

Research Article



Fingerling beluga sturgeon, *Huso huso* (Linnaeus, 1758) growth, hematological, biochemical parameters and opercular respiratory rate under hypoxia challenge with levels of dietary folic acid

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Abstract

Folic acid (FA) is an essential water-soluble vitamin, that is unstable in environmental condition and does not regard in vitamin premixes in spite of its importance. In this study 270 beluga sturgeon fingerlings with weight of 5-8 g were fed by various levels of FA: 0.6 (Control/treatment 1), 2.68 (treatment 2), 3.72 (treatment 3), 4.84 (treatment 4) mg kg⁻¹ feed with three replications for 56 days. Effect of treatment groups on growth, hematological, biochemical parameters before and after stress and opercular respiratory rate (ORR) during stress was investigated in fish fingerlings. The results showed the treatments has significant differences on FCR, SGR, feed efficiency and weight gain ($p < 0.05$). the treatments has significant differences with the control group on red blood cells (RBC) count, hemoglobin (Hb) and Hct ($P < 0.05$). Before stress, cortisol and glucose levels among treatment groups had no significant differences, but after stress they had. Minimum and maximum responses in cortisol levels were observed in treatment two (427.92 ± 6.05 ng mL⁻¹) and control groups (830.32 ± 5.97 ng mL⁻¹), respectively. Minimum glucose levels (43.75 ± 1.49 ng mL⁻¹) in control group and the maximum in treatment two (58.25 ± 1.49 ng mL⁻¹) were observed. There were significant differences for ORR in 0-5, 5-10, 10-20, 30-40 minutes during oxygen challenge among treatment groups ($p < 0.05$). But this indicator in 20-30 minutes was not affected by treatments. The results of this study, showed that the fish with FA 3.72 mg kg⁻¹ diet have more resistance against environmental oxygen challenge, therefor can be stated FA may lessen the negative effects of stress in beluga sturgeon fingerling.

Key words: Folic acid, Blood serum, Opercular respiratory rate, Oxygen challenge, Beluga sturgeon

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Introduction

Caspian Sea is the largest lake around the world and between Europe and Asia (Leroy *et al.*, 2020) and is one of the important habitat for acipenserid fishes (Mirrasooli *et al.*, 2019). Natural sturgeons stocks is decreasing (Abtahi *et al.*, 2007). Some of scientists believe that sturgeons survival depends on the artificial culture and propagation resulting commercial activities (Sudakova *et al.*, 2018). Beluga sturgeon (*Huso huso*) is the biggest one in freshwater. Folic acid (FA) in fish feed has an important role in fish health and growth promotion (Amri *et al.*, 2020; Poolsawat *et al.*, 2020; Roudsari *et al.*, 2021). At the present time, making a perfect formulated diet is aquaculture challenge for fish growth and health (Naidoo *et al.*, 2006). Vitamin B₉ or FA is vitamin B-complex known member and essential nutrient for fish (Khan and Jialal, 2022). FA is an completely oxidated small molecule that has a key role in many physiological process such as cell propagation, regulation of gene expression, cell wall function, chemical material synthesis, regulation of brain function, Amino acids and nucleotides metabolism (Lin *et al.*, 2011; Asaikkutti *et al.*, 2016; Wei *et al.*, 2016; Sesay *et al.*, 2017). It also has a key role in normal RBC formation (Miao *et al.*, 2013). FA supplement may cause growth promotion, immunophysiological response and anti-oxidant condition in fish (Jamalzar Falah *et al.*, 2020). FA deficiency can cause inappetance resulting growth retard, anxiety, fin brittling, anemia,

black pigmentation in skin, ecchymosis in spleen and erythropenia (Smith and Halver, 1969; John and Mahajan, 1979; Badran and Ali, 2021).

Fishes are constantly exposed to the several environmental stressors (Anish *et al.*, 2021). One of the stressors in commercial fish farming is hypoxia that caused by different reasons and enforce many loses. One of trusted indices in fish health and physiology is hematological parameters measurement that affected by nutrition, environmental factors and age (Swann, 1997; Fanouraki *et al.*, 2007; Birnie-Gauvin *et al.*, 2017; Sawecki *et al.*, 2019). Hypoxia in water can be harmful for aquatics. Generally, decrease of oxygen concentration between %1-3 of saturation names Hypoxia. This means dissolved oxygen (DO) is less than 2.8 mg/L (Diaz and Rosenberg, 1995). Hypoxia has substantial effects on physiological and immunological responses in fish and predispose them to diseases (Abdel-Tawwab *et al.*, 2019).

Jamalzar Falah *et al.* (2020) in a research showed that FA in diet of fingerling Siberian sturgeon (*Acipenser baerii*) can cause of growth improvement, immunophysiological response and anti-oxidant condition. Delsoz *et al.* (2017) were studied bilateral affection of FA and *Pediococcus acidilactici* on growth, hematological parameters and nonspecific immune response in *Acipenser nudiventris* fingerlings and showed such diet can cause significant increase in Body Weight Index (BWI), Specific Growth Rate (SGR), Feed

Conversion Rati (FCR), and Condition factor (CF). In several researches on other fish species such as low FA levels in diet of grass carp (*Ctenopharyngodon idella*) may cause immune deficiency in gills (Shi *et al.*, 2016). Sesay *et al.* (2017) indicated Diet containing folic acid on growth, active digestive enzymes and immune response and active antioxidant enzymes on *Megalobrama amblycephala*, FA supplement showed significant differences in Body weight, Growth rate and Specific Growth Rate. Also in researches (Abdel-Tawwab *et al.*, 2019), fish response to hypoxia were studied in growth, physiology and immunity as vital markers that showed hypoxia resulting much stress in fish. The aim of this study was the evaluation of different levels of FA in diet of beluga sturgeon fingerlings on hematological parameters and opercular respiratory rate.

