Iranian Journal of Fisheries Sciences
http:doi.org/ 10.22092/ijfs.2025.134645 2025, 24(6) 1451-1476

Research Article

Dietary effects of ascorbic acid and cobalt chloride on some biological parameters and gene expression in *Acipenser baerii*

Roudbaraki S.M.S.[1], Ershad Langroudi H.[1*], Zamini A.A.[1], Falahatkar B.[2], Safari R.[3]

1 Fisheries Department, Lahijan Branch, Islamic Azad University, P.O. Box 1616, Lahijan, Iran
2 Fisheries Department, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, P.O. Box 1144, Guilan, Iran
3 Fisheries Department, Faculty of Fisheries and Environment, University of Agricultural Sciences and Natural Resources, P.O. Box 4913815739, Gorgan, Golestan, Iran
*Correspondence hadi.ershad@iau.ac.ir

Keywords

Micronutrients,
Growth,
Physiological indices,
Gene expression,
Acipenser baerii

Article info

Received: December 2024
Accepted: March 2025
Published: November 2025

Abstract

This research investigated the effects of dietary cobalt chloride ($CoCl_2$) and ascorbic acid (AA) on growth, physiological, and molecular parameters in Siberian sturgeon (*Acipenser baerii*). Two hundred seventy juveniles were divided into eight groups fed diets containing 2 or 4 mg/kg $CoCl_2$ and 200 or 800 mg/kg AA for 12 weeks. Results showed significant differences in final weight, length, weight gain, and growth rate among treatments ($p<0.05$), indicating differences when compared to the control group. However, no significant difference was observed in specific growth rate, condition factor, Average daily growth, or protein efficiency ratio compared to the control ($p>0.05$). The lowest and highest AA/cobalamin levels were found in the control and the 4 mg/kg $CoCl_2$ with 200 mg/kg AA, and the 2 mg/kg $CoCl_2$ groups, respectively ($p<0.05$). Maximum serum iron level was observed in fish fed 800 mg/kg $CoCl_2$, and the lowest glucose was in the control ($p<0.05$). The cortisol level was significantly higher in 2 mg/kg $CoCl_2$ with 200 mg/kg AA group compared to the control, which had the lowest levels ($p<0.05$). The highest *hsp70* and *p450* gene upregulation, as well as the highest *GH* and *igf-1* expression, occurred in the 4 mg/kg $CoCl_2$ with 200 mg/kg AA group, with the lowest expression in the control ($p<0.05$). Overall, 4 mg/kg $CoCl_2$ and 200 mg/kg AA supplementation had more positive effects on growth performance, hematological and biochemical indices, and the modulation of genes associated with growth, stress response, and immunity in Siberian sturgeon.

Introduction

Micronutrients, encompassing vitamins and minerals, play a pivotal role in various physiological and biological processes in aquatic animals. These essential nutrients contribute significantly to health maintenance and growth enhancement (Dawood and Koshio, 2018; Khanjani and Sharifinia, 2020). Vitamins, particularly AA, are vital micronutrients known for their immune-stimulating properties. They promote macrophage activity, cell proliferation, and the production of cytokines and antibodies (Rahman *et al*., 2019; Rahmati, 2020). Unlike mammals that synthesize AA, the majority of fish species, including those unable to effectively convert L-gluconolactone, lack this ability due to a deficiency in the essential enzymes required for AA production. Consequently, dietary AA supplementation is necessary for normal growth and physiological processes (NRC, 2011; Khan *et al.,* 2015; Trichet *et al.,* 2015). The AA requirement of fish is influenced by various factors, including species-specific physiology, environmental conditions, age, diet, and genetics. This variability necessitates species-specific studies for optimal dosing. Research has identified diverse optimal AA levels across different species. For instance, studies indicated 150-250 mg/kg for rainbow trout (*Oncorhynchus mykiss*) (Adel and Khara, 2016), 400 mg/kg for red pacu (*Piaractus brachypomus*) (Hosseini *et al.,* 2015), 45.3 mg/kg for grouper (*Epinephelus malabaricus*) (Lin and Shiau, 2005), 120 mg/kg for barred knifejaw (*Oplegnathus fasciatus*) (Wang *et al.,* 2003), 160 mg/kg for puffer (*Takifugu rubripes*) (Eo and Lee, 2008), 60 mg/kg for tilapia (*Oreochromis karongae*) (Nsonga *et al.,* 2009), 100 mg/kg for vundu (*Heterobranchus longifilis*) (Ibiyo *et al.,* 2007), and 200 mg/kg for beluga sturgeon (*Huso huso*) (Falahatkar *et al.,* 2006). These variations highlight the diverse nutritional requirements among species and the importance of species-specific studies.

