

Research Article

Dietary effect of different thiamin levels on the growth performance, body composition, and hematological parameters of Beluga (*Huso huso*) in low and high-fat diets

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Keywords	Abstract
<i>Huso huso</i> , Feed supplement, Fat, Vitamin, B1	The present research evaluated the effects of dietary thiamin levels in low and high-fat diets on the growth performances, body composition, and hematology of Beluga (<i>Huso huso</i>) juveniles. A total number of 480 juveniles with an average weight of 44.98 ± 1.96 g were distributed in 24 tanks and fed with diets containing 180 g/kg fat (high-fat, HF), 180 g/kg fat+7 mg/kg thiamin (HF-7B1), 180 g/kg fat+15 mg/kg thiamin (HF-15B1), 180 g/kg fat+25 mg/kg thiamin (HF-25B1), 90 g/kg fat (low fat, LF), 90 g/kg fat+7 mg/kg thiamin (LF-7B1), 90 g/kg fat+15 mg/kg thiamin (LF-15B1), 90 g/kg fat, 25 mg/kg thiamin (LF-25B) for 8 weeks. At the end of the feeding trial, five fish from each tank were randomly sampled and the blood samples were collected to evaluate hematological parameters. The results showed that dietary fat and thiamin levels had no significant interaction effect on the final weight, weight gain, specific growth rate, feed conversion rate, and survival rate ($p > 0.05$). The fat had a significant effect on growth performance and high-fat diets substantially increased final weight, weight gain, and specific growth rate compared to low-fat diets ($p < 0.05$). Different thiamin levels had notable differences in the growth performance in low and high-fat diets and growth significantly were higher in fish fed with thiamine compared to control group ($p < 0.05$). The interaction effect of fat and thiamine on body composition was not significant ($p < 0.05$). The effect of fat on protein is significant and beluga fed with high-fat diet had a higher protein content than beluga fed with the low-fat diet. There were significant differences in WBC, Hb between treatments and the highest level was obtained in HF-15B1 and HF-25B1 ($p < 0.05$). The results showed that diets supplemented with thiamin improved growth performance, body composition, and hematological index in beluga sturgeon.
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Introduction

One of the most economical species of Caspian Sea is Beluga (*Huso huso*), a good candidate for aquaculture (Mohseni *et al.*, 2013). The effective cultivation of sturgeon depends on providing their nutritional needs with a balanced and adequate diet. Therefore, the deficiency in one or more essential nutrients reduces the efficiency of fish and causes disease and even death. One of essential nutrients is the vitamins which are necessary for different physiological function (Lonsdale, 2006). Vitamin B1 or thiamin acts in the central role as a co-factor for various enzymes involved in amino acid, carbohydrate, and fatty acid metabolism (NRC, 2011). It may be converted into the phosphoester forms thiamine monophosphate, thiamine pyrophosphate, and thiamine triphosphate in animal tissues (Zhao *et al.*, 2020). They serve many functions in enzymatic pathways and are vital coenzymes for the fish body (Jenco *et al.*, 2017). Optimal requirements of dietary thiamine were reported for various cultured fish species (Lim *et al.*, 2011; Huang *et al.*, 2011; Xiang *et al.*, 2016; Zehra and Khan, 2017; Mohseni *et al.*, 2023). Thiamin deficiency has different symptoms, including poor appetite, low growth performance, high mortality rate, muscle atrophy, abnormal color, and neurological disturbance (NRC, 2011; Zhao *et al.*, 2020). Fish should obtain thiamin from their diet and the need for thiamin depends on the energy level of the food (Woodbury, 1943; Niimi *et al.*, 1997). Since the net energy value of lipids is more than that of proteins, the requirement for thiamin depends on the dietary lipid content (Keinanen *et al.*, 2018). Thiamine deficiency induced by fat-rich diets was reported in Atlantic salmon (*Salmo salar*)

(Keinanen *et al.*, 2018). Numerous research on salmon revealed that consuming a high-fat diet increased the amount of fat oxidative stress, and thiamine deficiency in the salmon tissues (Ostbye *et al.*, 2011). Thiamine deficiency in these fish was reported in terms of the peroxidation and free radical oxidation of fatty acids (Gibson and Zhang, 2002).

