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Effect of thiamine injection on growth performance, hematology and germinal vesicle migration in sterlet sturgeon *Acipenser ruthenus* L.

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Abstract The present study aimed to investigate the effect of thiamine on growth, hematological, egg thiamine content and oocyte nucleus migration indices in sterlet sturgeon (*Acipenser ruthenus*). A total of 45 female fish (698.6 ± 8.9 g) were distributed in nine fiberglass tanks with three treatments (each in three replicates). Experimental fish were fed once a day with practical diet supplemented with 1 g kg^{-1} amprolium hydrochloride (as the antithiamine) for 5 months before spawning. Thiamine hydrochloride was injected to fish with one of three doses at 0, 5 and 50 mg kg^{-1} body weight (BW) at three different stages. At the end of each month, fish were weighed and growth parameters such as weight gain, specific growth rate, feed efficiency and final weight were determined. At the end of the 5-month period, hematological parameters and egg thiamine content were measured. The results showed no significant differences in terms of growth performance. Hemoglobin, number of red blood cells and white blood cells were not significantly different among the treatments, but hematocrit was significantly higher in fish injected with 5 mg kg^{-1} BW thiamine. The mean corpuscular volume and mean corpuscular hemoglobin were not significantly different, but lower value of mean corpuscular hemoglobin concentration was observed in fish injected with 5 mg thiamine. Percentage of lympho-

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cytes, neutrophils, eosinophils and monocytes were not significantly different among treatments. The results of the germinal vesicle migration index at different stages showed no significant differences in different groups. Results showed that free thiamine, thiamine pyrophosphate and total thiamine in eggs significantly increased at 50 mg kg^{-1} injection dose compared with the control, but no significant different was found in thiamine monophosphate. The results revealed that thiamine injection to sterlet broodstocks has positive accumulation in egg thiamine content and some hematological parameters but have no effect on growth and ovarian maturation. We conclude that thiamine can affect some physiological features of broodstock, which needs to be specified in further studies.

Keywords Vitamin B_1 · Growth · Hematology · Germinal vesicle migration · Reproduction · Sturgeon

Introduction

Sturgeons are one of the most valuable fish species due to their quality of meat and market value of caviar. Stocks of sturgeons have been reduced due to overfishing, environmental pollution and damming the rivers resulting in the disturbance of reproduction (Birstein 1993; Pourkazemi et al. 1999; Billard and Lecointre 2001; Hurvitz et al. 2007). Therefore, most of the species of acipenserids are listed as endangered fish by IUCN (2012). Thus, it is necessary to improve propagation efficiency and enhance survival during embryonic development and larviculture of these species. Sterlet, *Acipenser ruthenus*, is the smallest species of sturgeon and considered the most suitable model among the sturgeons for biological, nutritional and genetic studies because of high early growth rate and earlier maturation compared with other sturgeons (Sokolov and Vasiliev 1989). Moreover, due to limitations in obtaining wild broodstock of other sturgeons, this species is considered as a biological model in many studies (Piros et al. 2002; Lahnsteiner et al. 2004; Williot et al. 2005). Despite advances in rearing methods during the recent years, there is no sufficient knowledge regarding vitamins requirement and their functions in sturgeons (Moreau et al. 1999; Hung and Deng 2002; Tatina et al. 2010).

Studies showed that nutrition has an important role in maturation and reproduction of fishes (Berglund 1995; Izquierdo et al. 2001; Watanabe and Vassallo-Agius 2003; Nyina-wamwiza et al. 2012; Bittencourt et al. 2012). Thiamine (vitamin B_1), the first described member of vitamin B family, is a water-soluble vitamin. This vitamin serves as a coenzyme and is essential for cellular metabolic processes, metabolism of fatty acids and carbohydrates (Brown et al. 1998), and neural activities (Amcoff et al. 2000). The enzyme thiaminase breakdown and destroy the thiamine in prey fish (Honeyfield et al. 2005) and results in reducing this vitamin in predatory fish. Deficiency sings such as poor appetite, lower growth rates (Halver 1972) and neural tissue degeneration have been described. Sensitivity to abrupt shocks and trunk whirling syndrome also has shown in eel *Anguilla japonica* (Hashimoto et al. 1970). In salmonids, thiamine deficiency leads to swimming disorders, loss of balance and fish death (Masumoto et al. 1987).

Previous studies showed that thiamine is an important nutrient in early life stages of fish (Fitzsimons et al. 2007; Lee et al. 2009). In this regard, some diseases including early mortality syndrome were identified as a result of thiamine deficiency, leading to major mortality in early life stages (Honeyfield et al. 2005). Most of the studies on thiamine and antithiamine have focused on salmonids, especially the effect of thiamine deficiency in eggs and alevins (Amcoff et al. 1998, 2000; Werner et al. 2006; Ketola et al. 2008;

Lee et al. 2009); however, no study has been performed in the effect of thiamine on egg thiamine content in sturgeons. Also less examined area was the influence of thiamine on hematological parameters. The positive effect of thiamine on hematological parameters in fish has been demonstrated previously (Ghazaly 1991; Feng et al. 2011).

Previous studies with salmonids showed that thiamine injection before spawning leads to positive effects on physiological indicators in broodstock (Borjeson et al. 1999; Amcoff et al. 2000; Ketola et al. 2000; Fitzsimons et al. 2005). No study has been performed in respect to thiamine effects on sturgeon broodstock. Therefore, this research aimed to investigate the effects of thiamine injection on growth, egg thiamine content, hematology and ovarian characteristics in sterlet sturgeon broodstocks.

Materials and methods

Fish and experimental conditions

This study was performed during a 5-month period from November 2011 to April 2012 in the Shahid Dr. Beheshti Sturgeon Fish Propagation and Rearing Complex (Guilan, Iran). One hundred and twenty females were weighed and examined for gonadal development characterized by germinal vesicle migration (GVM) (Dettlaff et al. 1993). Based on these indicators, 45 fish with similar weight and maturity stage were selected. Fish (697.8 ± 8.9 g; mean ± SE) were tagged, and 5 fish were introduced to each tank. Females were divided into three treatments with triplicate groups and allocated to 1,063 l squared fiberglass tanks (2 × 2 × 0.5 m). Water was supplied from Sefidrood River with non-circulated filtered water and the flow rate regulated to 13 ± 0.1 l min^{-1}. Throughout the experiment, fish were reared under natural photoperiod. During this period, the average water temperature and dissolved oxygen were 8.9 ± 0.3 °C and 7.5 ± 0.2 mg l^{-1}, respectively.

