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Dietary protein levels may affect the growth and physiological performance of Beluga sturgeon, *Huso huso* (Linnaeus, 1758) under wintering conditions

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ABSTRACT
Fish in an optimum temperature range seem to have different nutritional requirements compared to optimal conditions. To evaluate the effect of different dietary protein levels during winter feeding of Beluga sturgeon, *Huso huso* (334.1 ± 0.3 g initial body weight; mean ± SD) a feeding trial with 8 weeks duration was conducted. Four diets with three replicates were formulated containing different dietary protein levels of 32%, 36%, 40% and 44% with 14.5% crude fat at low temperature of 8.8 ± 2.0 °C in twelve circular concrete tanks (900 L volume; 5 fish per tank). Fish were fed 1% of body weight three times daily, and at the end of the experiment, fish were weighed and sampled for plasma biochemical indices. The results have shown that various dietary protein levels had no noteworthy effect on growth indices ($p > 0.05$). The highest numerical, but not significant, final weight, specific growth rate, protein and lipid efficiency ratios were found in diets comprising 36% protein. No mortality was recorded throughout the trial. The results of biochemical plasma parameters showed that triglyceride, cholesterol, and total protein were not influenced by dietary protein levels ($p > 0.05$), while the glucose in fish fed 44% protein was significantly lower than the other treatments ($p < 0.05$). Hematocrit was significantly increased by increasing dietary protein levels, and the highest value was observed in the 44% treatment ($p < 0.05$). Based on the findings, it is advised that a protein level of 36% would be beneficial for optimal growth and physiological conditions in winter feeding of juvenile Beluga sturgeon.

Keywords: Biochemical plasma indices, Growth performance, Low temperature, Protein, Sturgeon.
Article type: Research Article.

INTRODUCTION
Sturgeons are the oldest of the freshwater fishes, which have lived in large areas of the northern hemisphere since the Cretaceous period (Bemis 1997). These species have a high commercial value due to precious caviar and boneless meat (Carmona *et al.* 2009). Many sturgeon populations have decreased rapidly due to the high pressure of overfishing, blockage of spawning migration routes and habitat degradation, and nowadays sturgeons are among the most endangered freshwater fish (IUCN 2021). Among sturgeons, Beluga sturgeon *Huso huso* is a worthy candidate for sturgeon rearing because of its relatively high growth rate, possibility of captive reproduction, supply of larvae and juveniles, high resistance to unfavorable conditions and a valuable source of caviar (Falahatkar *et al.* 2013). Water temperature as an important abiotic parameter can affect feed consumption, growth, metabolism, and nutrients digestion and absorption of aquatic animals (Guerreiro *et al.* 2014; Hosseini Shekarabi *et al.* 2022). Previous research has shown that physiological processes are reduced when water temperature is below the optimum range, leading to decreased feed intake, decreased swimming activity, defective immune response, and ultimately weakness and reduced growth (Yoo & Lee 2016; Abd El-Wahab *et al.* 2020;