It is well known that fish obtain most of their energy needs from protein and lipid, and when non-protein sources are used as an energy source, protein will be partially used to improve growth. The use of non-protein sources in fish diets is useful in terms of improving growth, saving protein and cost. Due to the obvious requirement of beluga for thiamine and the relationship between thiamine and fat, there is a possibility of a change in the quantitative requirement in response to changes in lipid levels and dietary impurities. So this study was done to investigate the relationship between thiamine and lipid to evaluate the appropriateness of increasing dietary lipid as an energy source in isoprotein diets with similar metabolizable energy. In addition, potential changes in thiamine requirement due to increased lipid were investigated.

Material and methods

Fish and experimental condition

A total of 480 beluga juveniles with an initial weight of 44.98 ± 1.96 g were chosen from breeding portions of a sturgeon farm (Qarebroun Sturgeon Culture Center, Sari, Mazandaran, Iran) and transferred to a study part before being randomly stocked in 24 tanks with a 2000 L capacity, each containing 20 fish. Before starting feeding trial, beluga juveniles were adapted to

experimental condition for two weeks and fed a basal diet three times daily to apparent satiety. After two weeks, the tanks were randomly allocated to 8 treatments, each with three replications. Uneaten feeds were collected one hour after each feeding time, dried at 60°C, and used in the calculations of feed intake. During feeding experiment, fresh water was pumped into the tanks at a flow rate 13 ± 0.3 L min $^{-1}$. Temperature, dissolved oxygen levels, and pH of the water were measured using mercury thermometers from Zomorodazma

Company, Cyberscan Eutech instruments (DO 110), and Hanna instruments (8314) (temperature 21.82 ± 0.6 °C, dissolved oxygen 7.8 ± 0.5 mg/L, pH 7.3 ± 0.2 ; photoperiod was maintained at 12D:12L; total ammonia 0.43 ± 0.05 mg/L, nitrate 19.5 ± 1.5 mg/L, nitrite 0.017 ± 0.001 mg/L).

Experimental diets and feeding trial

Eight isonitrogenous and isocaloric (Table 1) diets were prepared with four levels of thiamin (0, 7, 15, and 25 mg/kg diet) and two levels of fat (90 and 180 g/kg diet).

Table 1: Dietary formulation and proximate composition of the experimental diets.

Ingredient ^a (g/kg diet)	HF	HF-7B1	HF-15B1	HF-25B1	LF	LF-7B1	LF-15B2	LF-25B1
Fishmeal ^a	400	400	400	400	400	400	400	400
Soybean meal ^b	150	150	150	150	230	230	230	230
Meat and bone meal ^c	120	120	120	120	0	0	0	0
Wheat gluten	50	50	50	50	50	50	50	50
Corn flour	119.3	119.3	119.3	119.3	119.3	119.3	119.3	119.3
starch	50	50	50	50	50	50	50	50
Fish oil	35	35	35	35	0	0	0	0
Soybean oil	30	30	30	30	0	0	0	0
Lecithin	20	20	20	20	20	20	20	20
Di-calcium phosphate	3	3	3	3	3	3	3	3
Vitamin premix ^d (B1 free)	10	10	10	10	10	10	10	10
Mineral premix ^e	10	10	10	10	10	10	10	10
Antifungal	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Thiamin (g kg $^{-1}$)	0	0.007	0.015	0.025	0	0.007	0.015	0.025
Chemical composition								
Crude protein	46.18	40.02	40.89	40.01	40.04	40.69	40.79	41.2
Crude fat	19.64	19.68	19.62	19.59	12.5	12.9	12.86	12.56
Moisture	10.25	10.15	10.05	10.215	10.12	10.00	10.02	10.07
Ash	11.5	11.86	11.03	11.96	11.25	11.01	11.76	11.12
Crude energy (kcal kg $^{-1}$)	3021	3021	3021	3021	3021	3021	3021	3021

^a Vitamins mixture was manually provided according to feed requirements of the fish (NRC, 2011) and ingredients were obtained from Hashtgerd Laboratories (Hashtgerd, Alborz, Iran); which each 1000 g vitamin mixture provides: vitamin A, 1,600,000 I.U; vitamin D3, 400,000 I.U; riboflavin, 8 g; niacin, 12 g; pantothenic acid, 40 g; pyridoxine, 4 g; folic acid, 2 g; cyanocobalamin, 8 mg; vitamin C, 60 g; vitamin K3, 2 g; biotin, 240 mg; inositol, 20 g, and vitamin E, 60 g.