Feeding regime and experimental diet

Thiamine concentration was estimated in all diet ingredients and the ones that have lower thiamine content were used (www.nutritiondata.self.com). Thiamine was obtained from Sigma-Aldrich (vitamin B_1 hydrochloride; Fluka Sigma-Aldrich, Stuttgart, Germany), and amprolium hydrochloride was purchased from Animal Drug (Animal Drug Production, Tehran, Iran). Dry ingredients were mixed together, then amprolium was weighed and mixed with vitamins and minerals premixes and binder. The vitamin premix was made according to the NRC (1993) devoid of thiamine. This blend was mixed with the diet, and then oil and water were also added. The resulting complex was processed in an industrial meat grinder. Diets were dried at 40 °C for 36 h to adjust the moisture below 15 %. Diets were crushed to obtain pellets of 0.5 ± 0.01 g and 0.8 ± 0.01 cm long. To maintain the diets quality, pellets were produced monthly and kept at 0 °C. Fish were hand-fed 0.5 % of body weight (BW) (Falahatkar et al. 2013) once a day (12 at noon). Dietary ingredients and approximate diet composition are shown in Table 1.

Experimental design and thiamine injection

During the experimental period, fish were fed the diet containing 1 g amprolium kg^{-1} dry diet (Fynn-Aikins et al. 1998) as the antithiamine. Fish were injected by thiamine with one

Table 1 Composition of the experimental diet (dry weight)

Ingredients	g kg^{-1}
Fish meal[a]	340
Meat powder[b]	200
Soybean cake[c]	200
Wheat flour[d]	50
Wheat bran[d]	90
Fish oil[e]	30
Canola oil[f]	30
Carboxy methyl cellulose[g]	29
Vitamin mixture (vitamin B_1 free)[h]	15
Mineral mixture[i]	15
Amprolium hydrochloride[j]	1
Proximate composition ($n = 3$)	(%)
Crude protein	43 ± 0.6
Crude lipid	13.1 ± 0.2
Moisture	13.4 ± 0.1
Ash	9.7 ± 0.1

[a] Etehad North Caspian Company, Babol, Iran

[b] Hamedan Meat Powder Factory, Hamedan, Iran

[c] Modalal Company, Tehran, Iran

[d] Nahavand Flour Factory, Nahavand, Iran

[e] Etehad North Caspian Company, Babol, Iran

[f] Bahr Oil Factory, Varamin, Iran

[g] Tehran Acid Company, Tehran, Iran

[h] Vitamin mixture was manually provided according to feed requirements of the fish (NRC, 1993), and ingredients were obtained from Hashtgerd Laboratories (Hashtgerd, Alborz, Iran); which each 1,000 g vitamin mixture provides: vitamin A, 1,600,000 I.U; vitamin D_3, 400 000 I.U; riboflavin, 8 g; niacin, 12 g; pantothenic acid, 40 g; pyridoxine, 4 g; folic acid, 2 g; cyanocobalamin, 8 mg; vitamin C, 60 g; vitamin K_3, 2 g; biotin, 240 mg and inositol, 20 g; and vitamin E, 60 g

[i] Aquatic mineral premix was manufactured by Science Laboratories (Ghazvin, Iran); which each 1,000 g contains mineral trace elements: ferrous, 6,000 mg; zinc, 10,000 mg; selenium, 20 mg; cobalt, 100 mg; copper, 600 mg; magnesium, 5,000 mg; iodine, 600 mg and choline chloride, 6,000 mg

[j] Supplemented as amprolium hydrochloride (Animal Drug Production, Tehran, Iran)

of three doses of 0 (T0), 5 (T5) and 50 mg (T50) thiamine kg^{-1} BW (Ketola et al. 2000; Fitzsimons et al. 2005) at three different times (30, 90 and 150 days of the feeding trial).

For the preparation of injection solutions, thiamine was dissolved in 0.9 % NaCl physiological saline solution and then 10 M NaOH was added to adjust the pH to 7. Feeding was ceased 24 h before handling, weighing and injecting. Fish were anesthetized with 400 mg l^{-1} of clove powder extract. Thiamine solutions were slowly injected intraperitoneally to fish at 2 ml kg^{-1} BW (Fitzsimons et al. 2005). Fish were injected in early morning, and all procedures were carried out according to fish ethics principles (Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching; Granstrom 2003). After injections, fish were immediately transferred to the tanks with fresh water for recovery.

Growth performance

All fish were weighed at the end of each month, to examine the effect of thiamine on growth. All fish were anesthetized with 400 mg l^{-1} of clove powder extract and then weighed individually.

Weight gain (WG), specific growth rate (SGR) and feed efficiency (FE) in each group were determined using the following formulas:

$$\mathrm{SGR}\,(\%\ \mathrm{day}^{-1}) = 100 \times (\mathrm{Ln}\,W_t - \mathrm{Ln}\,W_0)/t$$

$$\mathrm{WG}\,(\%) = 100 \times (W_t - W_0)/W_0$$

$$\mathrm{FE}\,(\%) = 100 \times [\mathrm{WG}\,(\mathrm{g})/\mathrm{feed\ provided}\,(\mathrm{g})]$$

Germinal vesicle migration (GVM)

To investigate the effect of thiamine on GVM toward the animal pole, index was measured at three stages including (1) prior to the initiation of the experiment (day 0), (2) at the mid (on day 75) and (3) at the end (day 150) of feeding trial. All fish were examined for GVM distance prior to the experiment, and then, 3 fish at each phase were randomly examined from each tank. Fish were anesthetized and placed on the worktable, and samples of oocytes from the ovary were collected by small catheter. Fifteen eggs from each female were placed in an Eppendorf tube fixed in 4 % formaldehyde solution, transferred to laboratory and boiled gently for about 2–3 min. After that, eggs were dissected along the animal—the vegetal axis with a razor blade. The distance of animal and vegetal axis was measured under the stereoscope (Chapman and Van Eenennaam 2007). GVM was estimated according to Dettlaff et al. (1993) using the following formula:

$$\mathrm{GVM} = (\text{distance between nucleus and animal pole/distance between vegetal and animal poles})$$

Blood sampling and hematological analysis

At the termination of the experiment on day 150, 3 fish per tank were randomly caught for hematological assays. They were anesthetized, and 2 ml of blood sample was taken from the caudal vein by a 5-ml heparinized syringe.