Liang *et al.* 2022). The results on sturgeons revealed the effect of low water temperature on growth and feed utilization of Russian sturgeon, *Acipenser gueldenstaedtii* (Celikkale *et al.* 2002), shovelnose sturgeon *Scaphirhynchus platorynchus* (Kappenman *et al.* 2009), and Beluga sturgeon (Falahatkar *et al.* 2013). The optimum temperature range for the growth of Beluga sturgeon is 18 to 22 °C (Falahatkar *et al.* 2016). However, Beluga sturgeon in outdoor culture systems is affected by seasonal changes in water temperature from < 10 °C during the winter season to > 28 °C during the summer season. Due to the annual temperature fluctuations in many fish farms that use natural water resources and are under environmental conditions, determining the nutritional requirements of fish in various conditions including winter seems necessary. Protein quantity and quality greatly affect feed cost, which is why protein is so important in aqua feeds. Given that dietary protein exceeding the amount needed for growth will simply be broken down (Cowey,1994) and protein insufficiency leads to feed inefficiency and reduced growth, so the protein content in the diet should be carefully adjusted. Therefore, for the optimal use of protein in tissue synthesis instead of energy expenditures, sufficient information is needed to understand the impact of environmental cues such as water temperature on protein utilization. Dietary protein requirement is related to various features such as water temperature, size of fish, feeding frequency, quantity of non-protein sources, and quality of protein sources (Shabihul & Imtiaz 2020). The proper level of protein is not the same for different species and under different conditions, especially in winter conditions (Hung, 2017), where low temperatures can create a special situation both in terms of energy expenditure and digestion and absorption of food (Bogevik *et al.* 2010). Using a balanced and appropriate diet plays a crucial role in supplying the nutritional requirements of aquatic animals and achieving success in their culture under different conditions. Being carnivorous, protein is an important and expensive nutrient in the diet that plays a key role in the growth and quality of meat produced. Therefore, to ensure optimal growth, protein should be sufficient in the diet. Adequate energy intake from lipids and carbohydrates can greatly reduce the use of protein sources as an energy resource (Mohseni *et al.* 2011). Conversely, if there are not enough dietary non-protein energy sources, protein can be utilized to provide energy, which is not economically viable (Brendan *et al.* 1988). In case of high protein consumption, instead of protein being used for growth and increasing fish weight, it is used to produce energy, which increases production costs due to the high price of protein sources. On the other hand, waste products such as ammonia can increase, which increases the load of environmental pollution and reduces water quality (Tibbetts *et al.* 2000). Increasing non-protein energy sources to meet the fish requirements leads to a decreased amino acids oxidation and thus increases dietary protein consumption for growth and decreases nitrogen excretion (De Silva & Gunasekera 1991). In fish, blood features are sensitive to exogenous and endogenous factors such as those caused by thermal stress, diseases and alterations of dietary nutrients (Hassan *et al.* 2019; Imtiaz *et al.* Fazio 2020). Therefore, hematological analyses are essential tools for the evaluation of fish health (Řehulka *et al.* 2004). Fish species have the maximum growth rate in an ideal temperature range (Oyugi *et al.* 2011). Any changes in the optimal water temperature have a direct impact on some physiological procedures and behavior activities (Jonassen *et al.* 2000; Sarma *et al.* 2010), which are detectable in hemato-biochemical parameters. Due to thermal stress, temperatures beyond the optimal levels have a negative impact on oxygen consumption, metabolic rate, physiological performance and the health status of aquatic species (Divya & Ranjeet 2014). To date, no data are available regarding the appropriate dietary protein level for the best performance of Beluga sturgeon under low water temperatures in the winter season. Therefore, the aim of the current study was to determine the effect of dietary protein levels on growth performance and biochemical plasma indices of sub-yearling Beluga sturgeon throughout the winter season. By conducting such a study, it is possible to obtain a basic understanding of a suitable diet for sturgeon farming in different conditions and the production of fish with cost-effective feed.

MATERIALS AND METHODS

Fish and rearing conditions

Sixty 10-month-old Beluga sturgeon (mean weight 334.1 ± 29.8 g; and mean total length 43.6 ± 1.3 cm) obtained from controlled reproduction of farmed broodstock in Shahid Dr. Beheshti Sturgeon Restocking and Genetic Conservation Center (Rasht, Guilan, Iran), after adaptation to manual feed and rearing conditions, were distributed into the 12 concrete tanks (185 cm in diameter, 30 cm in depth, and 900-L water volume) each with 5 fish per tank. During the experimental period, dissolved oxygen, water temperature, and pH were recorded as 8.8 ± 0.2 mg L^{-1}, 8.8 ± 2.0 °C, and 8.3 ± 0.1, respectively.

Experimental design

Four treatments with three replicates containing protein levels of 32, 36, 40 and 44% were considered for the present study. Ingredients and proximate analysis of all diets have shown in Table 1. To make the feeds, different ingredients were weighed after sieving with a 500 µ mesh according to the intended formulation and then mixed well. Afterward, oil sources and the proper quantity of water (≈ 25%) were added to create stiff dough and finally the prepared mixture was poured into a meat grinder and pellets with 3 mm diameter were made. Prepared diets were dried in a ventilated oven at 40 °C for 24 h, and kept at 4 °C. The animals were fed manually based on appetite three times daily at 9:00, 13:00 and 17:00 for 8 weeks.

Table1. Ingredients and proximate composition of experimental diets of Beluga sturgeon, *Huso huso* in the present study.