^b Aquatic minerals mixture was manufactured by Science Laboratories (Ghazvin, Iran); where each 1000 g contains mineral trace elements: ferrous, 6000 mg; zinc, 10000 mg; selenium, 20 mg; cobalt, 100 mg; copper, 600 mg; magnesium, 5000 mg; iodine, 600 mg, and choline chloride, 6000 mg

The levels of fat was chosen based on previous study in beluga sturgeon (Keramat Amirkolaie *et al.*, 2012; Najafi *et al.*, 2017). Feed items were obtained from a sturgeon feed company (Caspian Yaqoot Talaei, Eslami), and thiamin was acquired from Hashtgerd Laboratories (Hashtgerd, Alborz, Iran). The materials were transferred to Qarebroun Culture Center (Sari, Mazandaran, Iran) to produce experimental diets. Doses of thiamin in experimental diets were selected based on information available in other species (NRC, 2011). To make the experimental diets, the dry components of each diet were thoroughly mixed. After the liquid components were diffused into the mixture, deionized water was added (250 ml per kg of diet). The prepared mixture was extruded in an electric meat grinder and feeds were crashed 3 mm in diameter. The pellets were spread out and allowed to air dry at room temperature (for 24 hours). The pellets were then packaged and stored at -20°C till feeding. During the feeding period, fish were fed one of the eight experimental

diets, including 180 g/kg fat (High-fat, HF), 180 g/kg fat+7 mg/kg thiamin (HF-7B1), 180 g/kg fat+15 mg/kg thiamin (HF-15B1), 180 g/kg fat+25 mg/kg thiamin (HF-25B1), 90 g/kg fat (Low Fat, LF), 90 g/kg fat+7 mg/kg thiamin (LF-7B1), 90 g/kg fat+15 mg/kg thiamin (LF-15B1), 90 g/kg fat+25 mg/kg thiamin (LF-25B) by hand three times per a day to apparent satiety for 8 weeks. All experimental protocols were approved by the faculty of sciences of the University of Tehran (357; 8 November 2000).

Biometry

At the end of feeding trial, All beluga juveniles in each tank were given 400 mg L⁻¹ clove powder extract to make them unconscious after a 24-hour fast and their weights were recorded. The weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (%) were all measured as measures of growth performance as below:

Weight gain (WG, g) = Final weight (g) – initial weight (g)

SGR: Specific Growth Rate = ((Ln final weight – Ln initial weight) / during the total experimental period (56 days)) × 100

FCR: Feed Conversion Ratio = Dry feed consumed (g) / weight gain (g)

Survival rate (%) = 100 × (no. of fish stocked – no. of fish died) / no. of fish stocked

Sample collections and analysis

At the end of the feeding trial, feeding was stopped for 24 hr. Then, five fish per tank were randomly selected and anesthetized with 400 mg L⁻¹ clove powder extract (Najafi *et al.*, 2017) and blood samples were collected by venipuncture of the caudal vein using a sterile 5-ml syringe.

Blood samples were diluted with phosphate-buffered saline (PBS) for counting the red blood cells (RBC) and white blood cells (WBC) and they were calculated using Hayem and Turk solutions. Hemoglobin (Hb) was determined by using cyanmethemoglobin method.

Statistical analysis

SPSS was used to examine the data (version 16, Chicago, IL, USA). First, the Kolmogorov-Smirnov and Levene's test was used for normality and homogeneity of variances. Two-way analysis of variance was used to assess all of the data (ANOVA). The differences between the eight treatments were evaluated using Duncan's multiple-range tests. The data were presented as means \pm SD.