Material and methods

A total number of 270 beluga sturgeon fingerlings with weight of 6.58 ± 0.16 g in four treatments and three replications of different levels of FA (0.6, 2.68, 3.72 and 4.84 mg kg^{-1} feed) were cultured for 56 days. each group (20 fish) were maintained in indoor 200-litre 12 fish tanks for adaptation. During this time, fish were fed with no FA supplement diet. Before stocking, fish tanks were disinfected by 2 ppm potassium permanganate. Each fish tank had air pump and the water was got from river and well with $6.5 \pm 0.2 \text{ l.min}^{-1}$.

Experimental diets

The fish were fed three times a day with 2-3% body weight in each fish tank (7, 15, 23). For biomass determination, all of fish were weighed by an analytical scale with the precision of 0.01 g and measured by biometric ruler with the precision of 1 cm every fifty day. 24 hours before biometry feeding was stopped (Jamalzad Falah *et al.*, 2020). For making the diet in order the Table 1, all of the ingredients were completely ground, mixed for 20 minutes and stored in -20°C . Used FA was 96% and maded by Daejung Chemical & Metals Co, LTD (South Korea).

Food consumption was recorded daily in each fish tank. The food was weighed by an analytical scale with the precision of 0.1 g and poured on the water. Feces, dirt and debris were siphoned every day. Factors like as, temperature, dissolved oxygen, pH, CO_2 concentration, alkalinity, total hardness, ammonia and light period (twelve hrs light per day) regarded as control group variables and was recorded twice a day (Mohseni *et al.*, 2008). Also weight gain, daily mean growth, SGR, FCR were measured by Merrifield *et al.* (2011) method.

Moisture, crude protein, raw fat and ash in experimental diets was determined by AOAC standard process (2000) (Table 2).

Table 1: Experimental diets ingredients.

Composition	Experimental diets			
	4	3	2	1
Kilka powder ^a (%)	56	56	56	56
Wheat flour ^b (%)	19	19	19	19
Soya powder ^c (%)	7	7	7	7
Corn gluten ^d (%)	4	4	4	4
Fish oil ^e (%)	4	4	4	4
Soya oil ^c (%)	4	4	4	4
Cellulose (%)	3	3	3	3
Vitamin mixture ^f (%)	1.5	1.5	1.5	1.5
Mineral mixture ^g (%)	1.5	1.5	1.5	1.5

^a Gill powder Corporation, Anzali, Iran.

^b Golden flour Co., Tehran, Iran.

^c Behpak Industrial Co., Behshahr, Iran.

^d Mahshad Yazd Co., Yazd, Iran.

^e Refined fish oil of Black Sea sprat, *Clupeonella cultriventris*, Khazar Oil Co. Ltd., Anzali, Iran.

^f Composition per kg of mixture: vitamin A, 140,000 IU; vitamin D3, 40,000 IU; vitamin K3, 2 g; vitamin E, 40, g; vitamin B₁, 6, g; vitamin B₂, 8 g; pantothenic acid, 35 g; niacin, 12 g; vitamin B₆, 4 g; vitamin B₁₂, 8 mg; vitamin C, 60 g; inositol, 20 g; biotin, 240 mg (All ingredients were diluted with alpha cellulose to 1 kg)

^g Composition per kg of mixture: Fe, 4500 mg; Cu, 500 mg; Co, 50 mg; Mg, 5000 mg; Se, 50 mg; Zn, 6000 mg; I, 150 mg; choline chloride 150,000 mg (All ingredients were diluted with alpha cellulose to 1 kg)

Table 2: Composition of experimental diets.

Approximate composition of nutrients	Experimental diets			
	1	2	3	4
Moisture (%)	9.50	9.60	9.55	9.50
Protein (%)	47.65	47.84	47.38	47.36
Fat (%)	16.52	16.39	16.41	16.26
Ash (%)	10.05	10.10	10.10	10
FA (mg kg ⁻¹)	0.6	2.68	3.72	4.84

Growth index measurement formulas

Weight gain = (final weight – initial weight) (g)

Body Weight Index = (final weight – initial weight) * 100 / initial weight (% day⁻¹)

Feed Conversion Ratio = Fi / (W_f – W_i) (Feed intake) (g)

Condition Factor = Weight * 100 / Length³ (g)

Specific Growth Rate = (Ln W_f – Ln W_i) * 100 / T (% day⁻¹)

Blood index

RBC count was done by Neubauer's chamber manually. Hayem's solution was used for sample dilution. The red bulb pipette was filled up to 0.5 marks and diluted by Hayem's solution (1:200) for RBC count. Then it transferred to Neubauer's chamber, after 5 minutes

RBCs precipitation, they were counted in 5 medium squares of the big square in light microscope then multiplies 10,000. Thus the quantity of RBC was determined per mL. Measurement of hemoglobin (g/dl) was done by Cyanmethemoglobin Method and Spectrophotometer (Model 2100-VIS,

Unico, USA) in 540 nm. Then an standard curve and below formula were

used for determination of hemoglobin (Esmaeili Rad *et al.*, 2014):

$$\text{Hb (g/dl)} = (\text{sample optical density} / \text{standard optical density}) \times \text{standard concentration}$$

Hematocrit was measured by microhematocrit tube. Blood sample was centrifuged with 700 rpm for 5 min by microcentrifuge machine (Hettich, Germany) and hematocrit was read by the ruler.

Oxygen challenge

At the end of eighth week, three fish were selected randomly for primary bleeding before the challenge, then for oxygen challenge test 9 out of each treatment were selected randomly and transferred to another fish tank. Dissolved oxygen levels were determined 1-1.5 mg/L by two oxymeters. Over each fish tank a camera with the quality of 1080 pixel with 240 frame/sec for ORR was installed.