Ascorbic acid is also critical for growth performance, reproduction, and immunity in fish (Gouda *et al*., 2020). The confident effects of AA supplementation on growth and immune function in finfish have been well documented. Consequently, ensuring adequate AA levels in fish diets is crucial for achieving optimal health and productivity in aquaculture (Moreau and Dabrowski, 2000; Rahman *et al.,* 2018).

Fish hematology (Cytologic and Serologic indices) offers valuable insights into both physiological function and overall health, making it a valuable factor in aquaculture management (Percin and Konyalioglu, 2008; Percin *et al*., 2010). Biochemical studies, by measuring cortisol (as a stress indicator), glucose (as a carbohydrate metabolism indicator), albumin and total protein (as liver function and nutritional status indicators) in the blood, provide valuable information about internal organ function, metabolic status, and stress response in Siberian sturgeon. These parameters help us evaluate the effects of dietary supplements on the health and performance of this species (Roche and Bogé, 1996).

Cobalt, an essential trace mineral, has been recognized for its crucial role in various metabolic processes, particularly serving as a coenzyme in the conversion of

ribonucleotides to deoxyribonucleotides (Hall and Hall, 2020). It is important to note that the optimal levels of $CoCl_2$, supplementation reported in the literature can vary depending on factors such as the species of fish, age, size, sex, feed composition, feeding practices, and rearing conditions, reflecting differences in metabolic and functional demands for micronutrients (Biesalski and Jana, 2018). Deficiencies in essential micronutrients and vitamins can induce severe anemia in various fish species (Brunt and Austin, 2005; Cheng *et al*., 2006). Dietary inclusion of $CoCl_2$ and cobalt sulfate in fish diets has been shown to promote cobalamin (Vitamin B_{12}) synthesis by gut bacteria (Blust, 2012; Lall and Kaushik, 2021). The gut microbiome of fish utilizes cobalt for the production of vitamin B_{12}, a critical cofactor involved in metabolic pathways linked to lipid and carbohydrate energy metabolism (Hossain *et al*., 2022). Furthermore, $CoCl_2$ is essential for preventing pernicious anemia, and cobalt salts function as catalysts in the synthesis of various pigments in fish (Tonye and Sikoki, 2014). Vitamin B_{12} deficiency induces several deleterious outcomes in fish, including decreased hemoglobin levels, reduced appetite, growth impairment, and anemia (Stoica *et al.,* 2004; Boachie *et al.,* 2020). Moreover, cobalt supplementation is known to positively influence growth parameters, carcass composition, muscle manganese and iron content, and notably, the modulation of genes involved in growth regulation, as demonstrated in Golden mahseer (Younus *et al.,* 2020). Iron is an essential element for fish growth and metabolism, and its deficiency can lead to various physiological problems. Furthermore, cobalt is one of the most important elements that has the greatest interaction with dietary iron, such that during iron deficiency in the body, cobalt absorption increases, and the reverse is also true (González-Montaña *et al.,* 2020).

Growth in fish is regulated by the *gh/igf-1* axis. Growth hormone (GH) stimulates growth by affecting protein, carbohydrate, and fat metabolism (Rahimi *et al.,* 2012). However, insulin-like growth factor-1 (*igf-1*) is a more stable indicator of growth and is influenced by nutrition (Duan *et al.,* 2010). Therefore, *igf-1* gene expression can be used as an indicator of growth rate in fish (Larsen *et al.,* 2001). Heat shock protein (*hsp70*) genes play a role in response to various stresses, including heat shock and infection, and maintain cellular homeostasis (Basu *et al.,* 2002). cytochrome *p450* genes are involved in the metabolism of toxic substances and hormones (Arukwe and Goksoyr, 2003), and their expression can be altered by environmental and nutritional conditions (Van der Oost *et al.,* 2003). Therefore, changes in *p450* gene expression can be used as an indicator to assess the impact of pollutants and stressful conditions on fish health (Stegeman, 1994).

