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Research Article

Effects of Dietary Thiamine Supplementation on Growth Performance, Digestive Enzymes' Activity, and Biochemical Parameters of Beluga, *Huso huso*, Larvae

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Thiamine is a crucial nutrient in larval stage of fish, and thus, the present study aimed at evaluating the effects of dietary thiamine supplementation on survival, growth performance, and biochemical parameters of beluga, *Huso huso*, larvae. The fish larvae (50 ± 4.5 mg) were fed diets containing 0 (control), 5, 10, and 20 mg/kg thiamine for 26 days from 18 days after hatching to 44 days after hatching. Thiamine significantly increased the larvae survival, growth rate, digestive enzymes activity, and whole body protein and ash but decreased the whole body lipid and moisture ($P < 0.001$). Also, alanine amino transferase (ALT) and alkaline phosphatase (ALP) were decreased significantly in thiamine-treated groups ($P < 0.05$). The highest survival and whole body ash were observed in 20 mg/kg thiamine treatment; the highest whole body protein and lipase activity were observed in 10 mg/kg thiamine treatment. The highest growth rate, amylase, and lowest whole body lipid, alanine amino transferase (ALT), and alkaline phosphatase (ALP) were observed in 10 and 20 mg/kg thiamine treatments. All thiamine-treated fish exhibited similar pepsin and chymotrypsin activities, all above the control fish. There were no significant effects of dietary thiamine supplementation on trypsin, whole body lysozyme, alternative complement (ACH50), lactate dehydrogenase (LDH) activities, and IgM levels. According to the results, 10–20 mg/kg dietary thiamine supplementation can increase the performance of beluga larvae by improving the health of liver and the activity of digestive system.

1. Introduction

The aquaculture sector is responsible for producing food for human consumption, and as such, this industry is rapidly expanding to meet the growing global demand for protein. However, one of the major challenges in aquaculture practices is the process of larviculture, as fish larvae are highly susceptible to adverse conditions, which can result in significant mortality rates and economic losses [1]. Therefore, the study on this life stage is crucial to improve the performance of fish larvae. Nutrition is very important in larval stage in fish, because the larvae have less ability for swimming and predation and their digestive system is still developing [2, 3]. Insufficient quantities or subpar quality of larvae feed can result in a rapid decline in the population. It is imperative to provide fish larvae with high-quality feed that is suitable for their developing digestive systems. Failure to do so can impede proper digestion, leading to a decrease in energy intake and potential gastrointestinal issues [4]. Fish larvae have thin skin and large surface area, compared to juveniles and adults; hence, they are more vulnerable to waterborne pathogens and need to have a well-functioning immune system [5]. Moreover, fish larvae have high metabolic and oxygen consumption rate, compared to the juveniles and adults [6], so they should have a strong antioxidant system to protect them from harmful pro-

oxidants. A well-formulated diet can optimize the performance of the larvae. Among the nutrients, thiamine deficiency has been found to cause serious problems and mortality in fish larvae [7–9]. Thiamine is an essential nutrient that cannot be synthetized by fish body, thus must be supplied via diet. Thiamine has various important physiological roles in animals [10]. In fish, thiamine deficiency has been reported to cause hampered antioxidant capacity, immune strength, digestive enzymes' activities, and growth rate [11–14]. Therefore, adequate thiamine supply can be crucial for fish larval stages, as mentioned previously.

Sturgeons are important fish species with huge global demand in aquaculture industry [15, 16]. Thiamine deficiency in these fish can reduce their performance at larval stage, as shown in sterlet sturgeon, *Acipenser ruthenus* [9]. They represented that thiamine deficiency caused reduction in feed intake which lead to insufficient supply of adequate nutrients. Also, diseases symptoms such as yolk sac deformation, irregular swimming, and loss of equilibrium were reported in that investigation. Beluga, *Huso huso*, is an important aquaculture candidate due to its higher growth rate and valuable meat and caviar [17]. Therefore, it is artificially propagated in different countries. The survival rate of sturgeon larvae during the initial feeding stage is significantly lower compared to many other freshwater farmed fish. Low fry survival is generally ascribed to fish husbandry practices, especially feeding practices. However, diet quality may also be a determinant of sturgeon fry survival [18, 19]. While there are numerous studies on beluga diet formulations (for example, Razeghi Mansour et al. [20]), limited research has been conducted on the nutritional needs of beluga larvae [21, 22]. As a result, it is crucial to improve the diets of sturgeon larvae with nutritional supplements, as doing so can have a positive impact on their health and survival. Considering the importance of dietary thiamine in larval stage of fish, the present study aimed at assessing the effects of dietary thiamine supplementation on growth performance, digestive enzymes activity, and biochemical/immunological parameters of beluga larvae.

