ | $1.6 \pm 0.2^b$ | 0.001 | 0.065 | 0.544 |
| VSI (%) | $8.2 \pm 1.2$ | $10.7 \pm 1.4$ | $10.7 \pm 2.0$ | $11.9 \pm 2.5$ | 0.077 | 0.067 | 0.504 |

All values are means of four replicates (tanks)/treatment $\pm$ standard deviation

Different superscript letters show significant differences

both high-and low-fat diets. Dietary fat content and feeding level also did not change plasma protein in beluga sturgeon juveniles ($p > 0.05$; Table 4).

## Discussion

The present study shows that the growth-related parameters improved by fat content of the diets examined. This is in agreement with the results reported by Hung et al. (1997), who found a positive correlation between fat content of the diet (up to 350 g kg$^{-1}$) and growth parameters in *Acipenser transmontanus*. Mohseni et al. (2007) also reported that juvenile Persian sturgeon is capable of utilizing high levels of dietary fat (up to 250 g kg$^{-1}$). On the other hand, Medale et al. (1991) observed, in Siberian sturgeon (*Acipenser baerii*), that a dietary fat of 218 g kg$^{-1}$ did not improve growth performance compared with that of 125 g kg$^{-1}$, but increased fat content of the liver. This may suggest that the efficiency of fat utilization in sturgeon is related to species.

In this study, juvenile beluga sturgeon grew well (6.5- to 10-fold) within 3 months. This growth rate is comparable with or even higher than those reported by Jodun (2004) in Atlantic sturgeon, Vaccaro et al. (2004) in *A. naccarii* × *A. baerii*, and Mohseni et al. (2007) in Persian sturgeon. This finding may suggest that beluga sturgeon, as a fast grower species, can be a promising candidate for aquaculture. A lower growth rate of juvenile *H. huso* (threefold to fourfold within 4 months; Hosseini et al. 2010) feeding on diet containing 120 g kg$^{-1}$ fat may support the idea that this species requires a larger dietary fat to satisfy large energy demand for growth during juvenile stage.

High-fat diet did not lead to reduction in feed intake in *H. huso*. It appears that a difference of 3.5 kJ g$^{-1}$ in crude energy content between the high- and low-fat diets may not to be enough to cause a significant change in feed intake. However, this work as a preliminary study on feed intake regulation in relation to dietary fat has its drawbacks. Having only two levels of dietary fat is probably too small to induce a clear reaction of the test animal regarding their feed intake. Moreover, precise measurement of satiation feeding

**Table 3** Chemical composition of muscle, liver, and viscera without liver in juvenile beluga (g kg$^{-1}$ wet weight basis; mean $\pm$ standard deviation) fed with two dietary fat contents (high and low) and two feeding levels (60 and 100% satiation) over a 90-day experimental period

| Fat level: | High fat | High fat | Low fat | Low fat | $p$ values of the factors | | |
|---|---|---|---|---|---|---|---|
| Feeding level: | 100% | 60% | 100% | 60% | Fat | Feed level | Fat*level |
| Back muscle | | | | | | | |
| Moisture | 614.5 $\pm$ 24.1$^c$ | 631.5 $\pm$ 24.0$^{bc}$ | 676.4 $\pm$ 9.4$^a$ | 674.5 $\pm$ 22.2$^{ab}$ | 0.001 | 0.486 | 0.386 |
| Crude protein | 178.7 $\pm$ 3.2 | 181.7 $\pm$ 7.1 | 176.5 $\pm$ 11.7 | 198.1 $\pm$ 26.5 | 0.364 | 0.128 | 0.241 |
| Crude fat | 186.5 $\pm$ 2.1$^a$ | 170.2 $\pm$ 31.2$^{ab}$ | 144.1 $\pm$ 17.6$^{ab}$ | 127.1 $\pm$ 7.3$^b$ | 0.009 | 0.253 | 0.982 |
| Ash | 14.4 $\pm$ 01.9 | 16.9 $\pm$ 2.1 | 13.3 $\pm$ 0.3 | 14.5 $\pm$ 2.7 | 0.109 | 0.143 | 0.169 |
| Liver | | | | | | | |
| Moisture | 550.1 $\pm$ 10.1$^b$ | 529.0 $\pm$ 10.0$^c$ | 524.3 $\pm$ 6.7$^c$ | 574.1 $\pm$ 7.5$^a$ | 0.046 | 0.006 | 0.001 |
| Crude protein | 131.8 $\pm$ 3.4$^b$ | 161.5 $\pm$ 10.2$^a$ | 105.4 $\pm$ 4.5$^c$ | 99.3 $\pm$ 7.4$^c$ | 0.001 | 0.004 | 0.001 |
| Crude fat | 329.6 $\pm$ 2.7$^a$ | 294.3 $\pm$ 16.2$^b$ | 261.9 $\pm$ 16.2$^c$ | 252.4 $\pm$ 13.8$^c$ | 0.001 | 0.006 | 0.806 |
| Ash | 14.3 $\pm$ 1.2 | 15.4 $\pm$ 1.8 | 15.1 $\pm$ 1.9 | 13.8 $\pm$ 0.9 | 0.630 | 0.888 | 0.155 |
| Viscera | | | | | | | |
| Moisture | 710.2 $\pm$ 64.8 | 764.3 $\pm$ 28.8 | 731.4 $\pm$ 14.7 | 740.0 $\pm$ 24.9 | 0.939 | 0.127 | 0.258 |
| Crude protein | 219.5 $\pm$ 47.7 | 208.7 $\pm$ 7.2 | 253.3 $\pm$ 10.6 | 219.5 $\pm$ 14.7 | 0.109 | 0.109 | 0.391 |
| Crude fat | 60.4 $\pm$ 9.3 | 55.8 $\pm$ 9.2 | 50.7 $\pm$ 9.5 | 49.8 $\pm$ 5.7 | 0.093 | 0.534 | 0.674 |
| Ash | 16.0 $\pm$ 1.0 | 16.5 $\pm$ 2.6 | 16.3 $\pm$ 2.6 | 13.7 $\pm$ 0.8 | 0.202 | 0.271 | 0.119 |