A number of red blood cells (RBC) and white blood cells (WBC) were counted using a Neubauer hemocytometer according to Yuan et al. (2008). Hematocrit (Hct) value was measured using the standard microhematocrit method and expressed in percentage. Briefly, duplicate blood samples were loaded into standard heparinized capillary tubes, spun in a microhematocrit centrifuge (3,500*g* for 10 min) and measured on a microcapillary reader (Rey Vazquez and Guerrero 2007). Hemoglobin (Hb) concentration was assayed spectrophotometrically through cyanmethemoglobin method (Drabkin 1945). The following parameters were determined, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) according to the following formulas:

$$\mathrm{MCV}\,(\mathrm{fl}) = 10 \times (\mathrm{Hct/RBC})$$

$$\mathrm{MCH\,(pg\,cell^{-1})} = 10 \times (\mathrm{Hb/RBC})$$

$$\mathrm{MCHC\,(g\,dl^{-1})} = 100 \times (\mathrm{Hb/Hct})$$

Differential white blood cells were also counted, and the number of lymphocytes, neutrophils, eosinophils and monocytes were determined (Blaxhall and Daisley 1973).

Thiamine analysis

During the final maturation and ovulation, the eggs were obtained by cesarean section method (Falahatkar and Efatpanah Komaee 2011). Free thiamine (THCl), thiamine pyrophosphate (TPP) and thiamine monophosphate (TMP) were extracted from the sterlet eggs according to Brown et al. (1998). Vitamins were then quantified using a high-performance liquid chromatograph (HPLC) system as described by Brown et al. (1998) and Mancinelli et al. (2003) with slight modifications. The HPLC system consisted of a delivery system pump (1200 Series, Agilent Technologies, CA, USA) equipped with a 100-µl automatic injection unit connected to a 4.6 mm × 150 mm (Zorbax, Agilent Technologies, CA, USA) column coupled with NH_2 packed guard column. Fluorescent detector (G1321A FLD) was set at 375 nm for excitation and at 430 nm for emission. Mobile phase was composed of potassium phosphate buffer (pH 7.5, 85 mM) + acetonitrile (65:35, v:v). Flow rate was 0.5 ml per min. The column thermostat was set at 30 °C. Each external standard curve for THCl, TMP and TPP was prepared using 1 mM of each standard stock solution in 0.01 M HCl. Each standard concentration ranged from 1.0 to 100 nmol l^{-1} for linearity. Extraction recovery rates were 94.7 ± 3.0 % (n = 4) for THCl and 100 % for both TMP and TPP. For the recovery, known amounts of each thiamine hydrochloride, TMP and TPP standards were added into running samples at the beginning of the extraction and followed by extraction procedure as described above.

Statistical analysis

All assays were performed in triplicates, and data were shown as mean ± SE for each treatment. At first, Kolmogorov–Smirnov test was applied to check for the normality of data. One-way analysis of variance (ANOVA) was applied to study the effect of thiamine on growth, hematological and GVM indices using SPSS 16.0 (Chicago, IL). Because of non-homogeneity in variance of WG, SGR, MCV and MCHC, data were transformed to log 10 prior to statistical analysis. Differences between treatments' means were determined by Tukey's as a post hoc test. Differences were considered statistically significant when $P < 0.05$.

Results

Growth indices of broodstocks fed diets containing amprolium and injected with thiamine are shown in Table 2. There were no significant differences in FW, WG, SGR and FE ($P > 0.05$).

Hematological parameters of sterlet sturgeon broodstocks fed by amprolium and periodically injected with thiamine are shown in Table 3. T_5 treatment had the highest RBC; however, there were no significant differences among treatments ($P > 0.05$). The highest and lowest Hct levels were found in fish that were injected with 5 mg kg^{-1} BW and

Table 2 Growth indices of sterlet sturgeon fed diets supplemented with amprolium hydrochloride in parallel to periodical thiamine injection during 5 months

Treatment	Initial weight (g)	Final weight (g)	SGR (% day^{-1})	WG (%)	FE (%)
T0	700.6 ± 20.8	744.6 ± 22.6	0.04 ± 0.01	6.6 ± 2.4	1.1 ± 0.5
T5	690.6 ± 18.7	757.3 ± 17.7	0.06 ± 0.00	9.8 ± 1.2	1.6 ± 0.1
T50	704.6 ± 18.3	783.3 ± 24.2	0.06 ± 0.01	11.2 ± 1.9	1.8 ± 0.1

All data are expressed as mean ± SE

T0: 0 mg thiamine, T5: 5 mg thiamine, T50: 50 mg thiamine per kg body weight

SGR specific growth rate, *WG* weight gain, *FE* feed efficiency

No significant differences were observed ($P > 0.05$)

0 mg kg^{-1} BW thiamine ($P < 0.05$), respectively. No significant differences were observed in MCH, MCV and Hb ($P > 0.05$). The highest MCHC was observed in treatment when no thiamine was injected and the lowest in fish treated with 5 mg thiamine ($P < 0.05$).

No significant differences were observed among the treatments in WBC ($P > 0.05$; Table 4). Moreover, there were no significant differences among lymphocytes, neutrophils, eosinophils and monocytes ($P > 0.05$).

