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Research Article

Dietary thiamine supplementation for beluga sturgeon (*Huso huso*) broodstock: Impacts on sex steroids, reproductive performance, glucose metabolism, and egg quality

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Abstract

The present study examined the effects of dietary thiamine (vitamin B_1) on the physiological performance and egg quality of beluga sturgeon (*Huso huso*) broodstock. A total of 96 beluga sturgeon broodstocks (mean weight 39.65 ± 6.01 kg) were randomly distributed to 12 tanks and received diets containing 0 (control, C), 5 (TH1), 10 (TH2), and 20 (TH3) mg thiamine kg^{-1} for 24 months. After the feeding trial, plasma sex steroids, glucose-6-phosphate dehydrogenase (G6PD) enzyme activity, reproductive parameters, and the vitamin content of the egg were assessed. Plasma levels of progesterone, testosterone, and 17β-estradiol were significantly higher in the TH3 group than in the others (p<0.05). G6PD enzyme activity was significantly elevated in the TH2 and TH3 groups compared to the control group (p<0.05). The control group showed a significant increase in the egg number compared to the TH3 group. The egg diameter significantly increased in the TH3 group (p<0.05), while working and relative fecundities remained unaffected (p>0.05). Egg vitamin content significantly augmented in all three thiamine-fed groups compared to the control (p<0.05). This study concluded that dietary 20 mg thiamine kg^{-1} improved physiology, ovarian development, and egg quality of beluga sturgeon broodstock.

Introduction

As fascinating creatures, sturgeons are remarkable fish characterized by exceptional longevity and the high commercial value of their meat and roe, which set them apart from other fish species. Although these species were historically found in the Azov and Black Seas, the Caspian Sea currently supports approximately 90% of the world's commercially important sturgeon populations (Ermolin and Svolkinas, 2016; Falahatkar, 2024). Among the sturgeons of the Caspian basin, the beluga sturgeon (*Huso huso*) is recognized as the largest freshwater fish species and constitutes a principal species in sturgeon aquaculture (Ciulli *et al*., 2020). Rapid growth performance, together with substantial tolerance to environmental stressors and pathogenic agents, confers significant potential for participation in global aquaculture markets and demonstrates strong adaptability to captive rearing conditions (Falahatkar *et al*., 2009; Mohseni *et al*., 2021). Nevertheless, unsustainable fishing, aquatic environmental pollution, and dam construction on the habitats present substantial challenges to the beluga sturgeon and are the main reasons for many abnormalities in gonad development. The combined challenges led to the beluga sturgeon being categorized as a highly endangered species on the IUCN Red List (IUCN, 2025). To rehabilitate the depleted beluga sturgeon population in the Caspian Sea, reproduction management programs and selective breeding strategieshave been developed, along with the introduction of improved rearing and propagation techniques (Mohseni and Ozorio, 2014; Ruban *et al*., 2019). One key aspect of aquaculture practices is meeting the nutritional requirements of broodstock, which strongly influences maturation and reproductive performance (Bittencourt *et al*., 2012; Thiruvasagam *et al*., 2024).

Numerous studies have demonstrated that inadequate broodstock nutrition represents a major challenge in aquaculture, adversely affecting offspring survival (Izquierdo *et al*., 2001; Xu *et al*., 2019). Among the nutrients, vitamins play a crucial role in fish reproduction and influence important parameters such as gonad quality, fecundity, egg quality, embryonic development, fertilization rate, hatching success, and the survival of fish larvae (Lee and Dabrowski, 2004; El-Sayed and Izquierdo, 2022). To this point, most research has been conducted on vitamin A (Fontagné-Dicharry *et al*., 2010) and vitamin E (Nascimento *et al*., 2014; Erdogan and Arslan, 2019) or the combination effects of vitamins (Lee and Dabrowski, 2004) on reproduction performance of a variety of fish species; however, limited information is available on the role of vitamins in sturgeon.

Thiamine (vitamin B_1) is essential for cellular metabolic processes, lipid and carbohydrate metabolism, and neurological functions in fish (Keinänen *et al*., 2022; Xu *et al*., 2022). Thiamine deficiency in predatory fish, often caused by thiaminase in their prey, can result in anorexia, reduced growth, neural degeneration, impaired swimming, and even mortality (Harder *et al*., 2018). Furthermore, studies have shown that thiamine injection before spawning positively affects the

physiological indicators in broodstock of certain fish species (Ghiasi *et al*., 2017; Futia *et al*., 2017; Mitchell *et al*., 2021). Evidence suggeststhat thiamine depletion in salmonid eggs results in early mortality syndrome (EMS) in their offspring and an increased mortality rate (Fitzsimons *et al*., 2021). Consequently, thiamine administration is considered a vital strategy for augmenting thiamine reserves in broodstock. To date, empirical evidence regarding the specific effects of thiamine on beluga sturgeon remains scarce, and its influence on reproductive indices and maturation is yet to be fully elucidated. Therefore, the present study aimed to evaluate the effects of specialized formulated diets with varying thiamine concentrations on growth performance, reproductive characteristics, sex steroids, and egg vitamin content in beluga sturgeon broodstock.

Materials and Methods

Broodstock maintenance

All the research activities were conducted at the International Sturgeon Research Institute (Rasht, Iran). Initially, ovarian development was characterized in the female beluga sturgeon (n = 120) using the polarity index (Dettlaff *et al*., 1993). Ninety-six beluga sturgeon broodstocks were selected based on similar weight (mean 39.65±6.01 kg) and maturity stage (early stages III-IV) and marked by coded wire tags (CWT). Throughout the experiment, the water source was supplied by a combination of aerated groundwater and the Sefid-rud River (water flow rate 2.7 L s^{-1}). During this period, fish were reared under a natural photoperiod, and water quality parameters were monitored daily and continuously to ensure optimal conditions. The average water temperature, dissolved oxygen, ammonia, and pH were 14.30±0.90°C and 7.70±0.39 mg L^{-1}, 0.02±0.00 mg L^{-1}, and 7.70±0.18, respectively.

Diet formulation and chemical composition

This study utilized four isonitrogenous and isocaloric experimental diets, created by augmenting a basal diet with thiamine hydrochloride, including 0 (C), 5 (TH1), 10 (TH2), and 20 (TH3) mg kg^{-1} for a 24-month feeding trial. The experimental diets were formulated utilizing Lindo®, version 17 (LINDO Systems corporation based in Chicago, USA). Formulated diets were produced by Behdaneh Aquafeed Manufacture (Babolsar, Iran), and the formulation is represented in Table 1. Thiamine hydrochloride was purchased from Sigma-Aldrich (Stuttgart, Germany). Since cereals are the main sources of thiamineas the main ingredients of the experimental diets (Garg *et al*., 2021), the diet ingredients were analyzed for thiamine content (www.nutritiondata.self.com) to select cereals with lower thiamine content to achieve the desired thiamine concentration in the diet. The experimental diets were prepared with Kilka anchovy fishmeal processed at low temperatures and soybean meal as animal and plant protein resources, respectively. The thiamine-free vitamin premix was made according to the NRC (1993). All the dry ingredients were initially ground and thoroughly mixed with micro-ingredients consisting of thiamine supplements. Fish and soybean oil in equal proportions with water were then added. The homogenized mixture passed through the pellet mill (CPM, model CL series; USA) to form pellets (12 mm diameter).

Table 1: Composition of the four experimental diets supplemented with different levels of thiamine.