2. Materials and Methods

2.1. Larvae Maintenance and Feeding Live Food. This study was conducted in International Sturgeon Research Institute, Rasht, Iran. The larvae of beluga were provided by fertilization of cultured male and female fish in that organization. Fertilized eggs were incubated in the McDonald incubator at constant water exchange of 0.13 L/s. After 7 days, the larvae hatched. Then, larvae were distributed in 12 fiberglass rectangular tanks ($200 \times 70 \times 30$ cm size) with a volume of 210 L of water and a water flow of 0.13 L/s to design 4 treatments as follows. Water was aerated constantly. The storage density was 250 larvae per tank. The larvae yolk sac absorption lasted 6 to 7 days. Semiactive feeding of larvae was started approximately one week posthatching when 80% of the larvae were actively swimming. The larvae were first fed with *Artemia nauplius* for 12 times a day with an interval of 2 hours. Then, Chironomidae and Artemia biomass were added to the larvae diet in addition to the *Artemia nauplius*. The larvae were fed until satiety.

2.2. Diets and Larviculture. From the 18th day after hatching, the live foods were gradually replaced by artificial diets. Two experimental diets were formulated as dough and pellet according to specifications outlined in Tables 1 and 2. The main sources of protein were casein and fish meal (the Caspian Sea sprat low-temperature processed). Fish oil and soybean oil were the main sources of lipids in the diets. To prepare experimental diets containing 5 (TS5), 10 (TS10), and 20 (TS20) mg thiamine per kg, thiamine hydrochloride (Sigma-Aldrich, Stuttgart, Germany; T4625, reagent grade, 99%) was added to other ingredients in amounts of 6.36, 12.7, and 25.4 mg/kg, respectively. These levels were selected based on the only study available on sturgeons [9, 23] and studies on fish species fed energy-dense diets that feed on high energy diets [14, 24]. A control diet without thiamine incorporation was also included (TS0). All dry ingredients were completely grounded by laboratory mill (Iran Damikar Co, Tehran, Iran) to about 100 μm. Thiamine-free vitamins and premixes of minerals were primarily mixed with wheat flour for 15 min and then added to other ingredients. Finally, all the dry ingredients were thoroughly mixed for 10 min by an electric mixer (Raybony, Garma Electric, Amol, Iran). The thiamine supplement was dissolved in water, and then, a blend of water and oils was added to the mixture, resulting in a paste. The prepared dough was divided into small circular pieces and placed in several parts of the rearing tank to feed the larvae.

Since 27th day after hatching, the pellet diet was gradually added to the larvae diet. From 29th after hatching until the end of the experiment (44th day after hatching), the larvae were just fed with the pellet diet, until apparent satiation. The prepared dough, which was formulated ingredients in Table 2, was transferred to a pelletizing machine (CPM, model CL series; USA) to generate 1-mm diameter feed. Eventually, the pellets were dried at 70°C (in an oven) for 18 h and stored at refrigerator to use.

During the whole experiment, water exchange was 10 L/min, and the physicochemical parameters of water were monitored as follows: oxygen: 7.45 ± 0.34 mg/L, pH: 7.2 ± 0.18, temperature: 17.5 ± 0.5°C.

2.3. Diet Thiamine Content. Thiamine content of the diets was measured by fluorometric method. Thiamine is oxidized to thiochrome, which is a fluorescent compound, by alkaline potassium ferricyanide. The thiochrome was extracted in isobutyl alcohol and measured in a fluorometer (Elico fluorometer) at 366 nm. The standard substance used in the fluorometer was made by dissolving 50 mg of thiamine hydrochloride in 500 ml of 0.1 N sulfuric acid containing 25% alcohol (100 μg/mL) [25].

Table 1: Composition (g/kg) of the four concentrated diets supplemented at different levels thiamine.