Table 3 Hematological indices of sterlet sturgeon fed diets supplemented with amprolium hydrochloride in parallel to periodical thiamine injection during 5 months

Treatment	RBC ($\times 10^5$ mm^{-3})	Hct (%)	Hb (g dl^{-1})	MCH (pg $cell^{-1}$)	MCHC (g dl^{-1})	MCV (fl)
T0	1.32 ± 0.05	27.0 ± 1.3^{b}	6.3 ± 0.3	49.1 ± 3.6	24.2 ± 1.9^{a}	20.7 ± 1.3
T5	1.52 ± 0.1	32.0 ± 1.1^{a}	6.8 ± 0.4	50.2 ± 6.2	21.7 ± 1.5^{b}	23.2 ± 2.2
T50	1.32 ± 0.12	29.6 ± 1.4^{ab}	6.9 ± 0.3	58.1 ± 5.4	24.0 ± 2.1^{a}	24.8 ± 2.2

All data are expressed as mean ± SE

T0: 0 mg thiamine, T5: 5 mg thiamine, T50: 50 mg thiamine per kg body weight

RBC number of red blood cells, *Hct* hematocrit, *Hb* hemoglobin, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *MCV* mean corpuscular volume

Numbers with different superscripts indicating significant differences between treatments ($P < 0.05$)

Table 4 Number of white blood cells (WBC) and differential white blood cells counts of sterlet sturgeon fed diets supplemented with amprolium hydrochloride in parallel to periodical thiamine injection during 5 months

Treatment	WBC ($\times 10^3$ mm^{-3})	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)	Monocytes (%)
T0	62.3 ± 7.5	91.7 ± 1.4	6.8 ± 1.2	0.8 ± 0.3	0.3 ± 0.1
T5	56.4 ± 3.1	93.0 ± 1.0	5.4 ± 0.9	1.3 ± 0.4	0.2 ± 0.1
T50	67.4 ± 5.7	87.3 ± 2.7	11.2 ± 1.9	0.8 ± 0.3	0.6 ± 0.4

All data are expressed as mean ± SE

T0: 0 mg thiamine, T5: 5 mg thiamine, T50: 50 mg thiamine per kg body weight

No significant differences were observed ($P > 0.05$)

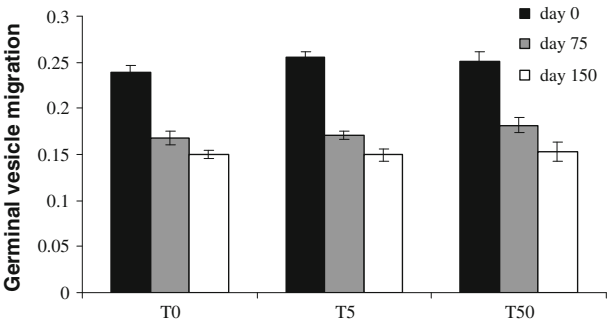

Fig. 1 Germinal vesicle migration (GVM) of sterlet sturgeon fed diets supplemented with amprolium hydrochloride in parallel to periodical thiamine injection during 5 months. GVM was measured in 3 stages at day 0, 75 and 150 of feeding trial. Data are expressed as mean ± SE. T0: 0 mg thiamine, T5: 5 mg thiamine, T50: 50 mg thiamine per kg body weight

Table 5 Egg thiamine concentration of sterlet sturgeon fed diets supplemented with amprolium hydrochloride in parallel to periodical thiamine injection during 5 months

Treatment	Free thiamine (nmol g^{-1})	Thiamine monophosphate (nmol g^{-1})	Thiamine pyrophosphate (nmol g^{-1})	Total thiamine (nmol g^{-1})
T0	0.2 ± 0.0^{b}	0.5 ± 0.1	2.1 ± 0.3^{b}	2.9 ± 0.4^{b}
T5	0.4 ± 0.0^{ab}	0.5 ± 0.0	3.2 ± 0.8^{ab}	4.2 ± 0.8^{ab}
T50	0.8 ± 0.2^{a}	0.7 ± 0.1	5.6 ± 1.3^{a}	7.3 ± 1.6^{a}

All data are expressed as mean ± SE

T0: 0 mg thiamine, T5: 5 mg thiamine, T50: 50 mg thiamine per kg body weight

Numbers with different superscripts indicating significant differences between treatments ($P < 0.05$)

GVM index was measured at different stages, and the results showed no significant differences in all periods ($P > 0.05$; Fig. 1). Mean of GVM in T0, T5, T50 treatments in day 0, day 75 and at the end of experiment (day 150) showed reduction trend in each treatments.

Egg thiamine concentrations of sterlet broodstocks fed amprolium and periodically injected with thiamine are shown in Table 5. Results showed that free, TPP and total thiamine in eggs significantly increased at T50 compared with the control ($P < 0.05$), but no significant difference was found in TMP ($P > 0.05$) among treatments. Also thiamine analysis in egg showed the TPP was the predominant form of thiamine in sterlet eggs.

Discussion

Results of this study showed no differences in growth indices among the treatments, but injection of thiamine to fish at T50 level resulted in the BW 40 g larger than in control group. Studies on the thiamine requirement in different fish species showed the positive effect of this vitamin on growth performance. The effect of thiamine in juvenile Jian carp *Cyprinus carpio* var. Jian with an average weight of 8.2 g showed that thiamine could increase growth in fish fed 0.79 and 1.06 mg kg^{-1} diet (Huang et al. 2010). In addition, the authors showed the lower WG in control fish. Anderson and Murai (1978) found out that

sufficient amount of thiamine for optimum growth in channel catfish *Ictalurus punctatus* is 1 mg kg^{-1} of diet. In tilapia (*Oreochromis niloticus*), the best growth was found when thiamine level amounted to 3.5 mg kg^{-1} (Lim and Yildirim-Aksoy. 2011). In general, fish require 1–15 mg kg^{-1} thiamine in the diet (NRC 1993).

Keeping fish under low water temperature conditions resulted in no differences in WG and other growth indices, but this temperature requirement is necessary to keep sturgeon breeders prior to spawning (Dettlaff et al. 1993). Most studies regarding determination of the thiamine requirements have focused on the juveniles and growing fish. This may magnify the effect of thiamine on growth. In the present study, fish were in prespawning conditions and many studies showed that during this period the dietary nutrients have less impact on gonadal growth and somatic growth (Rijnsdorp 1990; Jennings and Philipp 1992; Moltschaniwskyj 1995; Quince et al. 2008).