Ingredients	C	TH1	TH2	TH3
Meat powder[1] (g kg^{-1})	150.00	150.00	150.00	150.00
Wheat gluten[2] (g kg^{-1})	67.90	67.90	67.90	67.90
Fishmeal[3] (g kg^{-1})	446.50	446.50	446.50	446.50
Soybean meal[4] (g kg^{-1})	41.00	41.00	41.00	41.00
Wheat flour (g kg^{-1})	143.00	142.90	142.90	142.80
Soy lecithin (g kg^{-1})	21.00	21.00	21.00	21.00
Sodium chloride (g kg^{-1})	5.00	5.00	5.00	5.00
Methionine[5] (g kg^{-1})	20.00	20.00	20.00	20.00
Lysine[5] (g kg^{-1})	10.00	10.00	10.00	10.00
Choline chloride[6] (g kg^{-1})	2.00	2.00	2.00	2.00
Vitamin permix[7] (g kg^{-1})	20.00	20.00	20.00	20.00
Mineral permix[8] (g kg^{-1})	10.00	10.00	10.00	10.00
Fish oil (g kg^{-1})	30.00	30.00	30.00	30.00
Soybean oil (g kg^{-1})	30.00	30.00	30.00	30.00
L-carnitine (g kg^{-1})	0.60	0.60	0.60	0.60
Anzymite (g kg^{-1})	3.00	3.00	3.00	3.00
Thiamine (mg kg^{-1})	0.00	5.00	10.00	20.00
Proximate composition (n = 3)				
Moisture (%)	8.14	7.62	8.16	8.21
Crude protein (%)	46.57	46.66	46.29	46.57
Crude lipid (%)	13.50	13.90	13.80	13.60
Crude ash (%)	8.40	8.50	8.50	8.60
Crude fiber (%)	2.04	2.11	2.08	2.01
NFE[9] (%)	23.23	23.51	23.39	23.46
Gross energy[10] (KJ g^{-1})	20.28	20.49	20.33	20.29
Phosphorus (%)	10.80	10.70	10.60	10.80

[1] Cattle slaughter waste contains 483.9 g kg^{-1} (crude protein, CP) and 119.8 g kg^{-1} (crude lipid, CL) (Gilehdam Caspian Co., Rasht, Iran).

[2] Wheat gluten meal contains 826.8 g kg^{-1} (CP) and 17.2 g kg^{-1} (CL) (Sanat Afarin Sanyar Co., Esfehan, Iran).

[3]Fishmeal was obtained from Kilka (*Clupeonella* sp.) that includes 672.9 g kg^{-1} (CP), 200.2 g kg^{-1} (CL), and total volatile nitrogen 15.7 mg 100 g^{-1} (Gill Poodr Co., Bandar Anzali, Iran).

[4]Soybean meal contains 413.8 g kg^{-1} (CP) and 25.6 g kg^{-1} (CL) (Behpak Industrial Co., Mazandaran, Iran).

[5]Evonik industries (Essen, Germany): 99%;

[6]Liaoning Biochem Co. (Dalian, China): 60%;

[7] Vitamin mixture (thiamine free, as IU or mg kg^{-1}) was manually provided according to feed requirements of the fish (NRC, 1993) and ingredients were obtained from Hashtgerd Laboratories (Hashtgerd, Iran): Alpha-tocopherol 60 IU; Calciferol 3000 IU; Riboflavin 30 IU; Pyridoxine 15 IU; B_{12} 0.05 IU; Nicotinic acid 175 IU; Folic acid 5 IU; Ascorbic acid 500 IU; Inositol 1000 mg kg^{-1}; Biotin 2.5 mg kg^{-1}; Calcium pantothenate 50 mg kg^{-1}.

[8] Aquatic mineral mixture as mg kg or g kg^{-1}) was manufactured by Science Laboratories (Ghazvin, Iran): (Calcium carbonate (40%) 2.15 g kg^{-1}; Manganese oxide 1.24 g kg^{-1}; Ferric citrate 0.2 g kg^{-1}; Potassium iodide 0.4 mg kg^{-1}; Copper sulfate 0.3 g kg^{-1}; Manganese sulfate 0.3 g kg^{-1}; Calcium phosphate 5 g kg^{-1}; Cobalt sulfate 2 mg kg^{-1}; Sodium selenite 3 mg kg^{-1}; Potassium chlorate 0.9 g kg^{-1}; Sodium chloride 0.4 g kg^{-1}.

[9] Nitrogen Free Extract = dry matter - (CP + CL + crude fiber (CF) + ash).

[10] The calculation for gross energy was based on the following values: Protein provides an energy value of 23.6 KJ g^{-1}, lipid provides 39.5 KJ g^{-1}, and NFE provides 17.2 KJ g^{-1} energy NRC (2011). C: 0, control group, TH1: 5 mg thiamine kg^{-1}, TH2: 10 mg thiamine kg^{-1} and TH3: 20 mg thiamine kg^{-1}.

The resulting pellets were dried in a convection oven at 70°C (Mohseni *et al*., 2023) and stored in a sealed plastic container at -20°C before the feeding trial.

The chemical compositions of the diets were evaluated following the procedures described in AOAC (2012). The moisture content was estimated by drying the samples at 105°C until a constant weight

was achieved. The crude protein and lipid were determined using the Kjeldahl and Soxhlet methods, respectively. The ash content was calculated by incinerating the dried samples at 550°C for 8h.

Experimental design

The selected brooders (8 individuals per tank) were distributed to 12 cement tanks (8 m diameter, 1.60 m depth, 80000 L volume) and fed with one of the following four experimental diets for 24 months before spawning: a control diet (C, 0 mg kg^{-1} thiamine), and three diets containing 5 (TH1), 10 (TH2) and 20 (TH3) mg kg^{-1} thiamine. Fish were hand-fed daily at 9:00, receiving 0.20% of their body weight (Falahatkar *et al*., 2013). Remained feed and feces were siphoned to waste once a day.

Plasma sex steroid levels

This ensured that animal welfare was paramount throughout the study. At the end of the 24-month experimental period, 15 brooders were randomly fasted for 24 h and anesthetized using 300 ppm clove extract (Mohseni and Ozorio, 2014). Blood samples were then taken from the caudal vein using sterile 5-mL heparinized syringes. By centrifuging (Heraeus Labofuge, Hanau, Germany) the samples at 3000 rpm for 10 min, plasma was collected and kept at -20°C until hormonal and biochemical analysis (Webb *et al*., 2007). Steroid levels were measured following procedures provided by Immunotech commercial kits (Marseille, France) (Akhavan *et al*., 2019). Progesterone (P), testosterone (T), and 17β-estradiol (E2) concentrations were measured by ELISA. The intra- and inter-assay coefficients of variation were 5.10% and 9.60% for P, 14.80% and 11.90 for T, and 12.10% and 9.50% for E2, respectively. The validation of steroid levels involved confirming that the serial dilutions were parallel to standard curves. The concentrations were measured as ng mL^{-1}. Absorbance was read at 450 nm using an ELISA microplate reader (Tecan Sunrise, Tecan GmbH, Grödig, Austria). All samples and standards were analyzed in duplicates.

Measurement of G6PD enzyme activity

The G6PDH activity was determined using a commercially available kit from Bahar Afshan (Tehran, Iran) and measured using an auto-analyzer (RA-1000, Technicon Corp, Tarrytown, USA). The samples were read at 340 nm at 37 ˚C, and the results were expressed as nM of NADPH produced per min per sample (Amcoff *et al.,* 2000).