Cortisol level

Separation of serum from blood was done by Heraeus Labofuge 200 centrifuge, for 10 minutes at 3000 rounds (Thermo Fisher Scientific Inc, Germany). Cortisol levels (ng mL^{-1}) was measured by radioimmunoassay (RIA) method using LKB Gamma counter with immunotech hormone kit (France).

Glucose level

Glucose level was measured by Automatic analyser (Prestige 24i, Tokyo, Japan) with commercial kits (Pars Azmoon, Karaj, Iran) in order to the protocols by spectrophotometer

(Cary 100 UV-Vis, Agilent, Santa Clara, USA). Measurements of serum Alanine transaminase (ALT), Aspartate transaminase (AST) was done by

Statistical analysis

This study was regarded as a random research. Data analysis were calculated by SPSS ver. 19 with One-way ANOVA variance analysis and TUKEY method for means comparison. Differences between means of treatments was determined ($p < 0.05$).

Results

Using of different levels of FA showed significant differences in FCR ($p < 0.05$); so that the maximum was for the control group (1.6%) and the minimum was for the 3rd treatment group (1.33%). SGR, feed efficiency and weight gain were shown statistical differences ($p < 0.05$) between treatments. The minimum was for the control group. Different levels of FA in diet showed significant differences in all treatments CF, as the maximum was for the control group (0.61 g cm^{-3}). Survival Rate was similar in treatment groups (Table 3).

Table 4 shows the hematological parameters of beluga sturgeon fingerlings before and after the stress. Using of various levels of FA showed significant differences ($p < 0.05$) in RBC count before stress; so that the minimum was for the control group ($675 \pm 20.41 \times$

10^3mm^{-3}) and the maximum was for the 1st treatment group ($785\pm 20.45 \times 10^3\text{mm}^{-3}$). Pre-stress hemoglobin (Hb) level was significantly affected by treatment groups that FA-contained diets had more Hb compare with control

group, also there was a significant difference between control and treatment groups for hematocrit (HCT) levels and the in control group had lowest level ($p<0.05$).

Table 3: Growth performance of beluga sturgeon fingerlings using various levels of FA in diet for 56 days.

Growth Index	In-diet FA treatment groups (mg kg ⁻¹)			
	T1-0.6	T2-2.68	T3-3.72	T4-4.84
FCR(%)	1.6±0.06 ^a	1.52±0.07 ^{ab}	1.35±0.02 ^b	1.33±0.01 ^b
SGR (% d ⁻¹)	1.33±0.15 ^b	2.12±0.09 ^a	2.06±0.08 ^a	2.1±0.07 ^a
FE(%)	0.37±0.03 ^b	0.59±0.02 ^a	0.56±2.02 ^a	0.57±0.01 ^a
WG(%)	11±1.45 ^b	19.17±1.57 ^a	17.71±1.96 ^a	18.04±1.63 ^a
CF (g cm ⁻³)	0.61±0.03 ^a	0.51±0.1 ^a	0.35±0.04 ^b	0.31±0.02 ^b
Survival rate (%)	99	100	100	98

^a Levels (mean ± standard deviation) in 27 fish with 3 replications. Significant differences were indicated in various letters by Tukey method ($p<0.05$).

Table 4: Hematological parameters of beluga sturgeon fingerlings using various levels of FA in diet for 56 days and before the oxygen challenge stress.

Blood parameters	In-diet FA treatment groups (mg kg ⁻¹)				P-value
	T1-0.6	T2-2.68	T3-3.72	T4-4.84	
RBC ($\times 10^{-3} \text{mm}^{-3}$)	675 ± 20.41 ^c	785 ± 20.45 ^a	727.50 ± 14.36 ^b	748.75 ± 15.86 ^{ab}	$p<0.0001$
Hb (g dl ⁻¹)	5.17 ± 0.07 ^c	5.80 ± 0.09 ^a	5.27 ± 0.06 ^{bc}	5.50 ± 0.04 ^b	$p<0.0002$
HCT (%)	25.75±0.63 ^c	30 ± 0.41 ^a	27.25 ± 0.62 ^{bc}	28.50 ± 0.64 ^{ab}	$p<0.0017$

^a Levels(mean±standard deviation) in 27 fish with 3 replications. Significant differences were indicated in various letters by Tukey method ($p<0.05$).

Table 5 shows the blood biochemical markers values before stress. Glucose and cortisol levels did not indicate any significant difference among the groups ($p<0.05$). ALT was determined as maximum for the control group

($6.5\pm 0.64 \text{IU L}^{-1}$) and minimum for the 1st treatment group ($4.25\pm 0.47 \text{IU L}^{-1}$) before the stress. AST was also measured that the maximum was for the control group ($547\pm 11.33 \text{IU L}^{-1}$).

Table 5: Blood biochemical markers of beluga sturgeon fingerlings using various levels of FA in diet for 56 days and before the oxygen challenge stress.

Biochemical indicators	In-diet FA treatment groups (mg kg ⁻¹)				p-value
	T1-0.6	T2-2.68	T3-3.72	T4-4.84	
Glucose (mg dl ⁻¹)	45 ± 1.08	42.5 ± 0.64	44.25 ± 1.75	40.50 ± 0.85	$p<0.06$
Cortisol (g dl ⁻¹)	284.28 ± 11.83	291.15 ± 7.06	280.93 ± 9.02	273.85 ± 6.71	$p<0.59$
ALT (IU L ⁻¹)	6.50±0.64 ^a	4.25 ± 0.47 ^b	5 ± 0.41 ^{ab}	4.75 ± 0.48 ^{ab}	$p<0.04$
AST (IU L ⁻¹)	547 ± 11.33 ^a	351 ± 12.87 ^b	347.75 ± 6.98 ^a	355.75 ± 14.49 ^a	$p<0.0001$

^a Levels(mean±standard deviation) in 27 fish with 3 replications. Significant differences were indicated in various letters by Tukey method ($p<0.05$).