In fish, like in other animals, hematological factors indicate the physiological condition and they are mostly affected by nutrients (Lim et al. 2000; Chen et al. 2004; Garcia et al. 2007). In our study, significant differences in hematocrit level were observed between treatments and the lowest level was observed in T5 treatment. Lim and Yildirim-Aksoy (2011) showed that tilapia fed diet without thiamine had a lower hematocrit and RBC level than fish fed diet containing 2 and 8 mg kg^{-1} thiamine. As a result of reduction in appetite and feeding, the amounts of nutrient reaching the body may reduce the RBC and hematocrit. Lim and LeaMaster (1991) showed that an increase in hematocrit level in juvenile hybrid red tilapia was related to an increase of thiamine level in diets. Ghazaly (1991) used thiamine as a treatment for lead poisoned *Tilapia zillii* and found that there is no relationship between hematocrit and WBC with thiamine concentrations, but RBC and hemoglobin have been reduced in fish without thiamine. Twelve weeks study on thiamine requirement in rainbow trout *Oncorhynchus mykiss* showed that there is no difference in hemoglobin and hematocrit between fish fed diet without thiamine and 15 mg thiamine kg^{-1} diet (Morito et al. 1986). Feng et al. (2011) found that feeding with different levels of thiamine in common carp *Cyprinus carpio* juveniles increased Hct and RBC levels, and they suggested diet containing 3.5 mg thiamine kg^{-1} diet for normal RBC and hematocrit levels, and diet with 1.03 mg kg^{-1} for maximum WBC level and the increased immunity.

Regarding MCHC, in our study, there were significant differences between treatments, so that, T0 and T5 treatments had the highest and lowest levels, respectively. No study has been done in relation to thiamine effect on MCHC, but studies on other vitamins show that the MCHC was influenced by vitamin level (Agrawal and Mahajan 1983; Graff et al. 2002; Menezes et al. 2006; Andrade et al. 2007). The calculated blood indices of MCHC and MCV have a particular importance in anemia diagnosis in many animal species (Coles 1986). It seems that thiamine deficiency causes a mild anemia in fish and reduced the MCV in control treatment.

In this study, thiamine injection to sterlet broodstocks showed some differences in hematological parameters. Other studies showed that thiamine deficiency reduced the thiamine concentration in hematopoietic tissue such as kidney and spleen (Fitzsimons et al. 2005; Ketola et al. 2008). This consequently reduced the hematopoiesis and blood cells production. Therefore, in the present study, it seems that thiamine deficiency could have caused a mild anemia in fish of T0 group.

In the present study, GVM index was measured at three stages and the results showed no significant effect of thiamine on GVM in the middle and at the end of the trial. There is no information about the effects of feeding on GVM in sturgeons, and no study defined the effect of different vitamins on GVM. Results of Yue (2011) regarding the effect of feeding on GVM in white sturgeon (*Acipenser transmontanus*) showed no significant differences in

GVM of fish that were fed different lipid and protein levels. This study showed no significant difference in GVM, but control fish had a lower GVM compared with the vitamin injected fish. According to the long cycle of sturgeon reproduction, it seems that in a longer period of exposure, some positive effects will be observed in respect to thiamine impact on GVM.

Thiamine injection increased the thiamine content in the egg. Similar study on Atlantic salmon *Salmo salar* broodstock showed that when fish were fed amprolium before the spawning, thiamine level in the eggs was lower than in fish fed control diet (Fynn-Aikins et al. 1998). Also other study showed that reduction of thiamine in broodfish diet caused a decrease of thiamine in egg of salmon (Fitzsimons et al. 2009). Coho salmon *Oncorhynchus kisutch* breeders that were fed diets containing antithiamine and followed with the injection by thiamine showed increasing thiamine levels in the eggs (Fitzsimons et al. 2005). Carvalho et al. (2009) in a study of antithiamine effect on egg thiamine content in lake trout, *Salvelinus namaycush* reported that an increase of the antithiamine in broodstock diets caused reduced the thiamine concentration in eggs. Ketola et al. (2000) showed that injection of 7 mg kg^{-1} thiamine before spawning of Atlantic salmon increase the thiamine content in the eggs. Our results showed that the lowest and highest levels of eggs thiamine were in fish at T0 and T50, respectively.

Results of this study showed that TPP is the predominant form in the eggs and that is a similar result as in the study of Rinchard et al. (2011) on walleye *Sander vitreus* and in contrast to salmonid eggs, where free thiamine is the major form of thiamine in eggs (Koski et al. 2005). Dakshinamurti and Chauhan (1994) have suggested that thiamine is binding with protein and that this protein plays a role in the transport the free thiamine to egg. Therefore, it seems that the majority of thiamine can enter into the eggs with binding to the specific protein.

TPP is active and stored form of thiamine in the body. Based on this study, it seems that thiamine in eggs has a positive relationship with thiamine amount in broodfish diet and other studies reported similar phenomenon in relation to other vitamins (Watanabe 1985; Izquierdo et al. 2001; Furuita et al. 2009). Also, due to the possibility of vitamins stored in oocytes and gonadal tissue (Sandnes 1991; Lee and Dabrowski 2004), it seems thiamine stored in this tissue and is transferred to egg at the vitellogenesis stage.

In conclusion, present study showed that growth and GVM were not affected by thiamine injection in sterlet broodstocks. Therefore, due to factors such as short time of feeding and low feed intake under low water temperature, growth and gonadal development were not influenced. However, thiamine showed some positive effect on thiamine content in the egg and hematological parameters such as hematocrit and MCHC and therefore, it is necessary to use this vitamin in broodstock nutrition.