Reproductive performance

To determine the reproductive parameters at the end of the feeding period, the fish of each tank (n=8) were anesthetized separately with clove powder extract and rinsed with water to remove the anesthetic substance from the body before the operation. The fish were dried with a clean towel, and gentle pressure was applied to the abdominal region to pass the eggs into the plastic bowl. Eggs were collected and weighed individually after removing the ovarian fluid with 0.01 g precision, and egg diameter and the number of eggs per gram were calculated. To determine the number of eggs per gram, each sample weighing approximately 1.00 g was counted separately. Following the stripping, working and relative fecundities were assessed using the following formula:

Working fecundity (number of eggs per fish) = total weight of obtained eggs (g) × number of matured eggs in 1 g sub-sample
Relative fecundity (number of eggs per g fish) = working fecundity/total weight of fish

Assessment of thiamine content of egg

After the feeding trial, eggs (10 g) from fifteen fish were collected and immediately frozen in liquid nitrogen. The samples were then stored at -80°C until the analysis. High-performance liquid chromatography (HPLC) was used to detect thiamine hydrochloride (THCL), thiamine pyrophosphate (TPP), and thiamine monophosphate (TMP), as described by Mancinelli *et al*. (2003). The HPLC system was composed of a 4.6 mm×250 mm column (Zorbax, Agilent Technologies, CA, USA) coupled with an NH_2 packed guard column and a delivery system pump (1200 Series, Agilent Technologies, CA, USA) with a 100 µL automatic injection unit. AG1321A FLD fluorescent detector was calibrated to excite at 375 nm and emit light at 430 nm. Potassium phosphate buffer at pH 7.50 (85 mM) was mixed with acetonitrile in a 65:35 (v:v) ratio to prepare the mobile phase. There was a 0.50 ml per minute flow rate. At 30°C, the column thermostat was adjusted. External standard curves for THCl, TMP, and TPP were generated using stock solutions (1 mM) in 0.01 M HCl. For linearity, each standard concentration was in the range of 1.00-100.00 nM L^{-1}. The extraction recovery rates were 100% for TMP and TPP and 94.70±3.00% (n=4) for THCl. To improve the recovery process, samples were initially treated with standard concentrations of THCl, TMP, and TPP. The extraction technique was then carried out as previously mentioned.

Statistical analysis

All trials presented the data as mean ± standard error (SE). Before analysis, normality and homogeneity of variance were assessed using the Kolmogorov-Smirnov and Levene's tests, respectively. The means across different groups were compared using One-way ANOVA. Tukey's HSD was applied to identify specific differences between the means of various treatment groups. A significance level of $p<0.05$ was adopted for all statistical analyses. The data were analyzed using SPSS for Windows, Version 16.0 (SPSS Inc., Chicago, US).

Results

Plasma levels of P, T, and E2 in beluga sturgeon broodstock fed different dietary thiamine levels were shown in Figures 1 to 3, respectively. There was a positive trend observed between thiamine levels and sex steroid levels. A significant increase in progesterone levels was observed in the TH3 group compared to the other groups ($p<0.05$). The control group exhibited the lowest progesterone level ($p<0.05$). According to the analysis of plasma T levels in the broodstock, the group fed with the 20 mg kg^{-1} thiamine diet and the control had the highest and lowest levels, respectively ($p<0.05$). Peak plasma E_2 levels were shown in the TH3 group at the end of the 24-month trial ($p<0.05$). There were no statistically significant differences between the control and TH1 groups ($p>0.05$).

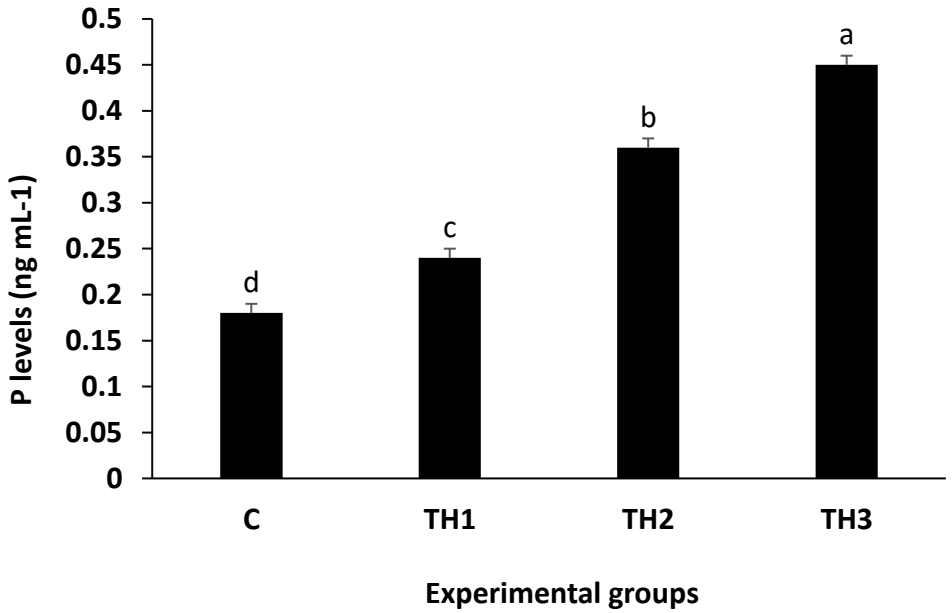

Figure 1: Plasma P (progesterone) level in beluga sturgeon (*Huso huso*) broodstock after 24 months of feeding with different thiamine concentrations (means ± SE, n = 8). C: 0, control group, TH1: 5 mg thiamine kg^{-1}, TH2: 10 mg thiamine kg^{-1} and TH3: 20 mg thiamine kg^{-1}. Different letters indicated significant differences among the treatments (p<0.05).

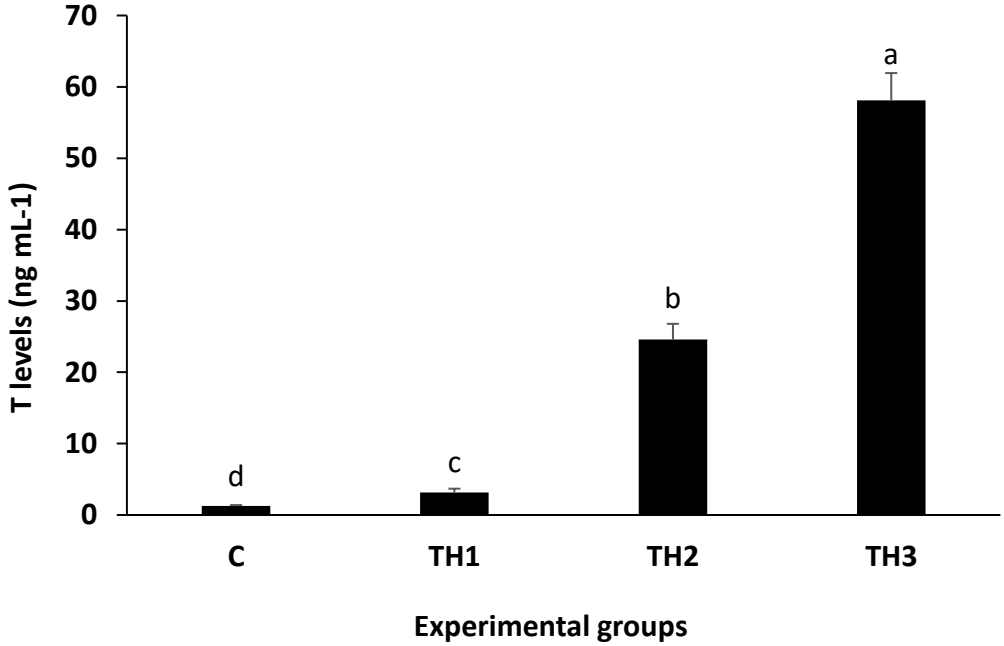

Figure 2: Plasma T (testosterone) levels in beluga sturgeon (*Huso huso*) broodstock after 24 months of feeding with different thiamine concentrations (means ± SE, n = 8). C: 0, control group, TH1: 5 mg thiamine kg^{-1}, TH2: 10 mg thiamine kg^{-1} and TH3: 20 mg thiamine kg^{-1}.Different letters indicated significant differences among the treatments (p<0.05).