	Protein level (%)			
Ingredients (%)	**32**	**36**	**40**	**44**
Fish meal	28.00	34.47	41.02	47.68
Soybean meal	15.04	16.00	17.00	18.00
Wheat meal	18.97	14.00	10.00	7.00
Wheat bran	18.00	16.07	13.07	8.99
Fish oil	4.45	4.18	3.90	3.62
Corn oil	4.45	4.18	3.90	3.62
Molasses	2.50	2.50	2.50	2.50
Vitamin premix*	2.00	2.00	2.00	2.00
Mineral premix**	1.50	1.50	1.50	1.50
Lecithin	2.00	2.00	2.00	2.00
Yeast	1.50	1.50	1.50	1.50
Methionine	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
DCP ($CaHPO_4$)	0.10	0.10	0.10	0.10
Proximate composition				
Moisture	7.62 ± 0.13	7.45 ± 0.28	7.51 ± 0.19	7.55 ± 0.43
Crude protein	31.93 ± 0.09	35.93 ± 0.07	40.10 ± 0.14	43.89 ± 0.25
Crude lipid	14.55 ± 0.11	14.18 ± 0.06	14.52 ± 0.08	14.49 ± 0.21
Total ash	8.79 ± 0.18	10.21 ± 0.23	11.40 ± 0.20	12.92 ± 0.12

Note: *vitamin A, 1600 IU g^{-1}; vitamin D_3, 40 IU g^{-1}; vitamin E, 40 mg g^{-1}; vitamin K_3, 2 mg g^{-1}; vitamin B_1 (thiamin mononitrate), 6 mg g^{-1}; vitamin B_2 (riboflavin), 8 mg g^{-1}; vitamin B_3, 12 mg g^{-1}; vitamin B_5, 40 mg g^{-1}; vitamin B_6 (pyridoxine hydrochloride), 4 mg g^{-1}; B_9 (pyridoxine hydrochloride), 2 mg g^{-1}; vitamin B_{12} (cyanocobalamin), 8 µg g^{-1}; vitamin C-stay, 60 mg g^{-1}; vitamin H_2, 240 µg g^{-1}; inositol, 20 mg g^{-1}; BHT, 200 µg g^{-1}.**manganese, 5 mg g^{-1}; copper, 6 mg g^{-1}; ferrous, 6 mg g^{-1}; zinc, 10 mg g^{-1}; selenium, 20 µg g^{-1}; iodine, 3 mg g^{-1}; cobalt, 100 µg g^{-1}; choline chloride, 6 mg g^{-1}.

Growth performance

At the initiation of the trial and every 2 weeks intervals all fish were individually weighed (with an accuracy of 1 g) and also total length of each fish was measured (with an accuracy of 1 mm). Feeding was ceased before any manipulation for 24 h and fish were anesthetized in 300 mg L^{-1} clove powder extract. Growth factors and feed utilization were calculated according to the following formulas:

Weight gain (WG; g) = final body weight (g) – initial body weight (g)

Body weight increase (BWI; %) = 100 × [final body weight (g) – initial body weight (g)] / initial body weight (g)

Specific growth rate (SGR; % day^{-1}) = 100 × [Ln final body weight (g) – Ln initial body weight (g)] / time (days)

Protein efficiency ratio (PER) = wet weight gain (g) / total protein intake (g)

Lipid efficiency ratio (LER) = wet weight gain (g) / total lipid intake (g)

Condition factor (CF) = 100 × [final body weight (g) / total body length $(cm)^3$]

Feed conversion ratio (FCR) = Feed intake (g) / weight gain (g)

Feed intake (FI; g $fish^{-1}$) = total feed intake (g) / total number of fish

Voluntary feed intake (VFI; % day^{-1}) = 100 × [feed intake (g) / days] × [2 / initial biomass (g) + final biomass (g)]

Survival rate (SR; %) = 100 × [final number of surviving fish / initial number of fish]

Blood collection and analyses

All handling and sampling were carried out based on the approved ethical approach (Bennett *et al.* 2016). At the end of the 8 weeks, three fish were randomly caught from each tank to measure hemato-biochemical parameters. Blood was taken from the caudal peduncle using a 5-mL heparinized syringe (18-G). Blood plasma was separated by a centrifuge (Universal, Tehran, Iran) at 1500 *g* for 10 min, transferred into the 1.5-mL tubes and preserved at

-20 °C for further analyses. Plasma triglyceride, cholesterol, glucose, and total protein concentrations were measured spectrophotometrically using standard kits (Pars Azmun, Karaj, Iran; Falahatkar 2015). The hematocrit (Hct) value was measured by centrifugation of the microhematocrit heparinized capillary tubes at 3500 *g* for 7 min (Falahatkar *et al.* 2006).