Ingredients	Dietary thiamine (g/kg)			
	0	5	10	20
Casein[1]	203	203	203	203
Wheat gluten[2]	330	330	330	330
Fish meal[3]	275	275	275	275
Dextrin[4]	50	50	50	50
Methionine[5]	8	8	8	8
Lysine[6]	7	7	7	7
Choline chloride[7]	2	2	2	2
Vitamin mix[8]	20	20	20	20
Mineral mix[9]	5	5	5	5
Fish oil	50	50	50	50
Soybean oil	50	50	50	50
Proximate composition analysis				
Moisture (g/kg)	103	100	105	104
Crude protein (g/kg DM basis)	631	628	633	637
Crude lipid (g/kg DM basis)	160	162	161	156
Crude ash (g/kg DM basis)	30	31	33	33
Gross energy (cal/kg)	4687	4680	4691	4681
Thiamine (mg/kg DM basis)	0.05	5.07	9.91	20.1

1 Vitamin free (Sigma Chemical Co., St. Louis, MO): protein 60%. 2 Protein 78%, lipid 1%. 3 Low-temperature processed fish meal. Protein 63%, lipid 15%. 4 United States Biochemical, Cleveland, OH, USA. 5 Evonik Industries (Germany): 99%. 6 Evonik Industries (Germany): 99%. 7 Liaoning Biochem Co. (China): 60%. 8 Vitamin premix: Alpha tocopherol 60 IU/kg; calciferol 3000 IU/kg; riboflavin 30 mg/kg; pyridoxine 15 mg/kg; B12 0.05 mg/kg; nicotinic acid 175 mg/kg; folic acid 5 mg/kg; ascorbic acid 500 mg/kg; inositol 1000 mg/kg; biotin 2.5 mg/kg; calcium pantothenate 50 mg/kg. 9 Mineral premix: calcium carbonate (40%) 2.15 g/kg; manganese oxide 1.24 g/kg; ferric citrate 0.2 g/kg; potassium iodide 0.4 g/kg; copper sulfate 0.3 g/kg; manganese sulfate 0.3 g/kg; calcium phosphate 5 g/kg; cobalt sulfate 2 g/kg; sodium selenite 3 g/kg; potassium chloride 0.9 g/kg; sodium chloride 0.4 g/kg.

Table 2: Composition (g/kg) of the four starter diets supplemented at different levels thiamine.

Ingredients	Dietary thiamine (g/kg)			
	0	5	10	20
Casein[1]	210	210	210	210
Wheat gluten[2]	250	250	250	250
Fish meal[3]	220	220	220	220
Dextrin[4]	187.4	187.4	187.4	187.4
Methionine[5]	8	8	8	8
Lysine[6]	7	7	7	7
Choline chloride[7]	2	2	2	2
Vitamin mix[8]	20	20	20	20
Mineral mix[9]	5	5	5	5
Fish oil	45	45	45	45
Soybean oil	45	45	45	45
L-carnitine	0.6	0.6	0.6	0.6
Proximate composition analysis				
Moisture (g/kg)	102	105	101	106
Crude protein (g/kg DM basis)	527	525	530	524
Crude lipid (g/kg DM basis)	139	140	141	144
Crude ash (g/kg DM basis)	35	33	31	36
Gross energy (cal/kg)	4570	4577	4574	4568
Thiamine (mg/kg DM basis)	0.03	4.95	10.4	19.1

1 Vitamin free (Sigma Chemical Co., St. Louis, MO): protein 60%. 2 Protein 78%, lipid 1%. 3 Low-temperature processed fish meal: protein 63%, lipid 15%. 4 United States Biochemical, Cleveland, OH, USA. 5 Evonik Industries (Germany): 99%. 6 Evonik Industries (Germany): 99%. 7 Liaoning Biochem Co. (China): 60%. 8 Vitamin premix: alpha tocopherol 60 IU/kg; calciferol 3000 IU/kg; riboflavin 30 mg/kg; pyridoxine 15 mg/kg; B12 0.05 mg/kg; nicotinic acid 175 mg/kg; folic acid 5 mg/kg; ascorbic acid 500 mg/kg; inositol 1000 mg/kg; biotin 2.5 mg/kg; calcium pantothenate 50 mg/kg. 9 Mineral premix: calcium carbonate (40%) 2.15 g/kg; manganese oxide 1.24 g/kg; ferric citrate 0.2 g/kg; potassium iodide 0.4 g/kg; copper sulfate 0.3 g/kg; manganese sulfate 0.3 g/kg; calcium phosphate 5 g/kg; cobalt sulfate 2 g/kg; sodium selenite 3 g/kg; potassium chloride 0.9 g/kg; sodium chloride 0.4 g/kg.