After oxygen challenge, RBC, Hb and hematocrit values showed significant differences among the groups (Table 6),

as there were significant differences in all treatments. And the maximum was for control group ($p < 0.05$).

Table 6: Hematological parameters of beluga sturgeon fingerlings using various levels of FA in diet for 56 days after stress challenge.

Blood parameters	In-diet FA treatment groups (mg kg ⁻¹)				P-value
	T1-0.6	T2-2.68	T3-3.72	T4-4.84	
RBC ($\times 10^{-3}$ mm ⁻³)	807.75 \pm 14.66 ^a	785 \pm 12.04 ^b	617 \pm 11.08 ^d	675 \pm 12.04 ^c	$p < 0.0001$
Hb (g dl ⁻¹)	6.37 \pm 0.08 ^a	5.07 \pm 0.04 ^b	5.17 \pm 0.07 ^c	5.25 \pm 0.05 ^b	$p < 0.0001$
HCT (%)	34 \pm 0.4 ^a	29.75 \pm 0.63 ^b	25.75 \pm 0.63 ^c	27 \pm 0.7 ^c	$p < 0.0001$

^aLevels(mean \pm standard deviation) in 27 fish with 3 replications. Significant differences were indicated in various letters by Tukey method ($p < 0.05$).

Using of different levels of FA showed significant differences in blood glucose after stress (Table 7), as minimum and maximum were for the 2nd treatment and control groups, respectively ($p < 0.05$), before the stress. After the stress, Cortisol level was significantly affected by treatment groups that maximum was for the control group (830.32) and

minimum was for the 2nd treatment group (427.92). ALT and AST were significantly affected by treatment groups that each treatment had significant difference with each other, so that the maximum and minimum were for the control and 2nd treatment groups, respectively ($p < 0.05$).

Table 7: Blood biochemical markers of beluga sturgeon fingerlings using various levels of FA in diet for 56 days after stress challenge.

Biochemical indicators	In-diet FA treatment groups (mg kg ⁻¹)				P-value
	T1-0.6	T2-2.68	T3-3.72	T4-4.84	
Glucose (mg dl ⁻¹)	58.25 \pm 1.49 ^a	49.75 \pm 0.47 ^b	43.75 \pm 1.49 ^c	45.75 \pm 0.85 ^{bc}	$p < 0.0001$
Cortisol (g dl ⁻¹)	830.32 \pm 5.97 ^a	738.77 \pm 6.41 ^b	427.92 \pm 6.05 ^d	489.85 \pm 4.5 ^c	$p < 0.0001$
ALT (IU L ⁻¹)	61 \pm 0.41 ^a	53.75 \pm 1.49 ^b	9.50 \pm 0.65 ^d	22.25 \pm 0.48 ^c	$p < 0.0001$
AST (IU L ⁻¹)	536.5 \pm 0.63 ^a	351.5 \pm 1/66 ^b	208 \pm 0.41 ^d	305.50 \pm 0.68 ^c	$p < 0.0001$

^a Levels(mean \pm standard deviation) in 27 fish with 3 replications. Significant differences were indicated in various letters by Tukey method ($p < 0.05$).

ORRs in 0-5 and 5-10 min. showed significant differences among control and treatment groups (Table 8), such that 2nd treatment group was different from others. In 10-20 min significant differences among in ORRs was observed ($p < 0.05$). But the treatments did not affect on ORRs in 20-30 min. ORRs in 30-40 min. showed statistical differences among the groups that the minimum and the maximum rates

belonged to the control and 2nd treatment groups, respectively.

Table 8: Serum biochemical markers of beluga sturgeon fingerlings using various levels of FA in diet for 56 days from the beginning of stress challenge.

Opercular respiratory rate (ORR)	In-diet FA treatment groups (mg kg ⁻¹)				P-value
	T1-0.6	T2-2.68	T3-3.72	T4-4.84	
Time 0-5 minutes	96 ± 0.91a	94 ± 1.68 ^a	83 ± 0.41 ^b	93 ± 0.91 ^a	p<0.0001
Time 5-10 minutes	93 ± 0.91a	92 ± 0.92 ^a	79 ± 0.91 ^b	90 ± 0.41 ^a	p<0.0001
Time 10-20 minutes	78 ± 0.41	81 ± 0.42 ^{ab}	76 ± 1.47 ^b	86 ± 2.12 ^a	p<0.001
Time 20-30 minutes	75 ± 1.78 ^b	77 ± 1.47	74 ± 1.08	73 ± 0/91	p<0.27
Time 30-40 minutes	63 ± 1.08 ^b	69 ± 0.91 ^a	71 ± 1.47 ^a	66 ± 1.49 ^{ab}	p<0.004

^a Levels (mean±standard deviation) in 27 fish with 3 replications. Significant differences were indicated in various letters by Tukey method ($p<0.05$).

Discussion

Disolved oxygen is one of the most important limiting factors in aquaculture, because the fishes have aerobic metabolism, they need various levels of water dissolved oxygen. Decrease of oxygen in water may cause increase of CO₂ and ammonia in water both are toxic for fish and increase of these materials simultanously can supress fish immunity against pathogens (Mwegoha *et al.*, 2010). Most fishes adapted to low or high dissolved oxygen, but if the hypoxia is severe, fish will die (Fitzgibbon *et al.*, 2007). Hypoxia negatively affects on fish behavior, physiology, immunity and growth (Thorarensen *et al.*, 2010; Burgos-Aceves *et al.*, 2018; Abdel-Tawwab *et al.*, 2019). Researches were shown hypoxia can develop the stress primary, secondary and tertiary responses (Bernier *et al.*, 2012; Segner *et al.*, 2012; Anish *et al.*, 2021).