Statistical analysis

Variances homogeneity and data normality were tested by Levene and Kolmogorov-Smirnov tests, respectively. The data were examined using One-way analysis of variance. By observing a significant difference, Tukey's test was performed to compare treatments. All tests were accomplished by SPSS software (IBM SPSS Statistics V22, Armonk, USA). Differences between the means were considered significant at $p < 0.05$. All data are shown as mean ± standard deviation (SD) throughout the text.

RESULTS

The growth trends of Beluga sturgeon during the trial are shown in Fig. 1, which revealed no difference among treatments throughout the experimental period. The growth indices of fish fed with different protein levels for 8 weeks are presented in Table 2. Based on the results, growth-related indices including WG, BWI, SGR, PER, LER, CF, FCR, FI and VFI in the experimental fish were not significantly changed by different dietary protein levels ($p > 0.05$). However, the numerical highest and lowest WG, BWI and LER were found in fish fed with 36% and 32% protein levels, respectively.

There was no death or any signs of deficiency during the experiment. The Hct values were significantly increased when dietary protein increased (Fig. 2; $p < 0.05$), with the highest value in fish fed 44% protein level. The results of biochemical factors are shown in Table 3. The lowest amount of glucose was observed in the Beluga sturgeon fed diet 44% protein, which was significantly dissimilar from the control group and fish fed with 40% protein level ($p < 0.05$). Furthermore, no significant difference in triglyceride, cholesterol and total protein were found among fish fed different levels of dietary protein ($p > 0.05$).

Table 2. The effects of dietary protein on growth performance and feed utilization of Beluga sturgeon, *Huso huso* after 8 weeks' experimental period during the wintering at below 10 °C (mean ± SD).

	Protein level (%)			
	32	**36**	**40**	**44**
W_i (g)	334.4 ± 35.7	333.8 ± 27.1	334.2 ± 31.1	334.0 ± 27.7
W_f (g)	387.1 ± 44.8	405.5 ± 36.7	403.7 ± 55.9	401.2 ± 30.3
TL_i (cm)	43.6 ± 1.4	43.4 ± 1.3	43.7 ± 1.1	43.7 ± 1.5
TL_f (cm)	44.5 ± 1.4	44.9 ± 1.3	45.2 ± 1.1	44.7 ± 1.4
WG (g)	52.7 ± 14.2	71.7 ± 5.8	69.4 ± 7.9	67.0 ± 10.5
BWI (%)	15.7 ± 4.2	21.5 ± 1.8	20.8 ± 2.3	20.0 ± 3.1
SGR (% day^{-1})	0.3 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.1
PER	2.2 ± 0.5	2.6 ± 0. 2	2.3 ± 0.3	2.0 ± 0.3
LER	4.7 ± 1.2	6.7 ± 0.5	6.2 ± 0.8	6.1 ± 0.9
CF	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.1
FCR	1.5 ± 0.4	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.2
FI (g $fish^{-1}$)	75.8 ± 1.9	75.7 ± 0.9	75.4 ± 2.3	75.7 ± 1.0
VFI (% day^{-1})	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
SR (%)	100	100	100	100

W_i = Initial Weight, F_i = Final Weight, TL_i = Initial Total Length, TL_f = Final Total Length, WG = Weight Gain, BWI = Body Weight Increase, SGR = Specific Growth Rate, PER = Protein Efficiency Ratio, LER = Lipid Efficiency Ratio, CF = Condition Factor, FCR = Feed Conversion Ratio, FI = Feed Intake, VFI = Voluntary Feed Intake, SR= Survival Rate.

Table 3. The effects of dietary protein levels on biochemical plasma indicators of Beluga sturgeon, *Huso huso* after 8 weeks' experimental period during the wintering at below 10 °C (mean ± SD).