Results

Growth performance

Table 2 displays the beluga sturgeon's growth performance after being given the

test diets. Dietary fat and thiamin levels had no discernible interaction effect on the beluga sturgeon's FW, WG, SGR, FCR, or survival rate ($p>0.05$). Fat had a significant effect on growth performance and in comparison, to low-fat diets, high-fat diets substantially increased FW, WG, and SGR ($p<0.05$). Different thiamin levels had notable differences in growth performance in low and high-fat diets and growth significantly were higher in fish fed with thiamine compared to control group ($p<0.05$).

Table 2: Growth performance of beluga juveniles fed with experimental diets.

Growth index	Initial weight (g)	Final weight (g)	Weight gain (g)	SGR	FCR	Survival rate
HF	46.6 \pm 1.7	190.8 \pm 0.9 ^c	157.8 \pm 22.5 ^c	2.3 \pm 0.1 ^b	1.57 \pm 0.05 ^a	100
HF-7B1	45.5 \pm 0.3	217.0 \pm 4.7 ^b	171.4 \pm 14.1 ^b	2.6 \pm 0.07 ^a	1.30 \pm 0.04 ^b	100
HF-15B1	45.9 \pm 2.9	233.6 \pm 3.5 ^a	187.7 \pm 6.9 ^a	2.71 \pm 0.09 ^a	1.18 \pm 0.05 ^{bc}	100
HF-25B1	42.2 \pm 1.5	227.3 \pm 4.5 ^{ab}	185.1 \pm 3.6 ^{ab}	2.69 \pm 0.04 ^a	1.11 \pm 0.05 ^c	100
LF	43.5 \pm 1.3	180.8 \pm 0.4 ^c	134.2 \pm 10.2 ^c	2.2 \pm 0.0 ^b	1.6 \pm 0.04 ^a	100
LF-7B1	45.7 \pm 2.3	212.7 \pm 3.4 ^b	167.1 \pm 5.7 ^b	2.5 \pm 0.1 ^b	1.3 \pm 0.02 ^b	100
LF-15B1	45.1 \pm 1.6	213.5 \pm 3.4 ^b	167.6 \pm 9.0 ^b	2.5 \pm 0.1 ^b	1.3 \pm 0.04 ^b	100
LF-25B1	44.8 \pm 1.6	217.3 \pm 0.7 ^b	172.1 \pm 1.1 ^b	2.6 \pm 0.0 ^a	1.1 \pm 0.005 ^c	100
<i>p</i> -value fat	-	*	**	ns	*	-
<i>p</i> -value thiamine	-	**	**	*	**	-
<i>p</i> -value fat \times thiamine	-	ns	ns	ns	ns	-

180 g/kg fat (High-fat, HF), 180 g/kg fat+7 mg/kg thiamin (HF-7B1), 180 g/kg fat+15 mg/kg thiamin (HF-15B1), 180 g/kg fat+25 mg/kg thiamin (HF-25B1), 90 g/kg fat (Low Fat, LF), 90 g/kg fat+7 mg/kg thiamin (LF-7B1), 90 g/kg fat+15 mg/kg thiamin (LF-15B1), 90 g/kg fat+25 mg/kg thiamin (LF-25B). Means with different superscripts indicate significant differences between treatments ($p<0.05$).

Body composition

The results related to the body composition of beluga fed with experimental diets were presented in Table 3. The interaction effect of fat and thiamine on body composition was not significant ($p<0.05$). The effect of fat on protein is significant and beluga fed with high-fat diet (HF, HF-7B1, HF-15B1,

and HF-25B1) have a higher protein content than beluga fed with low-fat diet (LF, LF-7B1, LF-15B1, and LF-25B1). No significant effect was observed between the treatments in fat, ash, and moisture.

Hematology

Experimental diets had no significant effect on RBC. There were significant differences in WBC, Hb between treatments and the

highest level was obtained in HF-15B1 and HF-25B1 ($p<0.05$, Table 4).

Table 3: Body composition of beluga juveniles fed with experimental diets.