At the end of the feeding trial, G6PD activity in TH2 and TH3 was significantly increased compared to the control group (Fig. 4, p<0.05). Moreover, there was no significant difference among thiamine-fed groups (p>0.05).

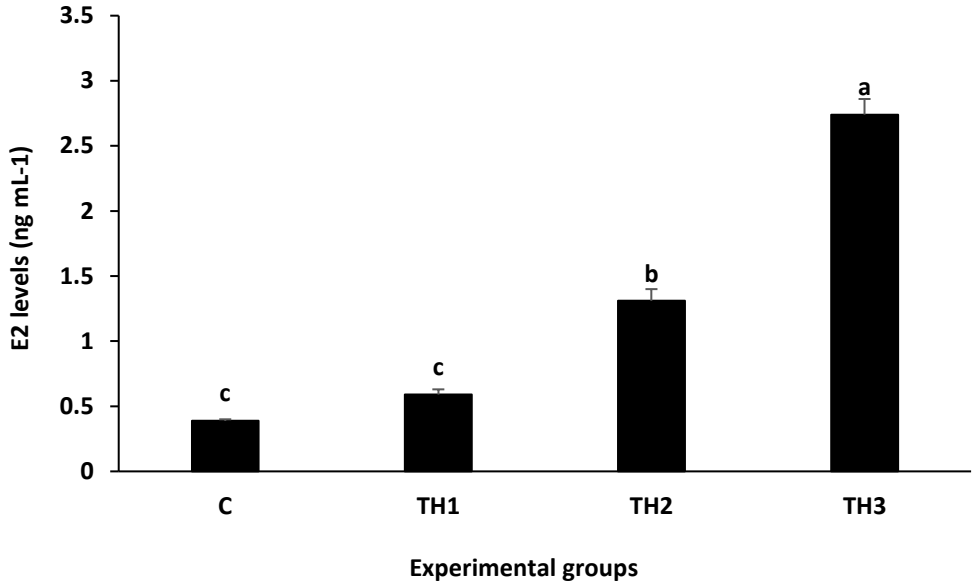

Figure 3: Plasma E2 (17-β estradiol) level in beluga sturgeon (*Huso huso*) broodstock after 24 months of feeding with different thiamine concentrations (means ± SE, n = 8). C: 0, control group, TH1: 5 mg thiamine kg^{-1}, TH2: 10 mg thiamine kg^{-1} and TH3: 20 mg thiamine kg^{-1}. Different letters indicated significant differences among the treatments (p<0.05).

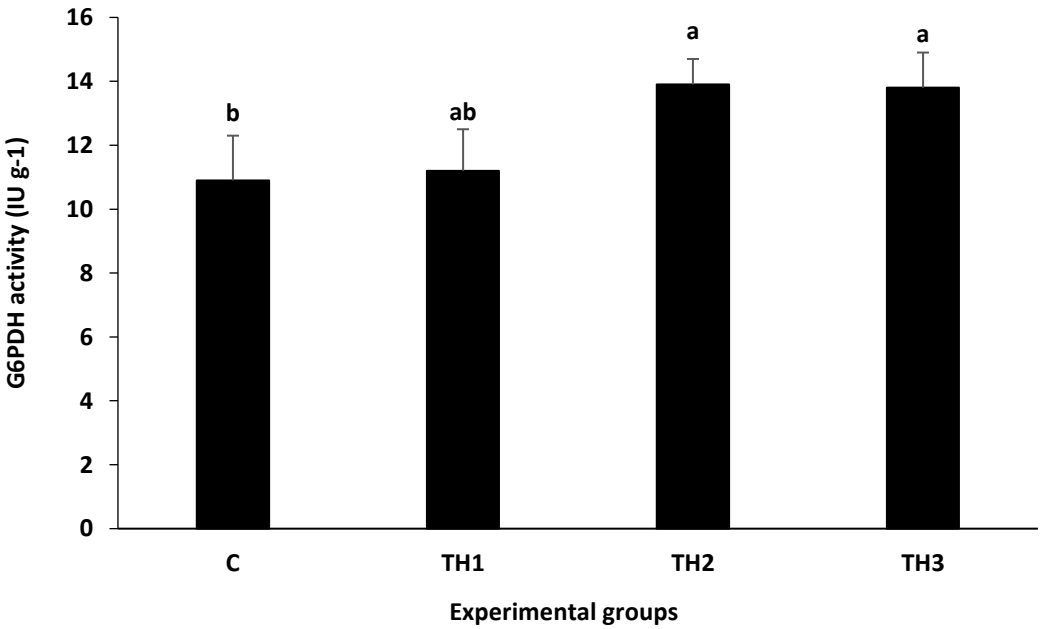

Figure 4: G6PDH enzyme activity in beluga sturgeon (*Huso huso*) broodstock after 24 months of feeding with different thiamine concentrations (means ± SE, n = 8). C: 0, control group, TH1: 5 mg thiamine kg^{-1}, TH2: 10 mg thiamine kg^{-1} and TH3: 20 mg thiamine kg^{-1}. Different letters indicated significant differences among the treatments (p<0.05).

Table 2 displays the reproductive performance of the beluga broodstock fed with different thiamine concentrations. The fish in the control group indicated the highest number of eggs per gram which it was significantly higher than the group fed with 20 mg kg^{-1} thiamine (p<0.05). The TH3 group fed the highest thiamine concentration, produced eggs with the largest mean diameter (p<0.05).

Table 2: Reproductive performance and egg parameters of the beluga sturgeon (*Huso huso*) broodstock after 24 months of feeding with different thiamine concentrations (means ± SE, n = 8).

Indices	C	TH1	TH2	TH3
Number of eggs per g	42.70 ± 0.56^{a}	41.30 ± 0.58^{ab}	41.30 ± 0.58^{ab}	39.70 ± 1.15^{b}
Egg diameter (mm)	3.21 ± 0.02^{c}	3.23 ± 0.11^{c}	3.35 ± 0.07^{b}	3.83 ± 0.23^{a}
Working fecundity (number of eggs per fish)	274067.00 ± 28014.00	251390.00 ± 30875.00	235560.00 ± 13161.00	239190.00 ± 34870.00
Relative fecundity (number of eggs per g fish)	5535.00 ± 145.20	5468.00 ± 127.10	5536.00 ± 97.10	5492.00 ± 184.70

Different superscripts in each column indicated significant differences among the treatments (p<0.05). C: 0, control group, TH1: 5 mg thiamine kg^{-1}, TH2: 10mg thiamine kg^{-1} and TH3: 20mg thiamine kg^{-1}.

However, working and relative fecundities showed no significant differences among the experimental groups (p>0.05).

According to Table 3, the assessment of vitamin contents in eggs obtained from beluga sturgeon exposed to the different thiamine concentrations showed that the control and the TH3 groups had the lowest and highest THCL content, respectively (p<0.05). No significant difference was detected among the treatments related to TMP content (p>0.05). Thiamine pyrophosphate also showed the highest level in the group fed with 20 mg kg^{-1} thiamine (p<0.05). Moreover, total thiamine exhibited a significant increase in the group that received the highest vitamin concentration (p<0.05).

Table 3: Thiamine content in the beluga sturgeon (*Huso huso*) egg after 24 months of feeding with different vitamin concentrations (means ± SE, n = 8).

Indices	C	TH1	TH2	TH3
THCL (nM g^{-1})	0.30 ± 0.00^{c}	0.60 ± 0.00^{b}	1.20 ± 0.00^{ab}	2.10 ± 0.20^{a}
TMP (nM g^{-1})	0.50 ± 0.10	0.60 ± 0.00	0.70 ± 0.00	0.80 ± 0.10
TPP (nM g^{-1})	2.00 ± 0.30^{c}	3.40 ± 0.70^{b}	3.70 ± 0.30^{b}	6.20 ± 0.90^{a}
Total thiamine (nM g^{-1})	2.80 ± 0.30^{c}	4.90 ± 0.60^{b}	6.40 ± 0.80^{ab}	9.30 ± 1.20^{a}

Different superscripts in each column indicated significant differences among the treatments (p<0.05). THCL: thiamine hydrochloride, TPP: thiamine pyrophosphate, and TMP: thiamine monophosphate. C: 0, control group, TH1: 5 mg thiamine kg^{-1}, TH2: 10 mg thiamine kg^{-1} and TH3: 20 mg thiamine kg^{-1}.