	Protein level (%)			
	32	**36**	**40**	**44**
Triglyceride (mg dL^{-1})	363.2 ± 47.9	295.7 ± 42.6	322.2 ± 65.2	350.9 ± 61.5
Cholesterol (mg dL^{-1})	75.3 ± 14.9	70.3 ± 21.1	67.4 ± 16.9	68.1 ± 14.2
Glucose (mg dL^{-1})	61.1 ± 7.4[a]	57.3 ± 5.8[ab]	61.7 ± 6.6[a]	50.0 ± 3.6[b]
Total protein (mg dL^{-1})	3.5 ± 0.3	3.6 ± 0.5	3.6 ± 0.3	3.4 ± 0.5

Note: Different superscripts show significant difference among the experimental treatments in each row ($p < 0.05$).

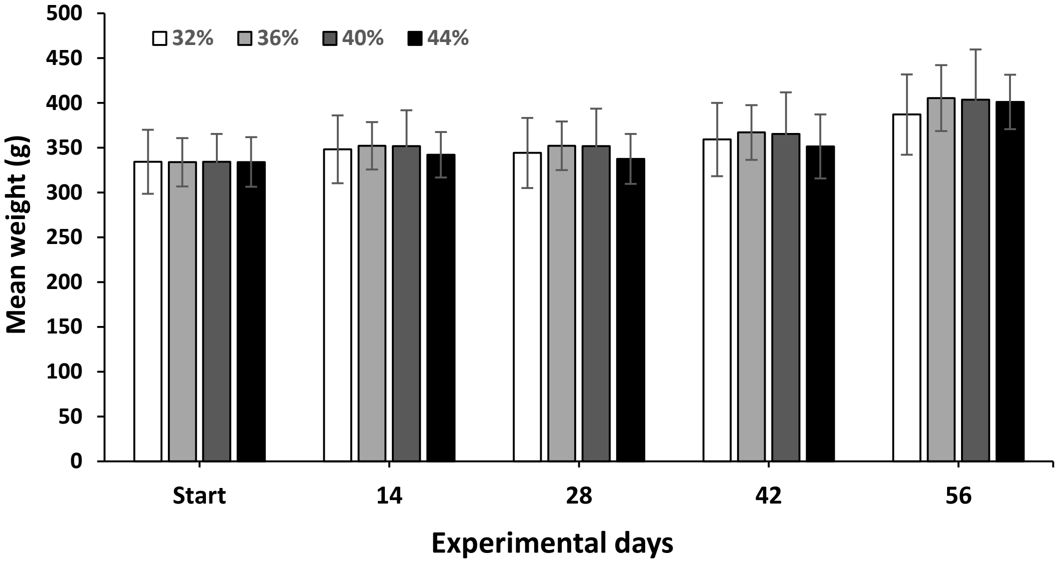

Fig. 1. The growth trends of Beluga sturgeon, *Huso huso* fed with different levels of dietary protein during the experimental period at below 10 °C (mean ± SD).

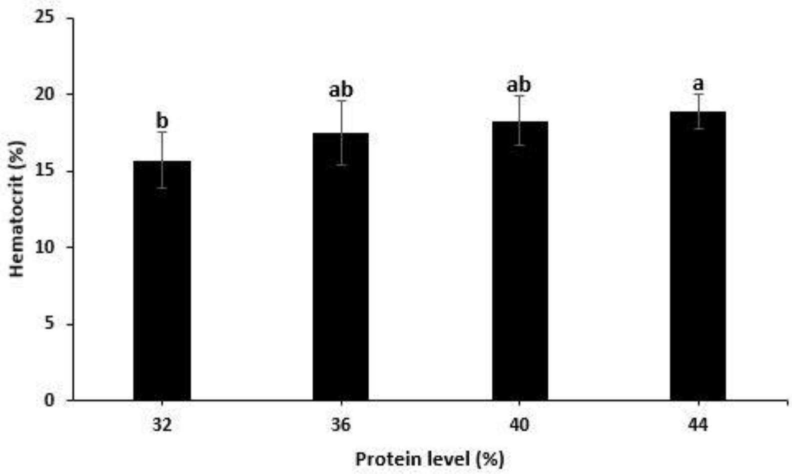

Fig. 2. The effects of dietary protein levels on hematocrit values of Beluga sturgeon, *Huso huso* after 8 weeks' experimental period during the wintering at below 10 °C **(**mean ± SD). Bars with different letters on columns represent significantly different values ($p < 0.05$).