Body composition	Crude protein (%)	Crude fat (%)	Ash (%)	Moisture (%)
HF	13.9 ± 0.8 ^a	11.4 ± 1.4	2.5 ± 0.5	73.1 ± 3.1
HF-7B1	14.0 ± 0.8 ^a	11.8 ± 1.1	2.3 ± 0.6	72.4 ± 2.5
HF-15B1	14.7 ± 0.4 ^a	11.1 ± 1.3	2.6 ± 0.4	72.48 ± 1.7
HF-25B1	14.5 ± 1.2 ^a	11.9 ± 1.1	2.4 ± 0.3	72.16 ± 1.4
LF	11.5 ± 0.02 ^b	12.5 ± 1.2	2.1 ± 0.2	72.8 ± 0.3
LF-7B1	12.8 ± 0.04 ^b	11.04 ± 2.2	2.8 ± 0.3	73.9 ± 0.4
LF-15B1	13.7 ± 0.04 ^b	11.8 ± 1.3	2.9 ± 0.6	73.8 ± 0.1
LF-25B1	12.4 ± 0.04 ^b	11.5 ± 1.3	2.7 ± 0.5	72.35 ± 0.5
<i>p</i> -value fat	*	ns	ns	ns
<i>p</i> -value thiamine	ns	ns	ns	ns
<i>p</i> -value fat × thiamine	ns	ns	ns	ns

180 g/kg fat (High-fat, HF), 180 g/kg fat, 7 mg/kg thiamin (HF-7B1), 180 g/kg fat, 15 mg/kg thiamin (HF-15B1), 180 g/kg fat, 25 mg/kg thiamin (HF-25B1), 90 g/kg fat (Low Fat, LF), 90 g/kg fat, 7 mg/kg thiamin (LF-7B1), 90 g/kg fat, 15 mg/kg thiamin (LF-15B1), 90 g/kg fat, 25 mg/kg thiamin (LF-25B). Means with different superscripts indicate significant differences between treatments ($p<0.05$).

Table 4: Hematological indices of beluga sturgeon juveniles fed with experimental diets.

Hematology indices/treatments	RBC (10^4 /ml)	WBC (10^3 /ml)	Hb (g/dL)
HF	61.5 ± 9.5	15.7 ± 0.94 ^c	1.8 ± 0.1 ^c
HF-7B1	60.5 ± 5.8	19.8 ± 1.2 ^b	2.9 ± 0.2 ^b
HF-15B1	64.0 ± 7.6	27.4 ± 3.1 ^a	3.5 ± 0.2 ^a
HF-25B1	63.3 ± 3.0	28.7 ± 1.9 ^a	2.4 ± 0.2 ^{bc}
LF	56.6 ± 5.5	14.8 ± 1.6 ^c	2.1 ± 0.8 ^c
LF-7B1	55.1 ± 4.5	10.9 ± 0.9 ^c	2.3 ± 0.0 ^{bc}
LF-15B1	52.5 ± 2.5	11.3 ± 0.9 ^c	2.5 ± 0.3 ^{bc}
LF-25B1	59.66 ± 3.0	10.9 ± 2.3 ^c	2.5 ± 0.5 ^{bc}
<i>p</i> -value fat	ns	**	**
<i>p</i> -value thiamine	ns	**	**
<i>p</i> -value fat × thiamine	ns	**	**

180 g/kg fat (High-fat, HF), 180 g/kg fat+7 mg/kg thiamin (HF-7B1), 180 g/kg fat+15 mg/kg thiamin (HF-15B1), 180 g/kg fat+25 mg/kg thiamin (HF-25B1), 90 g/kg fat (Low Fat, LF), 90 g/kg fat+7 mg/kg thiamin (LF-7B1), 90 g/kg fat+15 mg/kg thiamin (LF-15B1), 90 g/kg fat+25 mg/kg thiamin (LF-25B). Different lowercase letters indicate significant interaction among the groups ($p<0.05$).