2.4. Sampling, Survival, and Growth Calculation of Larvae. At the end of the experiment (44th day posthatching), the number of larvae in each tank was counted to calculate the survival rate. Then, twelve larvae were randomly sampled from each tank and anesthetized at 400 mg/L clove powder extract and sacrificed by spinal cord transection. Following formula was used to calculate growth and survival rates:

$$\begin{aligned}
&\text{PWG (percent weight gain): } 100 \times \frac{(\text{final weight} - \text{initial weight})}{\text{initial weight}},\\
&\text{SGR (specific growth rate): } 100 \times \frac{[(\text{Ln (final weight)} - \text{Ln (initial weight)}]}{\text{da ys}},\\
&\text{survival percentage: } 100 \times \left(\frac{\text{survived fish}}{\text{initial fish}}\right).
\end{aligned} \tag{1}$$

2.5. Sampling Arrangement for Enzymatic and Biochemical Assay. All of the parameters were measured at the end of the experiment (third sampling) in four thiamine concentrations (TS0, TS5, TS10, and TS20) except digestive enzymes. Digestive enzymatic analysis was performed in three times during the experiment to assay the larvae digestive development trend. The three sampling times were performed as follows: first sampling (FS): when the larvae weight was 34 mg (1 day after semiactive feeding or 8 days after hatching), second sampling (SS): when the larvae weight was 50 mg (10 days after semiactive feeding or 18 days after hatching), and third sampling (TS): when the larvae weight was 3 g (26 days after semiactive feeding or 44 days after hatching). As the larvae were fed with artificial diets from 18th until 44th day after hatching, third sampling was divided into four treatments: feeding with diets contain 0, 5, 10, and 20 mg thiamine per kg which were, respectively, called TS0, TS5, TS10, and TS20.

2.6. Preparation of Larvae Extract. To prepare the larvae extract, four out of twelve larvae which collected per tank were homogenized in an electric homogenizer to provide sufficient tissue for determination of required indices [26]. For enzymatic extraction, 2 gr of the homogenized tissue was separated while the rest was used to determine the proximate composition.

2.7. Diet and larvae Proximate Composition. Kjeldahl method was used to measure the protein of diet and larvae, by determining the amount of nitrogen content ($N \times 6.25$) through digestion in strong acid, distillation, and titration. Soxhlet extraction method was used to determine the lipid content of diet and larvae. The moisture content of the diet and larvae was measured by drying the samples in an oven at 105°C for 24 hours to reach a constant weight. To measure the diet and larvae ash content, the samples were combusted at 550°C in a furnace for 3 h [27].

2.8. Enzymatic Analysis. The homogenized tissues of each sampling were mixed at a ratio of 1 : 5 with 50 mM phosphate buffer for 1.5 minutes at 4°C, pH 7.4. The homogenates were centrifuged at 11200 g for 20 minutes at 4°C. Then, the supernatant was removed and was used for the analysis. Total soluble protein was calculated by the Bradford method using bovine serum albumin as a standard [28]. Lactate dehydrogenase (LDH) enzyme activity was calculated using Pars Azmoun quantitative assay kit (product code: 122400-Karaj, Iran) by colorimetric method using pyruvate substrate at a wavelength of 340 nm for 3 minutes. Alanine aminotransferase (ALT) activity was measured using Pars Azmoun quantitative assay kit (product code: 119400-Karaj, Iran) by colorimetric method using *L*-alanine and 2-oxoglutarate substrates at a wavelength of 340 nm for 3 minutes. Alkaline phosphatase (ALP) activity was determined using Pars Azmoun quantitative assay kit (product code: 102400-Karaj, Iran) by colorimetric method using para-nitrophenyl phosphate substrate at a wavelength of 450 nm for 3 minutes [29].

Zinc sulfate precipitation method was used to measure IgM [30]. Lysozyme activity was measured by turbidimetric assay based on Hoseini et al. [31]. Alternative complement (ACH50) activity was measured based on the sheep red blood cell hemolysis method [31].

The activity of amylase was measured using the method described by Bernfeld [32] using soluble starch as the substrate. The increase in reducing power of buffered starch solution was measured with 3–5 dinitro salicylic acid (DNS) at 540 nm. Lipase activity was determined spectrophotometrically by hydrolysis of p-nitrophenyl myristate at 37°C and wavelength 580 nm [33]. The activity of trypsin was calculated at 410 nm wavelength using DL-arginine-*p*-nitroanilide as substrate [34]. Chymotrypsin activity was evaluated spectrophotometrically by monitoring the hydrolysis of *N*-benzoyl-*L*-tyrosine ethyl ester (BTEE) [35]. Absorbance was measured at 256 nm for 20 min at 1-min intervals. Determination of the pepsin activity was performed using 2% hemoglobin solution (in 0.3 N HCl at pH 2.0) at 280 nm. One unit of pepsin activity was equivalent to μg tyrosine per mL in one min [36].