DISCUSSION

The objective of the present study was to determine the optimal dietary protein level during winter feeding of Beluga sturgeon to achieve the best growth performance. The results showed that in fish fed diets comprising 40% and 44% protein, the growth indices were decreased compared to the group fed on a diet containing 36% protein. Likewise, Breden *et al.* (1988) showed that increasing the dietary protein to a certain extent (40-42%) leads to an increase in food efficiency and growth rate of white sturgeon (*Acipenser transmontanus*), however, adding more protein leads to a decrease in growth performance. It is possible that by raising the dietary protein level from 36% to higher than what the fish needs, the body faces the problem of breaking down amino acids caused by protein digestion. By increasing the dietary protein level, the amino acid-decomposing enzyme activities in the fish liver increases to be able to oxidize excess amino acids (Seyed Hasani *et al.* 2013; Yi & Xie 2022). It is demonstrated that oxidizing amino acids and removing nitrogen caused by deamination requires a lot of energy consumption, which reduces protein efficiency, energy, and ultimately the growth performance (Kaushik *et al.* 1994). Therefore, future study is recommended to find the possible changes in amino acid profiles when the fish fed various dietary protein levels. In our study on Beluga sturgeon, the numerical values of growth indices including final weight, WG, BWI, PER and LER in the treatment containing 36% protein were higher than the other protein levels.

Probably, in the sturgeon fed 36% dietary protein, more protein was spent on growth, and in fish fed 40% and 44% dietary protein, some of the protein departed into the energy production, resulting in decreased growth indices. By the rise in dietary protein level, the extra amino acids in food are not directly deposited in tissues and are used for energy production (Stone *et al.* 2003). Furthermore, other studies have suggested that diets with high protein can activate oxidative stress and inflammation-related pathways, making fish unhealthy and reducing digestive enzyme activities, including protease (Hassan *et al.* 2021; Gao *et al.* 2022). Fluctuations in water temperature can significantly influence the physiological functions of fish, including growth (Jobling, 1997), metabolism (Katersky & Carter 2007), feed intake, and swimming activity (Peres & Oliva-Tales 1999; Ibarz *et al.* 2007). Unfavorable water temperature, inadequate food, as well as low levels of some ingredients have been shown to inhibit fish growth (Fine *et al.* 1996). The results of our study indicated that a diet containing 36% protein can perform better than the other levels. Guo *et al.* (2012) reported a similar protein level (34-37%) during the optimal water temperature at 21.4 ± 0.6 °C for juvenile hybrid sturgeon (*Acipenser baerii* ♀ × *A. gueldenstaedtii* ♂). The main reason for insignificant effect of protein on growth could be that low temperatures during winter reduce the feeding rate (Lozano *et al.* 2003). Temperature acts as a warning and controlling aspect, making it the most effective factor among the various biotic and abiotic dynamics that can affect the growth rate of fish (Imsland *et al.* 2020). Previous studies have shown the direct impacts of water temperature on metabolic rate, feeding, growth and health status of fish (Person-Le Ruyet *et al.* 2004; Lall & Tibbetts 2009). Bailey & Alanara (2006) have shown that the appropriate water temperature range causes faster growth while lower temperatures cause slowness by reducing the digestion rate in fish. In the present study on Beluga sturgeon, the water temperature was 8.8 ± 2.0 °C, while this species grows faster at 18-22 °C (Falahatkar *et al.* 2016). By increasing water temperature, food intake increases in fish (Imsland *et al.* 2020). The effect of temperature on feeding varies according to species, however in general, when the temperature is outside the optimal range, it adversely affects food intake (Volkoff & Rønnestad 2020). Therefore, it is possible that in the current study, the low temperature may have caused a reduction in feed intake by juvenile Beluga sturgeon. Low temperatures in winter inhibit the appetite of young sturgeon (Jin *et al.* 2012); in other words, fish have relatively little feeding activity in winter due to reduced energy requirements at cooler water temperatures. In our study, it seems that the utilization of nutrients was lower than the other findings at higher temperatures, suggesting that winter conditions might influence nutrient utilization due to low temperature, which includes lower digestive enzyme activities, gut-transit time, digestibility, and absorption of nutrients (Miegel *et al.* 2010; Guerreiro *et al.* 2012; Falahatkar *et al.* 2016). At the end of the experiment, biochemical parameters showed that the increased dietary protein levels exhibit an insignificant outcome on the plasma concentrations of triglycerides, cholesterol and total protein. The blood biochemical parameters are highly variable, however, the alterations do not seem to be related to the protein level in the diet (Daniel & Gallagher 2008). The obtained findings are similar to the results of Kikuchi *et al.* (1994a, b, 1997) for Japanese flounder (*Paralychthis olivaceus*) and Falahatkar *et al.* (2016) for Beluga sturgeon. Two possible elucidations are: i) low water temperature can lead to a reduction in metabolic rate; ii) dietary protein levels were not very high or low to produce noteworthy effects on biochemical features. In addition, feed intake was not different between treatments. Consequently, it is also possible that the amount of energy reached the fish in all groups was the same and could not display an effect on the abovementioned parameters. Plasma glucose value is considered a profound index of environmental/rearing stress in various fish species (Jiang *et al.* 2017; Makaras *et al.* 2020). During the environmental stress, fish may experience substantial alterations in metabolism, however, glucose is a straight and effective energy source in fish (Li *et al.* 2018). Liver glycogen is considered the primary source of energy in sturgeons (Gillis & Ballantyne 1996), and in this way it provides the glucose needed for tissues and cells (Yarmohammadi *et al.* 2013). Due to the vital role of glucose in the body, glycogenolysis and gluconeogenesis control the plasma glucose level from non-carbohydrate precursors (Furné *et al.* 2012). Our findings revealed that by increasing dietary protein up to 44%, the blood glucose levels decreased significantly compared to the 32% dietary protein. Some species including rainbow trout (*Oncorhynchus mykiss*), European eel (*Anguilla anguilla*), *Rhamdia quelen* and Nile tilapia (*Oreochromis niloticus*) showed an increase in glucose level with an increase in dietary protein level (Lone *et al.* 1982; Suárez *et al.* 1995; Melo *et al.* 2006; Abdel Tawwab 2012). The activation of gluconeogenesis shows an increase in the glucose and glycogen levels from non-carbohydrate sources such as amino acids, glycerol and lactate (Cowey *et al.* 1977; French *et al.* 1981). Our experimental diets contain increasing levels of glucose by increasing the wheat meal and wheat bran supplementation, hence blood glucose to rise. In general, plant materials contain high amount of carbohydrates.