Discussion

Vitamin B1 is a fundamental supplement for different species and plays a critical role in development, physiology, and metabolism (Lonsdale, 2006; Zhao *et al.*, 2020). In the present study, significant effects were observed between treatments on FW, WG, SGR, and FCR of beluga

sturgeon but the growth performance of beluga sturgeon improved by increasing dietary vitamin B1 levels in both low and high-fat diets. It has been reported that dietary vitamin B1 improves growth performance for various fish species like starlet sturgeon (*Acipenser ruthenus*), Indian major carp (*Cirrhinus mrigala*),

golden pompano (*Trachinotus ovatus*) yellow catfish (*Pelteobagrus fulvidraco*) (Ghiasi *et al.*, 2017; Zehra and Khan, 2017; Xun *et al.*, 2019; Zhao *et al.*, 2020). An increasing the FW, WG, and SGR of Beluga sturgeon by increasing vitamin B1 indicates that the addition of vitamin B1 is necessary for the diet of Beluga sturgeon. An improvement in growth performance with an increased in dietary vitamin B1 may be related to increasing feed utilization (Xiang *et al.*, 2016). In this study, fish fed with high-fat diets had appropriate growth. Similar to the results, high dietary fat levels improved the growth performance of Persian sturgeon (*Acipenser persicus*) and beluga sturgeon (Keramat Amirkolaie *et al.*, 2012; Najafi *et al.*, 2017). High-fat diets enhance dietary energy levels which may reduce both feed consumption and total protein intake and eventually fish growth (Wang *et al.*, 2005). Some species such as beluga can use fat levels of 24 % (Najafi *et al.*, 2017), as obtained by a better growth performance of the fish-fed HF diets in this study.

In the study, the interaction effect of fat and thiamine on body composition was not significant, however, the effect of fat on protein was significant and beluga fed with high-fat diets had a higher protein content than those fed with lowfat diets. Similar results were reported in bester by Mohseni *et al.* (2007) and carcass protein content was lower in fish fed with the low-fat diet than fish fed with the high-fat diet. It seems that in fishes that feed with the low-fat diets, due to the lack of fat, after fat in the body is used up as the first and most important source of energy, it is time to turn to other sources of energy, and then the

energy needed by the body through exposure protein is provided in the metabolic pathway, which has reduced the body's protein content and growth in these treatments (Mohseni *et al.*, 2007).

In fish, as in other animals, blood factors represent the physiological state and are mostly influenced by nutrients (Lim *et al.*, 2000; Garcia *et al.*, 2007; Ghiasi *et al.*, 2014). In the present study, the RBC, Hb, and WBC of beluga were highest for the fish fed the different thiamin levels and lowest for fish fed the unsupplemented diet. A similar result was observed in juvenile Jian carp, and Nile tilapia fed with different thiamin levels (Lim *et al.*, 2007; Feng *et al.*, 2011). Thiamine treatment was effectively prevented lead-induced reduction of red blood cells in *Tilapia zillii* (Ghazaly, 1991). Benfey and Biron (2000) showed that the number of red blood cells in fish may affect oxygen exchange and oxygen transport capacity, causing stress and making fish susceptible to diseases under certain conditions. Therefore, maintaining normal blood factors may be important for fish immunity. In this study, Hb was significantly lower in the control group compared to the supplemented groups with thiamin. It was shown that thiamine deficiency reasoned a decrease in feed intake, which led to an insufficient supply of nutrients and thus a decrease in hemoglobin (Ketola *et al.*, 2008; Ghiasi *et al.*, 2017). In contrast, a study on juvenile rainbow trout (*Salmo gairdneri*) showed that diets with varying levels of thiamine had no significant effect on hemoglobin levels (Morito *et al.*, 1986). Former researches have shown that thiamin can be stored in hematopoietic tissues (Ketola *et*

al., 2008; Ghiasi *et al.*, 2017); and in the present study, thiamine significantly increased Hb. This result suggested that thiamine deficiency in hematopoietic tissues leads to a decrease in the amount of hemoglobin in the control group compared to the supplemented groups and appears as a type of anemia caused by thiamine deficiency in the control group.

Conclusion

In conclusion, the results of this study demonstrated that diets containing thiamin in both low and high-fat diets improved growth performance, hematology and body composition of beluga sturgeon.

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Conflicts of interest

There are no conflicts to declare.

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