2.9. Statistical Analysis. All data of the experiment were subjected to one-way analysis of variance (ANOVA). The activity of digestive enzymes was calculated among three

times of sampling to estimate the effect of larvae stage and also among four thiamine concentrations. Duncan's test was used to compare the mean values among individual treatments. The level of significant difference was set at $P \leq 0.05$. Statistical analysis was carried out using the SPSS v.19 software, and the results are presented as mean ± SD.

3. Results

3.1. Growth Performance and Survival. According to Table 3, diets containing different levels of thiamine led to the improvement of the growth performance and survival of beluga larvae, so that with the increase in thiamine concentration, the growth indicators including final weight, weight gain, and specific growth rates increased significantly compared to the control group ($P < 001$). Although there was no significant difference in these indicators in TS10 and TS20 treatments, the percentage of survival also increased significantly by increasing the concentration of thiamin in the diet from 0 to 20 mg/kg.

3.2. Whole Body Composition. Significant differences were noticed in terms of body composition levels in the experimental treatments ($P < 0.001$). Percentage moisture of the control larvae was significantly higher than the thiamine fed larvae. The body fat content was similar to the moisture status; however, it was significantly higher in TS5 treatment than TS10 and TS20 treatments. The protein content of fish fed thiamine increased considerably compared to the control group, while the highest amount was in the TS10 treatment. According to the results, the ash content of fish fed thiamine significantly increased compared to the control group, and the highest level was in the TS20 treatment (Table 4).

3.3. Whole Body Digestive Enzymes' Activity. Digestive enzyme (pepsin, trypsin, chymotrypsin, lipase, and amylase) activities of three feeding stages beluga were compared, initially. Then, their activities assessed in fingerlings after feeding thiamine-supplemented diets (Figure 1).

3.4. Before Receiving Thiamine. Pepsin, lipase, and amylase activities increased significantly with beluga growth and development, which was the highest at TS ($P < 0.001$). According to the results, trypsin activity increased significantly in SS compared to the FS and then decreased significantly in TS stage. The activity of chymotrypsin in different stages of beluga nutritional development was completely opposite to that of trypsin, and the highest level of chymotrypsin activity was evaluated in TS.

3.5. After Receiving Thiamine. Results show that the activity of digestive enzymes was significantly increased in the fish fed with thiamine supplemented artificial diets (TS5-20) in comparison with the fish fed artificial diets without thiamine supplementation (TS0). However, thiamine had no effect on the trypsin activity. The activity of pepsin and chymotrypsin of fish fed with TS5-20 was the same, and they were significantly higher than the TS0 treatment ($P < 0.001$). The highest activity of lipase was estimated in TS10 ($P < 0.031$). Amylase activity of fish fed TS10-20 was significantly higher than that of in TS0 and TS5 treatments, while the lowest one was in TS0 treatment ($P < 0.001$).

3.6. Whole Body Biochemical Parameters. According to Table 5, there were no differences among the ALP activities of TS5-TS20 treatments, and they were significantly lower than the TS0 treatment ($P < 0.013$). Adding 10 and 20 mg/kg thiamine to the diets caused a significant reduction in ALT activity compared to the TS0 treatment ($P < 0.027$); however, there was no difference between TS5 and TS0. Dietary thiamine supplementation had no effect on LDH, IgM, ACH50, and lysozyme.

4. Discussion

The activity and profile of digestive enzymes undergo changes throughout the ontogeny and development of fish larvae. In sturgeons, it has been demonstrated that these enzymes are detectable after hatching, aiding the larvae in utilizing yolk nutrients [3, 37]. Pepsin, trypsin, and chymotrypsin are main proteolytic enzymes that involve in fish digestive tract [38]. The sturgeon larvae have been observed to transition from alkaline protein digestion, facilitated by trypsin and chymotrypsin to acidic protein digestion facilitated by pepsin, during early ontogeny. This shift occurs as the larvae transition from endogenous feeding to exogenous feeding and from live food to formulated diets [3, 19]. Accordingly, the increase in pepsin activity from FS to TS can be due to the development of gastric gland as suggested by Babaei et al. [3] and Asgari et al. [19]. The increase in chymotrypsin activity at TS is in line with Asgari et al. [19], in this species, but opposite to findings in other sturgeons such as Persian sturgeon, *Acipenser persicus* Babaei et al. [3] and stellate sturgeon, *Acipenser stellatus* [39]. The observed variations may be specific to the species and highlight the significance of chymotrypsin (or chymotrypsin-like enzymes) in the protein digestion process of beluga. Furthermore, studies using transcriptomic and inhibitor tools can address this topic in beluga. The increase in lipase activity may be due to the importance of lipids in energy source to spare protein for growth performance, as previously observed in this species [19], as well as other sturgeons [3, 39]. Moreover, it may be due to increase in total daily fat intake, when the fish feed on the concentrated diets [40]. A previous study on beluga has shown that amylase activity increases immediately after exogenous feeding and during the early stage of cofeeding remains stable but shows an upsurge during full feeding on formulated diet [19]. Amylase activity showed similar pattern, and this might be increment in the diets carbohydrate of daily carbohydrate intake.