Discussion

The inclusion of 20 mg kg^{-1} thiamine at the end of the second year affected sex steroids. While there is limited information on the effects of vitamins on sex steroids in fish and other organisms, a few studies have focused on vitamins D and C (Yang *et al*., 2024). For example, Dabrowski *et al*. (1995) found that an elevated concentration of dietary vitamin C in rainbow trout (*Oncorhynchus mykiss*) brooders caused a significant increase in testosterone levels, which was in line with the current research. In contrast, thiamine injection to sterlet sturgeon (*Acipenser ruthenus*) did not influence E2 but increased T level in the group receiving 50 mg kg^{-1} thiamine (Ghiasi *et al*., 2017). These results were likely attributed to the ovarian maturation state. Wang *et al*. (2020) similarly reported that testosterone levels may slightly increase in the final stage of vitellogenesis in the sterlet sturgeon.

Our study revealed significantly higher steroid levels in the group receiving the highest thiamine concentration. Due to a

paucity of research, the concise relationship between thiamine concentrations and sex steroid levels has not been clarified; however, it appears that higher thiamine storage in target tissues of the brain is more likely a reason for the hormone increase, as the brain is the main organ for storing thiamine and is the first component in linking the hypothalamus-pituitary-gonad (HPG) axis (Munro *et al*., 1990; Marrs and Lonsdale, 2021). Moreover, thiamine is the crucial coenzyme to produce ATP, providing the energy essential for gonadal development (Ghiasi *et al*., 2017). Therefore, increasing the thiamine levels is assumed to promote the synthesis of steroids, which is also reported in limited studies on fish and humans (Ghiasi *et al*., 2017; Mrowicka *et al*., 2023). Many studies have established the vital role of vitamins in regulating fish steroidogenesis. In Indian carp (*Labeo rohita*), elevated dietary vitamin E stimulated steroid hormone synthesis (Ciji *et al*., 2013). Yang *et al*. (2024) highlighted the effect of vitamin D_3 on increasing the oocyte maturation rate and plasma estradiol in female zebrafish (*Danio rerio*). As information on thiamine in terms of steroidogenesis is rare, future research should focus on discovering the interconnection between this vitamin and gonad capacity to release sex steroids in different fish species.

Our investigation into G6PD enzyme activity after a 24-month period revealed that the control group exhibited the lowest levels. This observation aligns with previous research on Baltic salmon (*Salmo salar*) alevins, where thiamine administration, either through injection or bathing, resulted in significantly higher G6PD activity compared to control groups (Amcoff *et al*., 2000). Furthermore, optimal thiamine levels were found to aid the glycolipid metabolism of oriental river prawn (*Macrobrachium nipponense*), affecting the expression of enzymes like glucose transporter 2 and pyruvate kinase (Sun *et al*., 2022). The importance of thiamine in glucose metabolism is further highlighted in studies, such as in rats where thiamine disulfide therapy was observed to decrease blood glucose and serum glucagon levels while increasing serum insulin levels (Ghanbari Rad *et al*., 2022). Evidence suggests that G6PD activity is connected to other elements involved in thiamine metabolism, such as pyruvate, lactate, and transketolase, and plays a crucial role in glucose metabolism by facilitating the conversion of glucose-6-phosphate to 6-phosphogluconic acid (Kumar *et al*., 2019). Additionally, it safeguards red blood cells from oxidative damage by transforming NADP+ into NADPH, which is vital for mitigating oxidative stress by sustaining elevated levels of reduced glutathione, a potent antioxidant (Chowdhury and Saikia, 2020). A lack of thiamine can negatively impact G6PD function by inhibiting transketolase and the pentose phosphate pathway (Zhou *et al*., 2022; Behbodi *et al*., 2024).

Conversely, a report by Ghiasi *et al*. (2017) indicated no alteration in G6PD activity following thiamine injection, a finding consistent with a study on rainbow trout broodstock (Åkerman *et al*., 2003). Similarly, a study on another B vitamin, pyridoxine (vitamin B_6) in juvenile largemouth bass (*Micropterus salmoides*) did not find a significant effect on the

gluconeogenesis-related gene *g6pase* (Zhang *et al*., 2025). This discrepancy could potentially be attributed to species or methodological differences, as well as the different routes of thiamine administration; dietary intake over a prolonged period might lead to a more sustained or widespread effect on enzyme activity compared to a single injection. Our findings suggest that including thiamine in the diet of beluga sturgeon broodstock might enhance G6PD activity, thereby improving their carbohydrate metabolism.

Regarding reproductive efficiency, our findings revealed no significant differences in either working or relative fecundity across the various treatments. This outcome aligns with earlier research on sterlet sturgeon that involved thiamine injection (Ghiasi *et al*., 2017). While there is limited information specifically on the impact of vitamin B on fecundity, studies involving other vitamins, such as dietary vitamins A, E, D, and C, have demonstrated a notable increase in fish fecundity (Palace and Werner, 2006; Sarmento *et al*., 2018; Zhang *et al*., 2021; Grzesiak *et al*., 2022). It has been suggested that enhanced fecundity may be a result of nutrient effects on the pituitary gland, potentially leading to elevated steroid levels and improved reproductive efficiency (Izquierdo *et al*., 2001).

Interestingly, despite the lack of significant differences in overall fecundity in our study, thiamine treatment did appear to influence other egg characteristics in beluga sturgeon. While the number of eggs per gram of ovarian tissue was reduced with thiamine treatment, the highest thiamine concentration correlated with an increase in egg diameter. This observation is consistent with findings in sterlet sturgeon (Ghiasi *et al*., 2017). Similarly, a negative relationship between egg thiamine concentration and egg size has been noted in wild walleye (*Sander vitreus*), which is consistent with our findings regarding increased egg size alongside thiamine supplementation (Wiegand *et al*., 2011). The increase in egg size is primarily attributed to the deposition of vitellogenin. In sturgeon, vitellogenin is a large carbohydrate-rich glycophospholipoprotein. Given that thiamine is a crucial nutrient for carbohydrate metabolism (Bettendorff, 2020), it is plausible that the enhanced thiamine levels contributed to increased vitellogenin production, subsequently resulting in the observed larger egg size. Therefore, while thiamine may not have directly impacted the total number of eggs, it appears to have played a role in improving egg quality, as indicated by the increased egg diameter.