Appropriate indices for stress measurement in fish are both cortisol and sugar in blood (Barton *et al.*, 1985). It was proven that cortisol is effective in energy metabolism, ion regulation and stress response and by stimulation glycolysis and conversion of lactic acid

to glucose in liver, during chemical process, blood glucose will increase through protein and lipid sources (Wendelaar Bonga, 1997). Under hypoxic stress, Hypothalamus ACTH enters the proximal kidney and causes cortisol secretion into blood by stimulating interrenal cells (Bradford *et al.*, 1992; Schreck, 2000). Elevation of blood glucose, cortisol, ALT and AST can be useful indicators for stress, in hypoxic stress and various levels of FA in diet. Studies on Siberian sturgeon (*Acipenser baeri*), crucian carp (*Carassius carassius*) and mrigal carp (*Cirrhinus mrigala*) showed immediate and sever cortisol increase after an acute hypoxic disturbance (Maxime *et al.*, 1995; Sula and Aliko, 2017; Anish *et al.*, 2021). The present study showed that the growth of beluga sturgeon fingerlings was significantly affected by FA concentration in diet. The highest SGR and weight gain belonged to the fish group fed with 2.71 mg FA kg⁻¹ feed. Besides these results, FCR indicates a controversial SGR amounts. Our results show similarity with other researches in blunt snout bream (*Megalobrama amblycephala*) (Sesay *et al.*, 2017), grouper (Lin *et al.*, 2011) and

Siberian sturgeon (Jamalzad Falah *et al.*, 2020). Lack of FA in animals will represent megaloblastic anemia and inappetance resulting growth retard (Jobling, 2012). FA can be useful in purines and pyrimidines biosynthesis that are necessary for RBC formation and maturation (Barros *et al.*, 2009). FA supplements can boost blood erythrocytes, so that FA has an important role in erythropoiesis in siberian sturgeon (Lee *et al.*, 2017; Jamalzad Falah *et al.*, 2020). According to the results, usage of FA various levels developed significant difference on FCR, so that the highest one was in control group (1.60) and the lowest one was in treatment 3 (1.33). SGR, feed efficiency and weight gain showed statistical differences among all treatments, and the lowest amount was in control group. Usage of FA had also significant difference on condition factor, that reported in control group as the maximum one (0.61). In some researches this amount was 0-0.3 for juvenile rainbow trout (Covey and Woodward, 1993), 0.8 for juvenile hybrid Nile tilapia (Lin *et al.*, 2011), 0.8 for juvenile grouper and 0.68 mg kg⁻¹ for blunt snout bream (Sesay *et al.*, 2017). Although, in other researches that was 2.5 for rainbow trout (Esmaeili and Khara, 2014) and 4 mg kg⁻¹ for fingerling ship sturgeon (Delsoz *et al.*, 2017). specific dynamic action and swimming are two metabolic activities in fish life (Dupont-Prinet *et al.*, 2009). On this research that respiration rate of fingerling beluga sturgeon was studied in hypoxic stress, It indicated using of

various levels of FA in diet may make significant differences on hematologic parameters. ORR was also closer to the normal rate with the least changes in 3.72 FA levels. In many studies, immediated erythrocytosis, Hb and Hct increase have happened after hypoxia in fish (Affonso *et al.*, 2002; Wells and Baldwin, 2006; Abdel-Tawwab *et al.*, 2019). Spleen contraction can be the cause of erythrocytosis after hypoxia that release substantial RBCs in blood circulatory system to make up oxygen shortage (Douxflis *et al.*, 2012). It seems RBC count has high metabolic maintenance cost that is provided by glucose and lactate (Wells and Baldwin, 2006). Consequently, glucose increase in hypoxic conditions as RBC energy support for oxygen transfer is defined. FA has a main role as immunity enhancer, antioxidant and animal bacterial infections resistance (Lin *et al.*, 2011). The results of this study have shown RBC, cortisol and glucose amounts had less changes in hypoxic conditions between FA treatments that caused decrease of ORR and stress too (because RBC quantity is enough for oxygen requirement). In order to fish species, swimming behavior changed because of lack of oxygen and some species prefer to hovering at the bottom of tank (Israeli and Kimmel, 1996; Wu, 2002; Wu *et al.*, 2007; Douxfils *et al.*, 2012).

There are several researches about fish swimming in swim tunnel but not for ORR. As was shown in results, ORR increased up to 96 opercular beats m⁻¹ at the beginning of the challenge that is a

compensatory activity for oxygen intake, but with time passing, this range decreased up to 63 opercular beats m^{-1} . Therefore, administration of 3.72 mg AF kg^{-1} feed is suggested for making optimum conditions and lessen the stress effects on nursing of fingerlings beluga sturgeon.