The improved growth rate in thiamine-treated fish is in line with previous studies on mrigal carp, *Cirrhinus mrigala* [41], Jian carp, *Cyprinus carpio* var. Jian [42], and grass carp, *Ctenopharyngodon idella* [43]. Such an improvement can be partly a consequence of increase in the digestive enzymes'

Table 3: Comparison of growth parameters in beluga fingerlings fed diets supplemented with thiamine.

	IW (g)	FW (g)	PWG (%)	SGR (%/d)	Survival (%)
TS0	0.49 ± 0.00	2.26 ± 0.13^{a}	356 ± 30.8^{a}	3.61 ± 0.16^{a}	46.4 ± 1.23^{d}
TS5	0.49 ± 0.00	2.63 ± 0.05^{b}	431 ± 4.73^{b}	3.97 ± 0.02^{b}	58.0 ± 0.04^{c}
TS10	0.50 ± 0.00	3.79 ± 0.15^{c}	658 ± 30.0^{c}	4.82 ± 0.09^{c}	65.8 ± 0.35^{b}
TS20	0.50 ± 0.00	3.55 ± 0.14^{c}	611 ± 29.0^{c}	4.66 ± 0.10^{c}	70.2 ± 0.11^{a}

Values are presented as (mean ± SD). Different letters indicate significant difference among groups ($n = 3$, $P < 0.001$). PWG (percent weight gain): 100 × (final weight – initial weight)/initial weight. SGR (specific growth rate): 100 × [(ln (final weight) – ln (initial weight)]/days. Survival percentage: 100 × (survived fish/initial fish).

Table 4: Comparison of whole body composition in beluga fingerlings fed diets supplemented with thiamine.

	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
TS0	75.1 ± 0.40^{c}	7.17 ± 0.09^{c}	12.4 ± 0.15^{a}	1.70 ± 0.04^{a}
TS5	74.1 ± 0.15^{b}	6.64 ± 0.32^{b}	13.2 ± 0.50^{b}	2.09 ± 0.12^{b}
TS10	73.3 ± 0.06^{a}	6.19 ± 0.02^{a}	14.5 ± 0.25^{d}	2.23 ± 0.08^{bc}
TS20	73.7 ± 0.30^{ab}	6.17 ± 0.02^{a}	14.1 ± 0.17^{c}	2.36 ± 0.09^{c}

Values are presented as (mean ± SD). Different letters show significant difference between pair groups at $P < 0.001$.

activity in the present study. The exact mechanisms of improvement in digestive enzymes' activity by thiamine are not known in fish; however, studies on mammals have demonstrated that thiamine is necessary for proper functioning of a certain region of the central nervous system that controls the gastrointestinal tract function [44]. A decline in hydrochloric acid secretion by the gastric glands has been reported as a symptom of thiamine deficiency in mammals [45, 46]. Therefore, it is speculated that the increase in pepsin activity in the treatments fed with thiamine may be due to the increase in hydrochloric acid secretion by the gastric glands and, as a result, the increase in the conversion rate of pepsinogen to pepsin. Moreover, the highest growth performance was accompanied by the highest amylase and/or lipase activities, suggesting a potential better carbohydrate and lipid digestion and absorption. Such elevations in these pancreatic enzymes (as well as chymotrypsin) can be due to proper functioning of the pancreatic cells. Supporting this, mammalian studies have revealed that pancreatic cells are the richest cells in the body [47], and thiamine deficiency leads to impaired dysfunction of these cells.