This study showed a similar trend between dietary supplementation and the THCL, TPP, and TT content of eggs in beluga sturgeon after 24 months. TMP concentration was not statistically varied among the treatments, but we observed a tendency for increased TMP in egg and dietary thiamine levels. Our findings exhibited that supplementing beluga aquafeed before spawning considerably elevated the vitamin level in the obtained eggs. Similar results were achieved in pre-spawned sterlet sturgeon injected with thiamine concentrations (Ghiasi *et al*., 2014). The upward trend of thiamine content in fish eggs as a consequence of the

vitamin supplementation before the spawning is reported in different species exposed to vitamin immersion and diet inclusion (Amcoff *et al*., 2000; Fitzsimons *et al*., 2005; Fontagné-Dicharry *et al*., 2010). Fitzsimons *et al*. (2009) found thiamine deficiency in the offspring of lake trout (*Salvelinus namaycush*) broodstock received lower thiamine concentration, and the same results were presented by Czesny *et al*. (2012). These authors also noted that the TPP and TMP were detected as dominant in fish eggs, which was similar to the studies by Carvalho *et al*. (2009). Thiamine pyrophosphate constitutes approximately 80% of the thiamine in fish eggs and is primarily produced via intracellular thiamine phosphorylation (Pacei *et al*., 2020), as shown in our study. A strong correlation between broodstock diet and egg nutrient composition has been widely reported (Izquierdo *et al*., 2001; Furuita *et al*., 2009). Before spawning, thiamine can be stored in the gonad tissue or transferred into the egg with particular proteins, leading to increased thiamine levels in the produced eggs (Lee and Dabrowski, 2004). By comparison of different procedures, thiamine content in the fish egg after exposing to the highest dose through the injection (50 mg kg^{-1}) was 2.5 times more than the control group (Ghiasi *et al*., 2017), whereas, in the present study, oral administration of pre-spawned beluga sturgeon with 20 mg kg^{-1} resulted in a 3.5 times higher thiamine content in the eggs compared to the control group, revealing the high potential of dietary thiamine supplementation on improving broodstock performance and obtained eggs. Further examination is required to find underlying mechanisms behind thiamine storage in sturgeon eggs before and after spawning.

Conclusion

This research demonstrates the importance of manipulating the diet of sturgeon in improving the egg quality and reproductive process of these valuable species. The research found that thiamine supplementation positively influenced steroid hormone levels, ovarian development, and the activity of enzymes involved in carbohydrate metabolism. Additionally, egg vitamin content increased linearly and in a dose-dependent manner with increasing dietary thiamine supplementation. The increased egg diameter suggests a beneficial effect of thiamine on caviar production. Taken together, the study concludes that a dietary thiamine level of 20 mg kg^{-1} is optimal for enhancing egg vitamin content and improving reproductive efficiency in beluga sturgeon.

Conflicts of interest

There is no conflict of interest for this study.

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References

Åkerman, G., **Amcoff, P.**, **Tjärnlund, U.**, **Fogelberg, K.**, **Torrissen, O. and Balk, L., 2003**. Paraquat and menadione exposure of rainbow trout

(*Oncorhynchus mykiss*): Effects on the pentose-phosphate shunt and thiamine levels in liver and kidney. *Chemico-Biological Interactions*, 142, 269-283. DOI:10.1016/S0009-2797(02)00181-9

Akhavan, S.R., **Salati, A.P.**, **Jalali, A.H. and Falahatkar, B.**, **2019**. Expression profile of *star* and *cyp19* and plasma sex steroid during gonad development from previtellogenesis to early atresia in captive sterlet sturgeon, *Acipenser ruthenus*. *Journal of Applied Ichthyology*, 35, 249-256. DOI:10.1111/jai.13832

Amcoff, P., **Åkerman, G.**, **Börjeson, H.**, **Tjärnlund, U.**, **Norrgren, L. and Balk, L.**, **2000**. Hepatic activities of thiamine-dependent enzymes, glucose-6-phosphate dehydrogenase, and cytochrome P4501A in Baltic salmon (*Salmo salar*) yolk-sac fry after thiamine treatment. *Aquatic Toxicology*, 48, 391-402. DOI:10.1016/S0166-445X(99)00047-1

AOAC, **2012**. Official Methods of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists. USA. 1263 P.

Behbodi, Z., **Bahram, S.**, **Bahrekazemi, M.**, **Javadian, S.R.**, **Bozorgnia, A. and Abdel-Tawwab, M.**, **2024**. Effects of dietary thiamin (vitamin B1) on the growth performance, serum biochemical factors, immune response, and antioxidant activity of great sturgeon (*Huso huso*) juveniles. *Veterinary Research Communications*, 48, 485-496. DOI:10.1007/s11259-023-10189-8

Bettendorff, L., **2020**. Thiamine. In P. B.P. Marriott, D.F. Birt, V.A. Stallings, and A.A. Yates (Eds), Present Knowledge in Nutrition. Academic Press, USA. pp. 171-188.

Bittencourt, F., **de Souza, B.E.**, **Lui, T.A.**, **Borella, M.**, **Boscolo, W.R.**, **Feiden, A. and Romagosa, E.**, **2012**. Protein diets promote the maturation of oocytes and spawning of *Piaractus mesopotamicus* kept in cages. *Journal of Applied Ichthyology*, 28, 886-893. DOI:10.1111/jai.12045

Carvalho, P.S.M., **Tillitt, D.E.**, **Zajicek, J.L.**, **Dale, R.A.**, **Honeyfield, D.C.**, **Fitzsimons, J.D. and Brown, S.B.**, **2009**. Thiamine deficiency effects on the vision and foraging ability of lake trout fry. *Journal of Aquatic Animal Health*, 21, 315-325. DOI:10.1577/H08-062.1

Chowdhury, S. and Saikia, S.K., **2020**. Oxidative stress in fish: A review. *Journal of Scientific Research*, 12, 145-160. DOI:10.3329/jsr.v12i1.41862

Ciji, A., **Sahu, N.P.**, **Pal, A.K. and Akhtar, M.S.**, **2013**. Nitrite-induced alterations in sex steroids and thyroid hormones of *Labeo rohita* juveniles: Effects of dietary vitamin E and L-tryptophan. *Fish Physiology and Biochemistry*, 39, 1297-1307. DOI:10.1007/s10695-013-9783-9

Ciulli, S., **Volpe, E.**, **Sirri, R.**, **Tura, G.**, **Errani, F.**, **Zamperin, G.**, **Toffan, A.**, **Silvi, M.**, **Renzi, A.**, **Abbadi, M. and Biasini, L.**, **2020**. Multifactorial causes of chronic mortality in juvenile sturgeon (*Huso huso*). *Animals*, 10, 1866. DOI:10.3390/ani10101866

Czesny, S.J., **Rinchard, J.**, **Lee, B.J.**, **Dabrowski, K.**, **Dettmers, J.M. and Cao, Y.**, **2012**. Does spatial variation in egg thiamine and fatty-acid concentration of Lake Michigan lake trout *Salvelinus namaycush* lead to differential early mortality syndrome and yolk oedema mortality in offspring? *Journal of Fish Biology*, 80, 2475-2493.

DOI:10.1111/j.1095-8649.2012.03300.x

Dabrowski, K., **Ciereszko, R.E.**, **Blom, J.H. and Ottobre, J.S.**, **1995**. Relationship between vitamin C and plasma concentrations of testosterone in female rainbow trout, *Oncorhynchus mykiss*. *Fish Physiology and Biochemistry*, 14, 409-414. DOI:10.1007/BF00004073

Dettlaff, T.A., **Ginsburg, A.S. and Schmalhausen, O.I.**, **1993**. Sturgeon Fishes: Developmental Biology and Aquaculture. Springer Verlag, Germany,. 265 P.