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References

Agrawal NK, Mahajan CL (1983) Haematological and haematopoietic studies in pyridoxine deficient fish, *Channa punctatus* Bloch. J Fish Biol 22:91–103. doi:10.1111/j.1095-8649.1983.tb04729.x

Amcoff P, Borjeson H, Lindeberg J, Norrgren L (1998) Thiamine (vitamin B1) concentrations in feral Baltic salmon exhibiting the M74 syndrome. In: McDonald G, Fitzsimons JD, Honeyfield DC (eds) Early life stage mortality syndrome in fishes of the Great Lakes and Baltic Sea. Proceedings of the 126th annual meeting of the Am Fish Soc, 25–29 Aug. Am Fish Soc Symp 21, Dearborn, Michigan pp 82–89

Amcoff P, Akerman G, Borjeson H, Tjarnlund U, Norrgren L, Balk L (2000) Hepatic activities of thiamine hydrochloride-dependent enzymes, glucose-6-phosphate dehydrogenase and cytochrome P4501A in Baltic salmon (*Salmo salar*) yolk-sac fry after thiamine treatment. Aquat Toxicol 48:391–402. doi:10.1016/S0166-445X(00)00089-8

Anderson JW, Murai T (1978) Thiamin requirement of channel catfish fingerling. J Nutr 108:176–180. doi:10.1016/0044-8486(88)90292-X

Andrade JI, Ono EA, Cruz G, Menezes GC, Brasil EM, Roubach R, Urbinati EC, Tavares-Dias M, Marcon JL, Affonso EG (2007) Influence of diets supplemented with vitamins C and E on pirarucu (*Arapaima gigas*) blood parameters. Comp Biochem Physiol 146A:576–580. doi:10.1016/j.cbpa.2006.03.017

Berglund I (1995) Effects of spring temperature and feeding regime on sexual maturation in Atlantic salmon *Salmo salar* L. male parr. In: Goetz FW, Thomas P (eds) Reproductive physiology of fish, general and comparative endocrinology. Fish Symposium 95, Austin, Texas, pp 170–172

Billard R, Lecointre G (2001) Biology and conservation of sturgeon and paddlefish. Rev Fish Biol Fish 10:355–392. doi:10.1023/A:1012231526151

Birstein VJ (1993) Sturgeons and paddlefishes: threatened fishes in need of conservation. Conserv Biol 7:773–787. doi:10.1046/j.1523-1739.1993.740773.x

Bittencourt F, Souza BE, Lui TA, Borella MI, Boscolo WR, Feiden A, Romagosa E (2012) Protein diets promote the maturation of oocytes and spawning of *Piaractus mesopotamicus* kept in cages. J Appl Ichthyol 28:886–893. doi:10.1111/jai.12060

Blaxhall PC, Daisley KW (1973) Routine haematological methods for use with fish blood. J Fish Biol 5:771–781. doi:10.1111/j.1095-8649.1973.tb04510.x

Borjeson H, Amcoff P, Ragnarsson B (1999) Reconditioning of sea-run Baltic salmon (*Salmo salar*) that have produced progeny with the M74 syndrome. Ambio 28:30–36

Brown SB, Fitzsimons JD, Palace VP, Vandenbyllaardt L (1998) Thiamine and early mortality syndrome in lake trout. Am Fish Soc Symp 21, 28 Aug, Dearborn, Michigan, USA

Carvalho PSM, Tillitt DE, Zajicek JL, Dale RA, Honeyfield DC, Fitzsimons JD, Brown SB (2009) Thiamine deficiency effects on the vision and foraging ability of lake trout fry. J Aquat Anim Health 21:315–325. doi:10.1577/H08-025.1

Chapman FA, Van Eenennaam JP (2007) Sturgeon aquaculture - specialized techniques: determining the stage of sexual maturity in female sturgeon for artificial spawning: the egg polarization index or PI. Fisheries and Aquatic Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida

Chen R, Lochmann R, Goodwin A, Praveen K, Dabrowski K, Lee K (2004) Effects of dietary vitamins C and E on alternative complement activity, hematology, tissue composition, vitamin concentrations and response to heat stress in juvenile golden shiner (*Notemigonus crysoleucas*). Aquaculture 242:553–569. doi:10.1016/j.aquaculture.2004.09.012

Coles EH (1986) Remove from marked records. In: Saunders WB (ed) Veterinary clinical pathology. Saunders Company, Philadelphia, pp 10–42

Dakshinamurti K, Chauhan J (1994) Binding proteins for a-tocopherol, L-ascorbic acid, thiamine, and vitamin B6. In: Dakshinamurti K (ed) Vitamin receptors: vitamins as ligands in cell communication. Cambridge University Press, New York, pp 190–193

Dettlaff TA, Ginsburg AS, Schmalhausen OI (1993) Sturgeon fishes; Developmental biology and aquaculture. Springer, Berlin, 300 pp

Drabkin DR (1945) Crystallographic and optical properties of human hemoglobin: a proposal for the standardization of hemoglobin. Am J Med Sci 209:268–270. doi:10.1126/science.108.2792.15

Falahatkar B, Efatpanah Komaee I (2011) Egg extraction of sterlet sturgeon, *Acipenser ruthenus*, through surgery. J Vet Res 4:349–353

Falahatkar B, Akhavan SR, Efatpanah I, Meknatkhah B (2013) Effect of winter feeding and starvation on the growth performance of young-of-year (YOY) great sturgeon, *Huso huso*. J Appl Ichthyol 29:26–30. doi:10.1111/j.1439-0426.2012.02017.x

Feng L, Huang HH, Liu Y, Jiang WD, Hu K, Li SH (2011) Effect of dietary thiamin supplement on immune responses and intestinal microflora in juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquac Nutr 17:557–569. doi:10.1111/j.1365-2095.2011.00851.x

Fitzsimons JD, Williston B, Amcoff P, Balk L, Pecor C, Ketola HG, Hinterkopf JP, Honeyfield DC (2005) The effect of thiamine injection on upstream migration, survival, and thiamine status of putative thiamine hydrochloride-deficient coho salmon. J Aquat Anim Health 17:48–58. doi:10.1577/H04-003.1

Fitzsimons JD, Williston B, Williston G, Brown L, Shaarawi AE, Vandenbyllaardt L, Honeyfield D, Tillitt D, Wolgamood M, Brown SB (2007) Egg thiamine status of Lake Ontario salmonines, 1995–2004, with emphasis on lake trout. J Gt Lakes Res 33:93–103. doi:10.3394/0380-1330(2007)33[93:ETSOLO]2.0.CO;2