Thiamine supplementation increased protein content in the whole body, which can be due to higher protein synthesis. Increase in the whole body protein content after dietary thiamine supplementation has been reported in golden pompano, *Trachinotus ovatus* [14] and Nile tilapia, *Oreochromis niloticus* [48]. This can be another explanation for improved growth rate in the thiamine-treated fish. A previous study has shown that thiamine deficiency leads to lower growth rate parallel to lower RNA/DNA ratio in mrigal carp [41], which is an indicator of hampered protein synthesis. This can be due to stress in the endoplasmic reticulum that decreases protein synthesis, as suggested in human [49]. Unlike previous studies on other fish species, dietary thiamine supplementation significantly increased the whole body ash content in beluga, which its exact reason is not clear. Although thiamine is primarily known for its role as a coenzyme in energy metabolism and proper nervous system functioning in fish, recent studies suggest that it may also have a non-coenzyme role in bone structuring and strength. Research conducted on mice has supported this theory [50]. However, further evaluations are needed to determine the extent of thiamine's impact on bone health in fish.

ALT, ALP, and LDH are cytosolic enzymes and indicators of tissues' health [51]. There are limited studies on the effects of thiamine on tissues' health in fish. A study on common carp, *Cyprinus carpio*, has shown that thiamine can mitigate elevations in ALT and ALP, when the fish exposed to waterborne lead, suggesting potential hepatoprotective properties of thiamine [52]. Thiamine deficiency can decrease NADPH concentration, a radical scavenger, due to decrease in transketolase activity [53]. Moreover, thiamine deficiency can hamper antioxidant signaling pathways (Nrf2 and Keap1) [12]. So improvement in tissues' health can be due to boosted antioxidant capacity in the thiamine-treated beluga.

The innate immune system of fish has an important role in preventing the occurrence of diseases in fish [54]. Thiamine treatment increased fish survival rate, which is in line with previous studies showing thiamine deficiency is detrimental for fish early life stages [7–9]. This may be attributed to the higher metabolic rate during early life stages, which increases the demand for thiamine [14]. However, despite the higher survivability, no benefits of thiamine have been observed on immunological parameters. In contrast, thiamine has been found to improve various immune parameters in different fish species. For example, thiamine deficiency significantly decreased immune-related genes' expression, lysozyme, and complement activities in the gills and gut of grass carp [12, 13]. Moreover, thiamine deficiency significantly decreased serum immunological parameters in Jian carp [55]. Leukocytes functions decreased in lake trout, *Salvelinus namaycush*, when experienced a thiamine deficiency state [56]. Therefore, it is speculated that no significant effects of thiamine supplementation on beluga immunological parameters may be due to measuring them in the whole body extract.