El-Sayed, A.F.M. and Izquierdo, M., **2022**. The importance of vitamin E for farmed fish: A review. *Reviews in Aquaculture*, 14, 688-703. DOI:10.1111/raq.12620

Erdogan, M. and Arslan, T., **2019**. Effects of vitamin E on growth and reproductive performance of pindani (*Pseudotropheus socolofi*). *Aquaculture*, 509, 59-66. DOI:10.1016/j.aquaculture.2019.05.032

Ermolin, I. and Svolkinas, L., **2016**. Who owns sturgeon in the Caspian? New theoretical model of social responses towards state conservation policy. *Biodiversity and Conservation*, 25, 2929-2945. DOI:10.1007/s10531-016-1213-6

Falahatkar, B., **Poursaeid, S.**, **Shakoorian, M. and Barton, B.**, **2009**. Responses to handling and confinement stressors in juvenile great sturgeon *Huso huso*. *Journal of Fish Biology*, 75, 784-796. DOI:10.1111/j.1095-8649.2009.02334.x

Falahatkar, B., **Akhavan, S.R.**, **Efatpanah, I. and Meknatkhah, B.**, **2013**. Effect of winter feeding and starvation on the growth performance of young-of-year (YOY) great sturgeon, *Huso huso*. *Journal of Applied Ichthyology*, 29, 26-30. DOI:10.1111/jai.12017

Falahatkar, B., **2024**. Ship sturgeon extinction risk in the Caspian Sea. *Science*, 383, 838. DOI:10.1126/science.adn7523

Fitzsimons, J.D., **Williston, B.**, **Amcoff, P.**, **Balk, L.**, **Pecor, C.**, **Ketola, H.G.**, **Hinterkopf, J.P. and Honeyfield, D.C.**, **2005**. The effect of thiamine injection on upstream migration, survival, and thiamine status of putative thiamine hydrochloride-deficient Coho salmon. *Journal of Aquatic Animal Health*, 17, 48-58. DOI:10.1577/H04-003.1

Fitzsimons, J.D., **Clark, M. and Keir, M.**, **2009**. Addition of round gobies to the prey community of Lake Ontario and potential implications to thiamine status and reproductive success of lake trout. *Aquatic Ecosystem Health & Management*, 12, 296-312. DOI:10.1080/14634980903136453

Fitzsimons, J.D., **Brown, S.B. and El-Shaarawi, A.H.**, **2021**. Reduced thiamine utilization by Seneca Lake lake trout embryos and potential implications to restoration of lake trout in the Great Lakes. *Environmental Biology of Fishes*, 104, 751-766. DOI:10.1007/s10641-021-01109-4

Fontagné-Dicharry, S., **Lataillade, E.**, **Surget, A.**, **Brèque, J.**, **Zambonino-Infante, J.L. and Kaushik, S.J.**, **2010**. Effects of dietary vitamin A on broodstock performance, egg quality, early growth and retinoid nuclear receptor expression in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 303, 40-49. DOI:10.1016/j.aquaculture.2010.03.012

Furuita, H., **Ishida, T.**, **Suzuki, T.**, **Unuma, T.**, **Kurokawa, T.**, **Sugita, T. and Yamamoto, T.**, **2009**. Vitamin content and quality of eggs produced by broodstock injected with vitamins C and E during artificial maturation in Japanese eel *Anguilla japonica*. *Aquaculture*, 289, 334-339. DOI:10.1016/j.aquaculture.2009.01.024

Futia, M.H., **Hallenbeck, S.**, **Noyes, A.D.**, **Honeyfield, D.C.**, **Eckerlin, G.E. and Rinchard, J.**, **2017**. Thiamine deficiency and the effectiveness of thiamine treatments through broodstock injections and egg immersion on Lake Ontario steelhead trout. *Journal of Great Lakes Research*, 43, 352-358. DOI:10.1016/j.jglr.2017.01.004

Garg, M., **Sharma, A.**, **Vats, S.**, **Tiwari, V.**, **Kumari, A.**, **Mishra, V. and Krishania, M.**, **2021**. Vitamins in cereals: A critical review of content, health effects, processing losses, bioaccessibility, fortification, and biofortification strategies for their improvement. *Frontiers in Nutrition*, 8, 586815. DOI:10.3389/fnut.2021.586815

Ghanbari Rad, M., **Sharifi, M.**, **Meamar, R. and Soltani, N.**, **2022**. The role of pancreas to improve hyperglycemia in STZ-induced diabetic rats by thiamine disulfide. *Nutrition & Diabetes*, 12, 32. DOI:10.1038/s41387-022-00211-5

Ghiasi, S., **Falahatkar, B.**, **Dabrowski, K. and Abasalizadeh, A.**, **2014**. Effect of thiamine injection on growth performance, hematology and germinal vesicle migration in sterlet sturgeon *Acipenser ruthenus* L. *Aquaculture International*, 22, 1563-1576. DOI:10.1007/s10499-014-9765-7

Ghiasi, S., **Falahatkar, B.**, **Arslan, M. and Dabrowski, K.**, **2017**. Physiological changes and reproductive performance of sterlet sturgeon *Acipenser ruthenus* injected with thiamine. *Animal Reproduction Science*, 178, 23-30. DOI:10.1016/j.anireprosci.2017.01.006

Grzesiak, M., **Tchurzyk, M.**, **Socha, M.**, **Sechman, A. and Hrabia, A.**, **2022**. An overview of the current known and unknown roles of vitamin D3 in the female reproductive system: Lessons from farm animals, birds, and fish. *International Journal of Molecular Sciences*, 23, 14137. DOI:10.3390/ijms232214137

Harder, A.M., **Ardren, W.R.**, **Evans, A.N.**, **Futia, M.H.**, **Kraft, C.E.**, **Marsden, J.E.**, **Richter, C.A.**, **Rinchard, J.**, **Tillitt, D.E. and Christie, M.R.**, **2018**. Thiamine deficiency in fishes: Causes, consequences, and potential solutions. *Reviews in Fish Biology and Fisheries*, 28, 865-886. DOI:10.1007/s11160-018-9538-x

IUCN (International Union for Conservation of Nature), 2025. The IUCN Red List of Threatened Species. Version 2025-06. Available at: https://www.iucnredlist.org (accessed on 30 March 2025)

Izquierdo, M.S., **Fernandez-Palacios, H. and Tacon, A.G.J.**, **2001**. Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture*, 197, 25-42. DOI:10.1016/S0044-8486(01)00581-6

Keinänen, M., **Nikonen, S.**, **Käkelä, R.**, **Ritvanen, T.**, **Rokka, M.**, **Myllylä, T.**, **Pönni, J. and Vuorinen, P.J.**, **2022**. High lipid content of prey fish and n-3 PUFA peroxidation impair the thiamine status of feeding-migrating Atlantic salmon (*Salmo salar*) and is reflected in

hepatic biochemical indices. *Biomolecules*, 12, 526. DOI:10.3390/biom12040526

Kumar, N., **Gupta, S.K.**, **Bhushan, S. and Singh, N.P.**, **2019**. Impacts of acute toxicity of arsenic (III) alone and with high temperature on stress biomarkers, immunological status and cellular metabolism in fish. *Aquatic Toxicology*, 214, 105233. DOI:10.1016/j.aquatox.2019.105233

Lee, K.J. and Dabrowski, K., **2004**. Long-term effects and interactions of dietary vitamins C and E on growth and reproduction of yellow perch, *Perca flavescens*. *Aquaculture*, 230, 377-389. DOI:10.1016/S0044-8486(03)00421-6

Mancinelli, R., **Ceccanti, M.**, **Guiducci, M.S.**, **Sasso, G.F.**, **Sebastiani, G.**, **Attilia, M.L. and Allen, J.P.**, **2003**. Simultaneous liquid chromatographic assessment of thiamine, thiamine monophosphate and thiamine diphosphate in human erythrocytes: A study on alcoholics. *Journal of Chromatography*, 789B, 355-363. DOI:10.1016/S1570-0232(03)00139-9

Marrs, C. and Lonsdale, D., **2021**. Hiding in plain sight: Modern thiamine deficiency. *Cells*, 10, 2595. DOI:10.3390/cells10102595

Mitchell, K.T., **Garner, S.R.**, **Houde, A.L.**, **Wilson, C.C.**, **Pitcher, T.E. and Neff, B.D.**, **2021**. Effects of a low-thiamine diet on reproductive traits in three populations of Atlantic salmon targeted for reintroduction into Lake Ontario. *Canadian Journal of Fisheries and Aquatic Sciences*, 78, 135-143. DOI:10.1139/cjfas-2019-0379

