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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 fishh 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 B_9 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 (Jamalzad Falah *et al.*, 2020). FA defeciency 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 disolved 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).

Jamalzad 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±0.2 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 exprimental 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 B_1, 6, g; vitamin B_2, 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 / $Length^3$ (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. Measurment of hemoglobin (g/dl) was done by Cyanmethemoglobin Method and Spectrophotometer (Model 2100-VIS,

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Unico, USA) in 540 nm. Then an standard curve and below formula were used for determination of hemoglobin (Esmaeili Rad *et al*., 2014):

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

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 transfered 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 protocolos by spectrophotometer (Cary 100 UV-Vis, Agilent, Santa Clara, USA). Measurments 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±20.41 ×

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10^3mm^{-3}) and the maximum was for the 1st treatment group (785±20.45 × 10^3mm^{-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})				
	T1-0.6	**T2-2.68**	**T3-3.72**	**T4-4.84**	***P*-value**
RBC (×10^{-3} 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±0.64 IU L^{-1}) and minimum for the 1st treatment group (4.25±0.47 IU L^{-1}) before the stress. AST was also measured that the maximum was for the control group (547±11.33 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})				
	T1-0.6	**T2-2.68**	**T3-3.72**	**T4-4.84**	**p-value**
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})				
	T1-0.6	T2-2.68	T3-3.72	T4-4.84	*P*-value
RBC ($\times10^{-3}$ mm^{-3})	807.75 ± 14.66^{a}	785 ± 12.04^{b}	617 ± 11.08^{d}	675 ± 12.04^{c}	p<0.0001
Hb (g dl^{-1})	6.37 ± 0.08^{a}	5.07 ± 0.04^{b}	5.17 ± 0.07^{c}	5.25 ± 0.05^{b}	p<0.0001
HCT (%)	34±0.4^{a}	29.75 ± 0.63^{b}	25.75 ± 0.63^{c}	27 ± 0.7^{c}	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).

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

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 thr groups that the minimum and the maximum rates belonged to the control and 2nd treatment groups, respectively.

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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})				
	T1-0.6	**T2-2.68**	**T3-3.72**	**T4-4.84**	***P*-value**
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 CO_2 and ammonia in water both are toxic for fish and increase of these materials simultanouly 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 (*Acipencer 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 amouts. Our results show similarity with other researches in blunt snout bream (*Megalobrama amblycephala*) (Sesay *et al.*, 2017), grouper (Lin *et al.*, 2011) and

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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 erythrogenesis 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 amout was 0-0.3 for juvenile rainbow trout (Cowey 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 (Douxfils *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

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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.

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