Refcence

- Abdel-Tawwab, M., Monier, M. N., Hoseinifar, S. H. and Faggio, C., 2019.** Fish response to hypoxia stress: growth, physiological, and immunological biomarkers. *Fish physiology and biochemistry*, 45(3), 997-1013. Doi: 10.1007/s10695-019-00614-9.
- Abtahi, B., Ghodrati, S. M., Esmaili, S. A., Rahnama, M., Sharifpour, I., Bahmani, M., Kazemi, R. and Halajian, A., 2007.** Concentration of some heavy metals in tissues of Stellate Sturgeon (*Acipenser stellatus*) in the South Caspian Sea. *Environmental Sciences*, 4(3), 77-84.
- Affonso, E. G., Polez, V., Corrêa, C. F., Mazon, A. d. F., Araujo, M., Moraes, G. and Rantin, F. T., 2002.** Blood parameters and metabolites in the teleost fish *Colossoma macropomum* exposed to sulfide or hypoxia. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 133(3), 375-382. Doi: [https://doi.org/10.1016/S1532-0456\(02\)00127-8](https://doi.org/10.1016/S1532-0456(02)00127-8).
- Amri, A., Kessabi, K., Bouraoui, Z., Sakli, S., Gharred, T., Guerbej, H., Messaoudi, I. and Jebali, J., 2020.** Effect of melatonin and folic acid supplementation on the growth performance, antioxidant status, and liver histology of the farmed gilthead sea bream (*Sparus aurata* L.) under standard rearing conditions. *Fish physiology and biochemistry*, 46(6), 2265-2280. Doi: <https://doi.org/10.1007/s10695-020-00879-5>.
- Anish, D., Nagesh, T., Das, S.K. and Abraham, T.J., 2021.** Stress responses of Indian major carps cultured in the East Kolkata Wetland, West Bengal, India. *Aquatic Research*, 4(4), 351-362.
- AOAC, 2000.** Official Methods of Analysis of Official Analytical Chemists . *Association of Official Analytical Chemists International Gaithersburg, MD.*, 2.
- Asaikkutti, A., Bhavan, P.S. and Vimala, K., 2016.** Effects of different levels of dietary folic acid on the growth performance, muscle composition, immune response and antioxidant capacity of freshwater prawn, *Macrobrachium rosenbergii*. *Aquaculture*, 464, 136-144. Doi: <https://doi.org/10.1016/j.aquaculture.2016.06.014>.
- Badran, M.F. and Ali, M.A.M., 2021.** Effects of folic acid on growth performance and blood parameters of flathead grey mullet, *Mugil cephalus*. *Aquaculture*, 536, 736459. Doi: <https://doi.org/10.1016/j.aquaculture.2021.736459>.
- Barros, M.M., Ranzani-Paiva, M.J.T., Pezzato, L.E., Falcon, D.R. and Guimarães, I.G., 2009.** Haematological response and growth

- performance of Nile tilapia (*Oreochromis niloticus* L.) fed diets containing folic acid. *Aquaculture Research*, 40(8), 895-903. Doi: <https://doi.org/10.1111/j.1365-2109.2009.02175.x>.
- Barton, B.A., Weirter, G.S. and Schreck, C.B., 1985.** Effect of prior acid exposure on physiological responses of juvenile rainbow trout (*Salmo gairdneri*) to acute handling stress. *Canadian Journal of Fisheries and Aquatic Sciences*, 42(4), 710-717. Doi: <https://doi.org/10.1139/f85-091>.
- Bernier, N.J., Gorissen, M. and Flik, G., 2012.** Differential effects of chronic hypoxia and feed restriction on the expression of leptin and its receptor, food intake regulation and the endocrine stress response in common carp. *Journal of Experimental Biology*, 215(13), 2273-2282. Doi: <https://doi.org/10.1242/jeb.066183>.
- Birnie-Gauvin, K., Costantini, D., Cooke, S.J. and Willmore, W.G., 2017.** A comparative and evolutionary approach to oxidative stress in fish: a review. *Fish and Fisheries*, 18(5), 928-942. Doi: <https://doi.org/10.1111/faf.12215>.
- Bradford, C.S., Fitzpatrick, M.S. and Schreck, C.B., 1992.** Evidence for ultra-short-loop feedback in ACTH-induced interrenal steroidogenesis in coho salmon: acute self-suppression of cortisol secretion in vitro. *General and comparative endocrinology*, 87(2), 292-299. Doi: [https://doi.org/10.1016/0016-6480\(92\)90034-H](https://doi.org/10.1016/0016-6480(92)90034-H).
- Burgos-Aceves, M.A., Cohen, A., Smith, Y. and Faggio, C., 2018.** MicroRNAs and their role on fish oxidative stress during xenobiotic environmental exposures. *Ecotoxicology and Environmental Safety*, 148, 995-1000. DOI: <https://doi.org/10.1016/j.ecoenv.2017.12.001>.
- Cowey, C.B. and Woodward, B., 1993.** The dietary requirement of young rainbow trout (*Oncorhynchus mykiss*) for folic acid. *The Journal of nutrition*, 123(9), 1594-1600. Doi: <https://doi.org/10.1093/jn/123.9.1594>.
- Delsoz, N., Khara, H., Shenavar, A.R. and Mohseni, M., 2017.** Interaction of dietary *Pediococcus acidilactici* and folic acid on growth performance, haematological parameters and non-specific immune response of finger barbel, *Acipenser nudiventris*. *Iranian Journal of Fisheries Sciences*, 16(3), 869-883.
- Diaz, R.J. and Rosenberg, R., 1995.** Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. *Oceanography and Marine Biology. An Annual Review*, 33, 245-203.
- Douxflis, J., Deprez, M., Mandiki, S., Milla, S., Henrotte, E., Mathieu, C., Silvestre, F., Vandecan, M., Rougeot, C. and Mélard, C., 2012.** Physiological and proteomic responses to single and repeated hypoxia in juvenile Eurasian perch under domestication—clues to