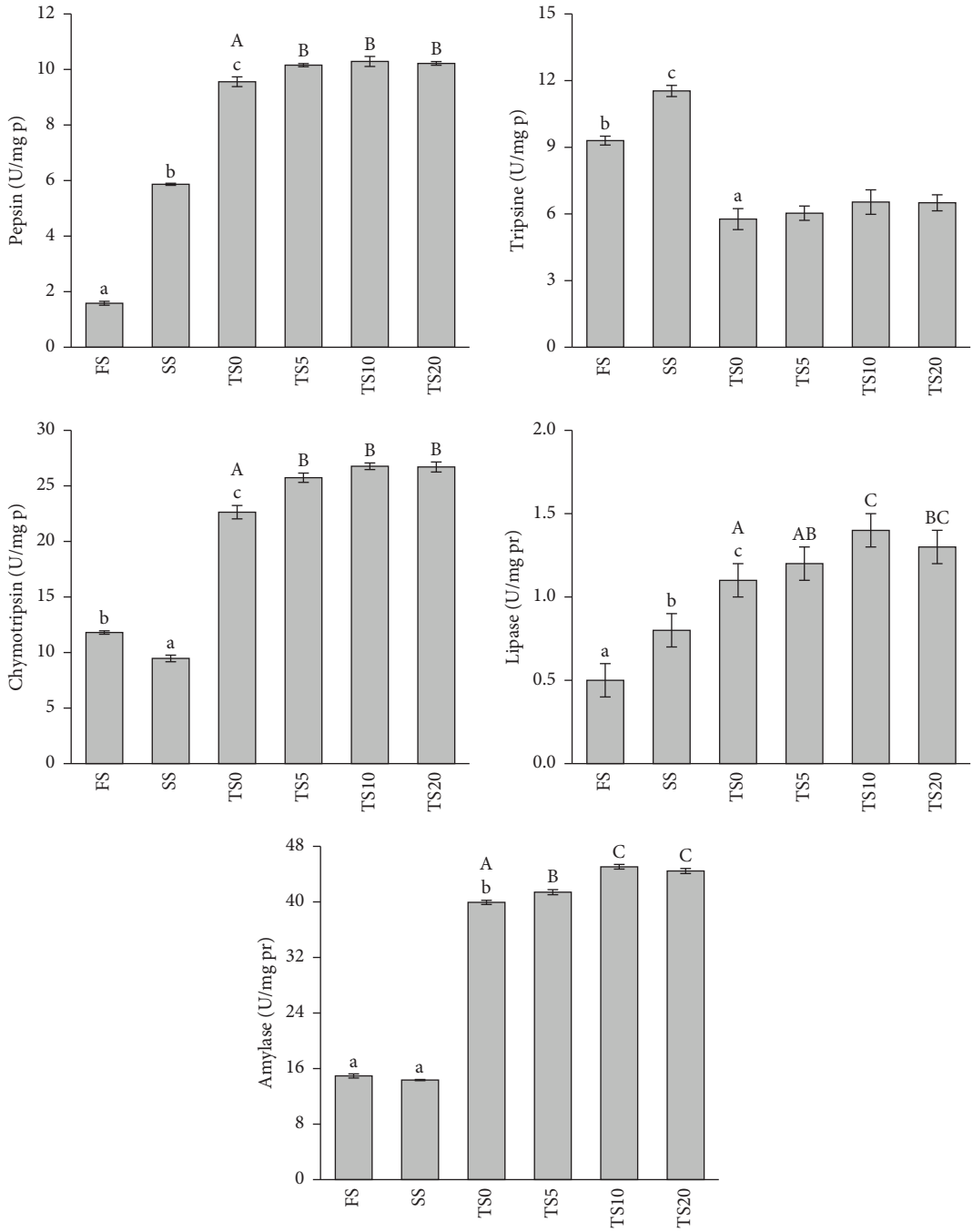

Figure 1: Comparison of digestive enzymes in beluga fingerling fed with the diets containing thiamine. FS: first sampling, SS: second sampling, TS: third sampling. TS0, TS5, TS10, and TS20 were related to third sampling of larvae were fed with diets contain 0, 5, 10, and 20 mg thiamine per kg diet. Values are presented as (mean ± SD). Different lowercase and uppercase letters indicate significant difference among different feeding stages without thiamine and different thiamine levels in artificial diet stage, respectively ($P < 0.05$).

Table 5: Comparison of whole body biochemical parameters in beluga fingerlings fed diets supplemented with thiamine.

	TS0	TS5	TS10	TS20
ALT (mU/mg protein)	61.1 ± 8.23^{b}	50.2 ± 8.82^{ab}	42.9 ± 1.77^{a}	44.5 ± 2.13^{a}
ALP (mU/mg protein)	860 ± 118^{b}	672 ± 109^{a}	587 ± 27.4^{a}	612 ± 13.1^{a}
LDH (mU/mg protein)	318 ± 55.6	263 ± 16.1	243 ± 36.9	221 ± 55.5
Lysozyme (U/mg protein)	33.8 ± 2.31	35.0 ± 3.92	37.2 ± 2.15	37.0 ± 3.09
ACH50 (%)	107 ± 6.56	103 ± 5.85	110 ± 2.88	108 ± 4.04
IgM (mg/100 g tissue)	43.6 ± 2.61	45.0 ± 3.26	46.7 ± 4.95	47.8 ± 1.25

Values are presented as (mean ± SD). Different show significant difference between pair groups at $P < 0.05$.

In conclusion, thiamine supplementation is beneficial in rearing beluga larvae. Thiamine supplementation improves the growth rate of the larvae, possibly due to an increase in digestive enzymes' activity and/or protein retention. Additionally, thiamine supplementation boosts larvae survival rate, possibly due to improve tissues' health, as evidence by lower ALT and ALP activities. For optimal results, it is recommended that thiamine be included at a concentration of 10–20 mg/kg during the beluga weaning stage until the fingerling stage.

Data Availability

The data of this study are available upon reasonable request from the corresponding author.

Conflicts of Interest

The authors declare they have no conflicts of interest.

Authors' Contributions

Mahmoud Mohseni contributed to conceptualization, supervision, and manuscript drafting. Melika Ghelichpour contributed to data analyzing and manuscript drafting. Mir Hamed Sayed Hassani performed data collection, visualization, and methodology. Zabih Ollah Pajand collected resources and data. Reza Ghorbani Vaghei performed data collection, visualization, and methodology.

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