Fitzsimons JD, Brown SB, Williston B, Williston G, Brown LR, Moore K, Honeyfield DC, Tillitt DE (2009) Influence of thiamine deficiency on lake trout larval growth, foraging, and predator avoidance. J Aquat Anim Health 21:302–314. doi:10.1577/H08-019.1

Furuita H, Ishida T, Suzuki T, Unuma T, Kurokawa T, Sugita T, 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.2009.01.032

Fynn-Aikins K, Bowser P, Honeyfield DC, Fitzsimons J, Ketola HG (1998) Effect of dietary amprolium hydrochloride on tissue thiamin and Cayuga syndrome in Atlantic salmon. Trans Am Fish Soc 127:747–757. doi:10.1577/1548-8659(1998)127<0747:EODAOT>2.0.CO;2

Garcia F, Pilarski F, Onaka EM, de Moraes FR, Martins ML (2007) Hematology of *Piaractus mesopotamicus* fed diets supplemented with vitamins C and E, challenged by *Aeromonas hydrophila*. Aquaculture 271:39–46. doi:10.1016/j.aquaculture.2007.06.021

Ghazaly KS (1991) Influences of thiamin on lead intoxication, lead deposition in tissues and lead hematological responses of *Tilapia zillii*. Comp Biochem Physiol C 3:417–421. doi:10.1016/0742-8413(91)90018-O

Graff E, Waagbo R, Fivelstad S, Vermeer C, Lie O, Klundeby A (2002) A multivariate study on the effects of dietary vitamin K, vitamin D3 and calcium, and dissolved carbon dioxide on growth, bone minerals, vitamin status and health performance in smolting Atlantic salmon *Salmo salar* L. J Fish Dis 25:599–614. doi:10.1046/j.1365-2761.2002.00403.x

Granstrom DE (2003) Agricultural (non biomedical) animal research outside the laboratory: a review of guidelines for institutional animal care and use committees. ILAR 44:206–210. doi:10.1093/ilar.44.3.206

Halver JE (1972) The vitamins. In: Halver JE (ed) Fish nutrition. Academic Press, New York, pp 29–103

Hashimoto Y, Arai S, Nose T (1970) Thiamine deficiency symptoms experimentally induced in the eel. Bull Jpn Soc Sci Fish 36:791–797

Honeyfield DC, Hinterkopf JP, Fitzsimons JD, Tillitt DE, Zajicek JL, Brown SB (2005) Development of thiamine deficiencies and early mortality syndrome in lake trout by feeding experimental and feral fish diets containing thiaminase. J Aquat Anim Health 17:4–12. doi:10.1577/H03-078.1

Huang JW, Feng L, Liu Y, Jiang J, Jiang WD, Hu K, Li SH, Zhou X (2010) Effect of dietary thiamin supplementation on growth, body composition and intestinal enzyme activities of juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquac Nutr 17:233–240. doi:10.1111/j.1365-2095.2010.00756.x

Hung S, Deng D (2002) Sturgeon, *Acipenser* spp. In: Webster CD, Lim C (eds) Nutrient requirement and feeding of finfish for aquaculture. Kluwer Academic, New York, pp 344–357

Hurvitz A, Jackson K, Degani G, Levavi-Sivan B (2007) Use of endoscopy for gender and ovarian stage determinations in Russian sturgeon (*Acipenser gueldenstaedtii*) grown in aquaculture. Aquaculture 270:158–166. doi:10.1016/j.aquaculture.2007.05.020

IUCN (2012) IUCN Red List of Threatened Species. Available at: www.iucnredlist.org. Downloaded on 12 Nov 2012

Izquierdo MS, Fernandez-Palacios H, Tacon AGJ (2001) Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197:25–42. doi:10.1016/S0044-8486(01)00581-6

Jennings MJ, Philipp DP (1992) Reproductive investment and somatic growth rates in longear sunfish. Environ Biol Fish 35:257–271. doi:10.1007/BF00001892

Ketola HG, Bowser PR, Wooster GA, Wedge LR, Hurst SS (2000) Effects of thiamine on reproduction of Atlantic salmon and a new hypothesis for their extirpation in Lake Ontario. Trans Am Fish Soc 129:607–612. doi:10.1577/1548-8659(2000)129<0607:EOTORO>2.0.CO;2

Ketola HG, Isaacs GR, Robins JS, Loyd RC (2008) Effectiveness and retention of thiamine and its analogs administered to steelhead and landlocked Atlantic salmon. J Aquat Anim Health 20:29–38. doi:10.1577/H07-012.1

Koski P, Bäckman C, Pelkonen O (2005) Pharmacokinetics of thiamine in female Baltic salmon (*Salmo salar* L.) broodfish. Environ Toxicol Pharmacol 19:139–152. doi:10.1016/j.etap.2004.06.001

Lahnsteiner B, Berger F, Horvath A, Urbanyi B (2004) Studies on the semen biology and sperm cryopreservation in the sterlet, *Acipenser ruthenus* L. Aquac Res 35:519–528. doi:10.1111/j.1365-2109.2004.01034.x

Lee KJ, 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

Lee B, Jaroszewska M, Dabrowski K, Czesny S, Rinchard J (2009) Effects of vitamin B1 (thiamine hydrochloride) deficiency in lake trout alevins and preventive treatments. J Aquat Anim Health 21:290–301. doi:10.1577/H07-053.1

Lim C, LeaMaster B (1991) Thiamin requirement of red hybrid tilapia grown in seawater. In: 22nd annual conference and exposition, world aquaculture society, San Juan, Puert Rico, June 16–20

Lim C, Yildirim-Aksoy M (2011) Thiamin requirement of Nile tilapia, *Oreochromis niloticus*. J World Aquac Soc 42:824–833. doi:10.1111/j.1749-7345.2011.00531.x

Lim C, Klesius PH, Li MH, Robinson EH (2000) Interaction between dietary levels of iron and vitamin C on growth, hematology, immune response and resistance of channel catfish (*Ictalurus punctatus*) to *Edwardsilla ictaluri* challenge. Aquaculture 185:313–327. doi:10.1016/S0044-8486(99)00352-X