Mohseni, M. and Ozorio, R.O.A., **2014**. Effects of dietary L-carnitine level on growth performance, body composition and antioxidant status of beluga, *Huso huso*. *Aquaculture Nutrition*, 20, 477-485. DOI:10.1111/anu.12105

Mohseni, M., **Hamidoghli, A. and Bai, S.C.**, **2021**. Organic and inorganic dietary zinc in beluga sturgeon (*Huso huso*): Effects on growth, hematology, tissue concentration and oxidative capacity. *Aquaculture*, 539, 736672. DOI:10.1016/j.aquaculture.2021.736672

Mohseni, M., **Ghelichpour, M.**, **Hassani, M.H.S.**, **Pajand, Z.O. and Vaghei, R.G.**, **2023**. Efficacy of dietary thiamine supplementation on growth performance, digestive enzymes, immunological responses, and antioxidant capacity of juvenile beluga (*Huso huso*). *Aquaculture Reports*, 31, 101664. DOI:10.1016/j.aqrep.2023.101664

Mrowicka, M., **Mrowicki, J.**, **Dragan, G. and Majsterek, I.**, **2023**. The importance of thiamine (vitamin B1) in humans. *Bioscience Reports*, 43, 20230374. DOI:10.1042/BSR20230374

Munro, A.D., **Scott, A.P. and Lam, T.J.**, **1990**. Reproductive Seasonality in Teleosts: Environmental Influences. CRC Press, USA. 49 P.

Nascimento, T.S.R., **De Stéfani, M.V.**, **Malheiros, E.B. and Koberstein, T.C.D.**, **2014**. High levels of dietary vitamin E improve the reproductive performance of female *Oreochromis niloticus*. *Acta Scientiarum Biological Sciences*, 36, 19-26. DOI:10.4025/actascibiolsci.v36i1.19381

NRC., 1993. Nutrient Requirements of Fish. National Academies Press, USA. 370 P.

NRC., 2011. Nutrient Requirement of Fish and Shrimp. National Research Council. National Academy Press, USA. 376 P.

Pacei, F., **Tesone, A.**, **Laudi, N.**, **Laudi, E.**, **Cretti, A.**, **Pnini, S.**, **Varesco, F. and Colombo, C.**, **2020**. The relevance of thiamine evaluation in a practical setting. *Nutrients*, 12, 2810. DOI:10.3390/nu12092810

Palace, V.P. and Werner, J., **2006**. Vitamins A and E in the maternal diet influence egg quality and early life stage development in fish: A review. *Scientia Marina*, 70, 41-57. DOI:10.3989/scimar.2006.70s241

Ruban, G., **Khodorevskaya, R. and Shatunovskii, M.**, **2019**. Factors influencing the natural reproduction decline in the beluga (*Huso huso*), Russian sturgeon (*Acipenser gueldenstaedtii*), and stellate sturgeon (*Acipenser stellatus*) of the Volga–Caspian basin: A review. *Journal of Applied Ichthyology*, 35, 387-395. DOI:10.1111/jai.13885

Sarmento, N.L., **Martins, E.F.**, **Costa, D.C.**, **Mattioli, C.C.**, **da Costa Julio, G.S.**, **Figueiredo, L.G.**, **Luz, M.R. and Luz, R.K.**, **2018**. Reproductive efficiency and egg and larvae quality of Nile tilapia fed different levels of vitamin C. *Aquaculture*, 482, 96-102. DOI:10.1016/j.aquaculture.2017.09.022

Sun, M., **Li, X.F.**, **Ge, Y.P.**, **Zhang, L.**, **Liu, B. and Liu, W.B.**, **2022**. Dietary thiamine requirement and its effects on glycolipid metabolism in Oriental River prawn (*Macrobrachium nipponense*). *Aquaculture*, 550, 737824. DOI:10.1016/j.aquaculture.2021.737824

Thiruvasagam, T., **Chidambaram, P.**, **Ranjan, A. and Komuhi, N.B.**, **2024**. Significance of fatty acids in fish broodstock nutrition. *Animal Reproduction Science*, 268, 107573. DOI:10.1016/j.anireprosci.2024.107573

Wang, W., **Zhu, H.**, **Tian, Z.**, **Sun, A.**, **Dong, Y.**, **Dong, T. and Hu, H.**, **2020**. Effects of 11-ketotestosterone on development of the previtellogenic ovary in the sterlet, *Acipenser ruthenus*. *Frontiers in Endocrinology*, 11, 115. DOI:10.3389/fendo.2020.00115

Webb, M.A., **Allert, J.A.**, **Kappenman, K.M.**, **Marcos, J.**, **Feist, G.W.**, **Schreck, C.B. and Shackleton, C.H.**, **2007**. Identification of plasma glucocorticoids in pallid sturgeon in response to stress. *General and Comparative Endocrinology*, 154, 98-104. DOI:10.1016/j.ygcen.2007.06.002

Wiegand, M.D., **Johnston, T.A.**, **Brown, L.R.**, **Brown, S.B.**, **Casselman, J.M. and Leggett, W.C.**, **2011**. Maternal influences on thiamine status of walleye *Sander vitreus* ova. *Journal of Fish Biology*, 78, 810-824. DOI:10.1111/j.1095-8649.2010.02894.x

Xu, H., **Turkmen, S.**, **Rimoldi, S.**, **Terova, G.**, **Zamorano, M.J.**, **Afonso, J.M.**, **Sarih, S.**, **Fernández-Palacios, H. and Izquierdo, M.**, **2019**. Nutritional intervention through dietary vegetable proteins and lipids to gilthead sea bream (*Sparus aurata*) broodstock affects the offspring utilization of a low fishmeal/fish oil diet. *Aquaculture*, 513, 734402. DOI:10.1016/j.aquaculture.2019.734402

Xu, C., **Li, Y.Y.**, **Brown, P.B.**, **Liu, W.B.**, **Gao, L.L.**, **Ding, Z.R.**, **Li, X.F. and Xie, D.Z.**, **2022**. Interactions between dietary carbohydrate and thiamine: Implications on the growth performance and intestinal mitochondrial biogenesis and function of *Megalobrama amblycephala*. *British Journal of*

Nutrition, 127, 321-334. DOI:10.1017/S000711452100101X

Yang, G., **Cheng, K.**, **Huang, Y. and Wang, C.**, **2024**. Vitamin D3 promotes fish oocyte development by directly regulating gonadal steroid hormone synthesis. *Biology of Reproduction*, 110, 521-535. DOI:10.1093/biolre/ioad176

Zhang, X., **Ma, Y.**, **Xiao, J.**, **Zhong, H.**, **Guo, Z.**, **Zhou, C.**, **Li, M.**, **Tang, Z.**, **Huang, K. and Liu, T.**, **2021**. Effects of vitamin E on the reproductive performance of female and male Nile tilapia (*Oreochromis niloticus*) at the physiological and molecular levels. *Aquaculture Research*, 52, 3518-3531. DOI:10.1111/are.15193

Zhou, D., **Wang, C.**, **Zheng, J.**, **Zhao, J.**, **Wei, S.**, **Xiong, Y.**, **Limbu, S.M.**, **Kong, Y.**, **Cao, F. and Ding, Z.**, **2022**. Dietary thiamine modulates carbohydrate metabolism, antioxidant status, and alleviates hypoxia stress in oriental river prawn *Macrobrachium nipponense* (de Haan). *Fish and Shellfish Immunology*, 131, 42-53. DOI:10.1016/j.fsi.2022.09.062

Zhang, L., **Huang, D.**, **Gu, J.**, **Liang, H. and Ren, M.**, **2025**. Evaluation of dietary vitamin B6 requirement of juvenile largemouth bass (*Micropterus salmoides*) on the basis of growth performance, transaminase activity, and nutrient metabolism. *Aquaculture Reports*, 42, 102743. DOI:10.1016/j.aqrep.2025.102743