At present, there is no clear explanation for the blood glucose rise when fish fed the lowered protein diets, and the possible mechanism behind the phenomenon needs to be studied in the future. In addition to biochemical analysis, Hct was also measured to investigate the effect of dietary protein levels, which is known as a suitable tool to monitor fish health status (Lundstedt *et al.* 2004). Hematological parameters such as Hct are used to evaluate the oxygen carrying capacity in the bloodstream (Shah & Atindag 2004) as well as the physiological assessment of animals under unfavorable conditions (Fernandez & Mazon 2003). In the current study, Hct value increased significantly by increasing the dietary protein levels from 32% to 44%. Similar findings were observed in summer flounder *Paralichthys dentatus* (Daniel & Gallagher 2008) and common carp *Cyprinus carpio* (Khan & Maqbool 2017). It appears that raising the dietary protein level can boost the number of red blood cells and thereby Hct by affecting erythropoiesis. Contrary to these results, Rosenlund *et al.* (2004) reported that Hct and blood glucose in Atlantic cod (*Gadus morhua*) were not influenced by protein levels. Differences between the results of various studies may depend on factors such as diet composition, fish weight, digestion efficiency, species characteristics and water temperature. More studies related to the specific effect of proteins on hematological parameters in fish are needed.

CONCLUSION

Although some fish culturists believe that fish will not feed more during the winter season, fish can feed and also grow. So, it is important to know which kind of feed is needed during the season to reduce costs and also save the weight of fish. This study showed that taking into account the prevailing temperature conditions in this experiment, to achieve maximum growth or to prevent the weight loss of juvenile Beluga sturgeon in winter feeding, the diet containing 36% protein has the best performance. The findings of such study can be used to improve feeding and managing of farmed sturgeon during the winter season. However, additional studies are desired to approve these results and achieve a proper diet for optimum growth and physiological status. In the future, studies about the nutritional requirements of sturgeon in different seasonal conditions can provide new and valuable findings in different climates.

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