- physiological acclimation and humoral immune modulations. *Fish & shellfish immunology*, 33(5), 1112-1122. Doi: <https://doi.org/10.1016/j.fsi.2012.08.013>.
- Dupont-Prinet, A., Claireaux, G. & McKenzie, D. J. 2009.** Effects of feeding and hypoxia on cardiac performance and gastrointestinal blood flow during critical speed swimming in the sea bass *Dicentrarchus labrax*. *Comparative Biochemistry and Physiology A* 154(2), 233–240. Doi: [10.1016/j.cbpa.2009.06.01](https://doi.org/10.1016/j.cbpa.2009.06.01)
- Esmaili Rad, A., Alishahi, M., Ghorbanpour, M. and Zarei, M., 2014.** The effects of oral administration of extracted chitosan from white leg shrimp (*Litopenaeus vannamei*) on hematological and growth indices in common carp (*Cyprinus carpio*). *Journal of Veterinary Research*, 69(4), 385-393. Doi: [10.22059/jvr.2014.52281](https://doi.org/10.22059/jvr.2014.52281).
- Esmaili, B. and Khara, H., 2014.** Growth performance, hematology and immunological parameters of rainbow trout, *Oncorhynchus mykiss*, fed with diets containing different levels of vitamin E and folic acid. *Iranian Journal of Fisheries Sciences*, 13(4), 931- 943.
- Fanouraki, E., Divanach, P. and Pavlidis, M., 2007.** Baseline values for acute and chronic stress indicators in sexually immature red porgy (*Pagrus pagrus*). *Aquaculture*, 265(1-4), 294-304. Doi: <https://doi.org/10.1016/j.aquaculture.2007.01.006>.
- Fitzgibbon, Q., Strawbridge, A. and Seymour, R., 2007.** Metabolic scope, swimming performance and the effects of hypoxia in the mullet, *Argyrosomus japonicus* (Pisces: Sciaenidae). *Aquaculture*, 270(1-4), 358-368. Doi: <https://doi.org/10.1016/j.aquaculture.2007.04.038>.
- Israeli, D. and Kimmel, E., 1996.** Monitoring the behavior of hypoxia-stressed *Carassius auratus* using computer vision. *Aquacultural engineering*, 15(6), 423-440. [https://doi.org/10.1016/S0144-8609\(96\)01009-6](https://doi.org/10.1016/S0144-8609(96)01009-6).
- Jamalzad Falah, F., Rajabi Islami, H. and Shamsaie Mehrgan, M., 2020.** Dietary folic acid improved growth performance, immuno-physiological response and antioxidant status of fingerling Siberian sturgeon, *Acipenser baerii* (Brandt 1896). *Aquaculture Reports*, 17, 100391. <https://doi.org/10.1016/j.aqrep.2020.100391>.
- Jobling, M., 2012.** National Research Council (NRC): Nutrient requirements of fish and shrimp: Springer.
- John, M. and Mahajan, C., 1979.** The physiological response of fishes to a deficiency of cyanocobalamin and folic acid. *Journal of Fish Biology*, 14(2), 127-133. <https://doi.org/10.1111/j.1095-8649.1979.tb03502.x>.
- Khan, K.M. and Jialal, I., 2022.** Folic acid (folate) deficiency. In:

- StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. PMID: 30570998.
- Lee, S., Sonmez, O., Hung, S.S. and Fadel, J.G., 2017.** Development of growth rate, body lipid, moisture, and energy models for white sturgeon (*Acipenser transmontanus*) fed at various feeding rates. *Animal Nutrition*, 3(1), 46-60. <https://doi.org/10.1016/j.aninu.2016.10.005>.
- Leroy, S.A.G., Lahijani, H.A.K., Crétaux, J.F., Aladin, N.V. and Plotnikov, I.S., 2020.** Past and Current Changes in the Largest Lake of the World: The Caspian Sea. In S. Mischke (Ed.), *Large Asian Lakes in a Changing World: Natural State and Human Impact* (pp. 65-107). Cham: Springer International Publishing.
- Lin, Y.H., Lin, H.Y. and Shiau, S.Y., 2011.** Dietary folic acid requirement of grouper, *Epinephelus malabaricus*, and its effects on non-specific immune responses. *Aquaculture*, 317(1-4), 133-137. <https://doi.org/10.1016/j.aquaculture.2011.04.010>.
- Maxime, V., Nonnotte, G., Peyraud, C., Williot, P. and Truchot, J., 1995.** Circulatory and respiratory effects of an hypoxic stress in the Siberian sturgeon. *Respiration physiology*, 100(3), 203-212. [https://doi.org/10.1016/0034-5687\(95\)00003-V](https://doi.org/10.1016/0034-5687(95)00003-V).
- Merrifield, D., Bradley, G., Harper, G., Baker, R., Munn, C. and Davies, S., 2011.** Assessment of the effects of vegetative and lyophilized *Pediococcus acidilactici* on growth, feed utilization, intestinal colonization and health parameters of rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquaculture Nutrition*, 17(1), 73-79. <https://doi.org/10.1111/j.1365-2095.2009.00712.x>.
- Miao, S., Zhang, W., Xu, W. and Mai, K., 2013.** Dietary folic acid requirement of juvenile abalone *Haliotis discus hannai* Ino. *Aquaculture*, 400-401, 73-76. <https://doi.org/10.1016/j.aquaculture.2013.03.005>.
- Mirrasooli, E., Ghorbani, R., Gorgin, S., Aghilinejad, S. M. and Jalali, A., 2019.** Factors associated with illegal fishing and fisher attitudes toward sturgeon conservation in the southern Caspian Sea. *Marine Policy*, 100, 107-115. <https://doi.org/10.1016/j.marpol.2018.11.028>.
- Mohseni, M., Ozorio, R., Pourkazemi, M. and Bai, S., 2008.** Effects of dietary l-carnitine supplements on growth and body composition in beluga sturgeon (*Huso huso*) juveniles. *Journal of Applied Ichthyology*, 24(6), 646-649. <https://doi.org/10.1111/j.1439-0426.2008.01121.x>.
- Mwegoha, W., Kaseva, M. and Sabai, S., 2010.** Mathematical modeling of dissolved oxygen in fish ponds. *African Journal of Environmental Science and Technology*, 4(9), 625-638.
- Naidoo, K., Maneveldt, G., Ruck, K. and Bolton, J.J., 2006.** A