Mancinelli R, Ceccanti M, Guiducci MS, Sasso GF, Sebastiani G, Attilia ML, Allen JP (2003) Simultaneous liquid chromatographic assessment of thiamine, thiamine monophosphate and thiamine diphosphate in human erythrocytes: a study on alcoholics. J Chromatogr 789:355–363. doi:10.1016/S1570-0232(03)00139-9

Masumoto T, Hardy RW, Casillas E (1987) Comparison of transketolase activity and thiamin pyrophosphate levels in erythrocytes and liver of rainbow trout (*Salmo gairdneri*) as indicator of thiamin status. J Nutr 117:1422–1426. doi:10.1007/BF098690209

Menezes JC, Tavares-Dias M, Akifumi E, Andrade JA, Brasil LM, Roubach R, Urbinati EC, Marcon JL, Affonso AJ (2006) The influence of dietary vitamin C and E supplementation on the physiological response of pirarucu, *Arapaima gigas*, in net culture. Comp Biochem Physiol 145A:274–279. doi:10.1016/j.cbpa.2006.03.017

Moltschaniwskyj NA (1995) Multiple spawning in the tropical squid *Photololigo* sp: what is the cost in somatic growth? Mar Biol 124:127–135. doi:10.1007/BF00349154

Moreau R, Dabrowski K, Sato PH (1999) Renal L-gulono-1,4-lactone oxidase as affected by dietary ascorbic acid in lake sturgeon (*Acipenser fulvescens* R). Aquaculture 180:359–372. doi:10.1016/S0044-8486(99)00211-2

Morito CLH, Conrad DH, Hilton JW (1986) The thiamin deficiency signs and requirement of rainbow trout (*Salmo gairdneri*, Richardson). Fish Physiol Biochem 1:93–104. doi:10.1007/BF02290209

NRC (1993) Nutrient requirements of fish. National Academies Press, Washington DC, 114 pp

Nyina-wamwiza L, Defreyne PS, Ngendahayo L, Milla S, Mandiki SNM, Kestemont P (2012) Effects of partial or total fish meal replacement by agricultural by-product diets on gonad maturation, sex steroids and vitellogenin dynamics of African catfish (*Clarias gariepinus*). Fish Physiol Biochem 38:1287–1298. doi:10.1007/s10695-012-9616-2

Piros B, Glogowski J, Kolman R, Rzemieniecki A, Domagala J, Horvath A, Urbanyi B, Ciereszko A (2002) Biochemical characterization of Siberian sturgeon *Acipenser baeri* and sterlet *Acipenser ruthenus* milt plasma and spermatozoa. Fish Physiol Biochem 26:289–295. doi:10.1023/A:1026280218957

Pourkazemi M, Skibinski DOF, Beardmore JA (1999) Application of mtDNA d-loop region for the study of Russian sturgeon population structure from Iranian coastline of the Caspian Sea. J Appl Ichthyol 15:23–28. doi:10.1111/j.1439-0426.1999.tb00199.x

Quince C, Shuter BJ, Abrams PA, Lester NP (2008) Biphasic growth in fish II: empirical assessment. J Theor Biol 27:207–214. doi:10.1016/j.jtbi.2008.05.030

Rey Vazquez G, Guerrero GA (2007) Characterization of blood cells and hematological parameters in *Cichlasoma dimerus* (Teleostei, Perciformes). Tissue Cell 39:151–160

Rijnsdorp AJ (1990) The mechanism of energy allocation over reproduction and somatic growth in female North Sea plaice, *Pleuronectes platessa* L. Neth J Sea Res 25:279–289. doi:10.1016/0077-7579(90)90027-E

Rinchard J, Dabrowski K, Marschal E (2011) Egg thiamine concentration affects embryo survival in Lake Erie walleye. Environ Biol Fish 90:53–60. doi:10.1007/s10641-010-9717-7

Sandnes K (1991) Vitamin C in fish nutrition. Fisk Skr Ser Ernaer 4:3–32

Sokolov LI, Vasiliev VP (1989) *Acipenser ruthenus* Linnaeus. In: Holčik J (ed) The freshwater fishes of Europe. AULA-Verlag, Wiesbaden, pp 227–262

Tatina M, Bahmani M, Soltani M, Abtahi B, Gharibkhani M (2010) Effects of different levels of dietary vitamin C and E on some of hematological and biochemical parameters of sterlet (*Acipenser ruthenus*). J Fish Aquat Sci 5:1–11. doi:10.3923/jfas.2010.1.11

Watanabe T (1985) Importance of the study of broodstock nutrition for further development of aquaculture. In: Cowey CB, Mackie AM, Bell JG (eds) Nutrition and feeding in fish. Academic Press, London, pp 395–414

Watanabe T, Vassallo-Agius R (2003) Broodstock nutrition research on marine finfish in Japan. Aquaculture 227:35–61. doi:10.1016/S0044-8486(03)00494-0

Werner MR, Rook B, Greil R (2006) Egg-thiamine status and occurrence of early mortality syndrome (EMS) in Atlantic salmon from the St. Marys River, Michigan. J Gt Lakes Res 32:293–305. doi:10.3394/0380-1330(2006)32[293:ESAOOE]2.0.CO;2

Williot P, Bruna TR, Rouaulta T, Pelarda M, Merciera D, Ludwigb A (2005) Artificial spawning in cultured sterlet sturgeon, *Acipenser ruthenus* L., with special emphasis on hermaphrodites. Aquaculture 246:263–273. doi:10.1016/j.aquaculture.2005.02.048

Yuan CT, Pan XP, Gong Y, Xia AJ, Wu GH, Tang JQ, Han XD (2008) Effects of astragalus polysaccharides (APS) on the expression of immune response genes in head kidney, gill and spleen of the common carp, *Cyprinus carpio* L. Int Immunopharmacol 8:51–58. doi:10.1016/j.intimp.2007.10.009

Yue Z (2011) Husbandry and dietary effects on sturgeon (*Acipenser transmontanus*) farmed for caviar. MSc thesis in Animal Biology, University of California, Davis, 48 pp