All values are means of four replicates (tanks)/treatment $\pm$ standard deviation

Different superscript letters show significant differences

**Table 4** Plasma analyses in juvenile beluga fed with two dietary fat contents (high and low) and two feeding levels (60 and 100% satiation) over a 90-day experimental period. All values are means of four replicates (tanks)/treatment $\pm$ standard deviation

| Fat level | High fat | High fat | Low fat | Low fat | $p$ values of the factors | | |
|---|---|---|---|---|---|---|---|
| Feeding level | 100% | 60% | 100% | 60% | Fat | Feed level | Fat*level |
| Glucose (mmol L$^{-1}$) | 9.38 $\pm$ 2.59$^a$ | 8.50 $\pm$ 1.51$^a$ | 6.40 $\pm$ 1.66$^b$ | 6.35 $\pm$ 1.61$^b$ | 0.018 | 0.633 | 0.670 |
| Triglyceride (mg dL$^{-1}$) | 392.0 $\pm$ 22.6$^a$ | 410.8 $\pm$ 150$^a$ | 288.8 $\pm$ 13.5$^b$ | 242.3 $\pm$ 52.2$^b$ | 0.023 | 0.796 | 0.546 |
| Plasma protein (g dL$^{-1}$) | 1.62 $\pm$ 0.23 | 1.43 $\pm$ 0.29 | 1.41 $\pm$ 0.35 | 1.59 $\pm$ 0.36 | 0.892 | 0.899 | 0.273 |

Different superscript letters show significant differences

level in sturgeon is quite difficult as they are slow-eater fish (Cui et al. 1997). Therefore, the experimental design did not permit us to clearly determine the effects of dietary fat on performance and feed intake regulation. Further studies with bigger number of fat and feeding levels and also a longer experimental period are necessary to have a clear judgment about feed regulation in *H. huso*.

It appears that sturgeons in general accept better or even show a better performance by feeding on high-fat diets. Body proximate composition and fat content of muscle might explain such high-fat requirement. Sturgeon muscle contains larger levels of fat ranging from 10 to 18% (Table 3; Hung et al. 1997; Alipour et al. 2010) compared with those below 10% in a range of fish species such as rainbow trout (Smith et al. 1988; Rasmussen and Ostenfeld 2000; De Francesco et al. 2004), channel catfish, Atlantic salmon, and Atlantic cod (Jobling 2001). Increased body fat retention leads to a larger energy requirement. Therefore, energy content of the diet should be sufficiently high to satisfy large energy demand in juvenile beluga sturgeon.

Viscera fat content was not influenced by fat level of the diets, and the overall liver fat content was not very high in comparison with those found by Hung et al. (1997) and Furne et al. (2009). In addition, a larger plasma glucose concentration in *Huso huso* fed on high-fat diet (Table 4) suggests a higher metabolism rate (Hemre and Sandnes 1999). These conditions may support the idea that *H. huso* can utilize well high-energy diets and that organ function would not be threatened by the use of diet containing 300 g kg$^{-1}$ fat. This finding is similar to that reported by Hung et al. (1997), who found that fat contents of liver and viscera are not influenced by diet containing up to 350 g kg$^{-1}$ fat.

In conclusion, rapid growth of beluga sturgeon during juvenile stage renders this species a promising candidate for intensive aquaculture. This study also reveals that an increase in dietary energy induced by high-fat diet improves *Huso huso* growth performance, suggesting a higher energy requirement. It seems that the differences in energy content of the diets are too small to induce the adjustment of feed intake in *H. huso*. A relatively slight difference (5–7%) in fat content of liver between high-and low-fat diets may suggest that liver function is not threatened by high-fat diet.

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