- comparison of various seaweed-based diets and formulated feed on growth rate of abalone in a land-based aquaculture system. *Journal of Applied Phycology*, 18(3), 437-443. <https://doi.org/10.1007/s10811-006-9045-7>.
- Poolsawat, L., Li, X., He, M., Ji, D. and Leng, X., 2020.** Clostridium butyricum as probiotic for promoting growth performance, feed utilization, gut health and microbiota community of tilapia (*Oreochromis niloticus* × *O. aureus*). *Aquaculture Nutrition*, 26(3), 657-670. <https://doi.org/10.1111/anu.13025>.
- Roudsari, S.F., Islami, H.R., Mousavi, S.A. and Mehrgan, M.S., 2021.** Folic Acid-Coated Nanochitosan Ameliorated the Growth Performance, Hematological Parameters, Antioxidant Status, and Immune Responses of Rainbow Trout (*Oncorhynchus mykiss*). *Frontiers in Veterinary Science*, 8, 1-11. DOI: 10.3389/fvets.2021.647722.
- Sawecki, J., Miros, E., Border, S.E. and Dijkstra, P.D., 2019.** Reproduction and maternal care increase oxidative stress in a mouthbrooding cichlid fish. *Behavioral Ecology*, 30(6), 1662-1671. <https://doi.org/10.1093/beheco/arz133>.
- Schreck, C., 2000.** Accumulation and long-term effects of stress in fish. *The Biology of Animal Sstress*, 1, 147-158.
- Segner, H., Sundh, H., Buchmann, K., Douxfils, J., Sundell, K.S., Mathieu, C., Ruane, N., Jutfelt, F., Toften, H. and Vaughan, L., 2012.** Health of farmed fish: its relation to fish welfare and its utility as welfare indicator. *Fish physiology and biochemistry*, 38(1), 85-105. <https://doi.org/10.1007/s10695-011-9517-9>.
- Sesay, D.F., Habte-Tsion, H.M., Zhou, Q., Ren, M., Xie, J., Liu, B., Chen, R. and Pan, L., 2017.** The effect of dietary folic acid on biochemical parameters and gene expression of three heat shock proteins (HSPs) of blunt snout bream (*Megalobrama amblycephala*) fingerling under acute high temperature stress. *Fish Physiology and Biochemistry*, 43(4), 923-940.
- Shi, L., Feng, L., Jiang, W.D., Liu, Y., Jiang, J., Wu, P., Kuang, S.Y., Tang, L., Tang, W.N. and Zhang, Y.A., 2016.** Immunity decreases, antioxidant system damages and tight junction changes in the intestine of grass carp (*Ctenopharyngodon idella*) during folic acid deficiency: Regulation of NF-κB, Nrf2 and MLCK mRNA levels. *Fish & Shellfish Immunology*, 51, 405-419. <https://doi.org/10.1016/j.fsi.2016.02.029>.
- Smith, C.E. and Halver, J. E., 1969.** Folic acid anemia in coho salmon. *Journal of the Fisheries Board of Canada*, 26(1), 111-114. <https://doi.org/10.1139/f69-009>.
- Sudakova, N., Mikodina, E. and Vasilyeva, L., 2018.** sturgeon (acipenseridae) artificial reproduction paradigm changeover

- under conditions of natural stock deficit of sturgeon in the volga-caspian basin. *Sel'skokhozyaistvennaya Biologiya*, 53(4), 698-711. DOI: 10.15389/agrobiology.2018.4.698eng.
- Sula, E. and Aliko, V., 2017.** Effects of stressors on hematological and immunological response in the fresh water crucian carp fish, *Carassius carassius*. *Albanian Journal of Agricultural Sciences*, 583-590.
- Swann, L., 1997.** *A fish farmer's guide to understanding water quality*: Aquaculture Extension, Illinois-Indiana Sea Grant Program.
- Thorarensen, H., Gústavsson, A., Mallya, Y., Gunnarsson, S., Árnason, J., Arnarson, I., Jónsson, A.F., Smáradóttir, H., Zoega, G.T. and Imsland, A.K., 2010.** The effect of oxygen saturation on the growth and feed conversion of Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture*, 309(1-4), 96-102. <https://doi.org/10.1016/j.aquaculture.2010.08.019>.
- Wei, J., Zhang, F., Tian, W., Kong, Y., Li, Q., Yu, N., Du, Z., Wu, Q., Qin, J. and Chen, L., 2016.** Effects of dietary folic acid on growth, antioxidant capacity, non-specific immune response and disease resistance of juvenile Chinese mitten crab *Eriocheir sinensis* (Milne-Edwards, 1853). *Aquaculture Nutrition*, 22(3), 567-574. <https://doi.org/10.1111/anu.12275>.
- Wells, R.M. and Baldwin, J., 2006.** Plasma lactate and glucose flushes following burst swimming in silver trevally (*Pseudocaranx dentex*: Carangidae) support the "releaser" hypothesis. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 143(3), 347-352. Doi: 10.1016/j.cbpa.2005.12.015.
- Wendelaar Bonga, S.E., 1997.** The stress response in fish. *Physiological reviews*, 77(3), 591-625. DOI: 10.1152/physrev.1997.77.3.591.
- Wu, R., 2002.** Hypoxia: from molecular responses to ecosystem responses. *Marine pollution bulletin*, 45(1-12), 35-45. DOI: 10.1016/s0025-326x(02)00061-9.
- Wu, Y., Zhang, H., Zhao, H.H. and Li, T., 2007.** Effects of different dissolved oxygen concentration on metabolic level of juvenile rainbow trout (*Oncorhynchus mykiss*) in the recirculating systems. *Journal of Shanghai Fisheries University*, 5, 16(5), 438-442. DOI: 10.1016/j.jtherbio.2021.102928.