The Siberian sturgeon (*A. baerii*) is an introduced species of sturgeon to Iran and has gained attention from researchers due to its ability to live in freshwater, tolerance to environmental fluctuations, adaptation to low temperatures, acceptance of a wide range of food items, and high growth potential (Falahatkar, 2018). These characteristics make the Siberian sturgeon a suitable model for investigating the

effects of dietary supplements on growth and metabolism. Among various sturgeon species, the Siberian sturgeon is recognized as one of the most important and globally cultured species, making it a suitable option for aquaculture purposes (Eslamloo *et al.,* 2012). Additionally, it is used as a biological model in physiological and nutritional studies (Fontagné *et al.,* 2006). This species is widely cultivated due to its adaptability to temperature variations, rapid growth rate, and the fact that it reaches sexual maturity relatively early (Eslamloo *et al*., 2012; Falahatkar and Poursaeid, 2014).

Former studies have analyzed the individual implications pertaining to $CoCl_2$ and AA, impacts on growth, stress responses, and hemato-biochemical indices in fish (Falahatkar *et al*., 2006; Hernandez *et al*., 2012; Rahimi *et al*., 2012; Tonye and Sikoki, 2014; Guo *et al*., 2015; Abbas and Javed, 2016; Pourgholam *et al*., 2016; Akdemir *et al*., 2017; Djissou *et al*., 2019; Ibrahim *et al*., 2020; Li *et al*., 2021; Aboseif *et al*., 2022; Li *et al*., 2022).

Given the worldwide growth of sturgeon aquaculture, the rapid growth potential of Siberian sturgeon, and the known importance of AA and $CoCl_2$ in aquatic organisms nutrition, this research carried out aimed to determine the combined effects of dietary $CoCl_2$ and AA micronutrients on some growth parameters, hematological and biochemical indices, and the expression of *hsp70*, *p450*, *gh*, and *igf-1* genes in *A. baerii* for order suitable doses of these supplements to sturgeon fish diet.

Materials and methods

Ethical considerations

This research was performed in compliance with the Care or Use of Laboratory Animals Act. The experimental protocol received approval from the pertinent Local Ethics Committee for Animal Use in Iran (Ahmadi Noorbakhsh *et al*., 2021).

Rearing system

The current research was performed at the Research Center of Fisheries Sciences and Marine Techniques Lahijan Branch, Islamic Azad University (Langroud, Guilan, Iran), in the summer of 2021. In this study, 270 fish with a mean initial weight and initial total length of 11.5±0.3 g and 12.9±0.1 cm, respectively, were divided into 27 fiberglass tanks (each 350 L) with a stocking rate of 10 fish per tank. Each tank was supplied with an aeration system, and water inflow was set at 1 L/min. The physicochemical parameters of the water, including temperature, pH, and oxygen, were measured 22.5±0.5°C, 7.2±0.1, and 6.3±0.3 mg/L, respectively (WTW Company, Weilheim, Germany).

Experimental design

In this study, experimental subjects were fed diets supplemented with 2 and 4 mg/kg of $CoCl_2$, and 200 and 800 mg/kg of AA (Falahatkar *et al*., 2006) (Table 1). A commercial diet (Faradaneh Co., Shahre Kord, Iran) was used, with the following nutritional composition: 54% crude protein, 14% fat, 3% fiber, 11% ash, 11% moisture, and 2% phosphorus, with a diameter of 1.2 mm. The experiment consisted of eight experimental treatments and a control, with each having three replicates. The AA (powder) was obtained from Science Laboratory, Aras Bazar, Qazvin, Iran, and

$CoCl_2$ (CAS No. 7791-13-1) was from (Merck, Darmstadt, Germany). Ascorbic acid and $CoCl_2$ were dissolved in 10 mL water and then sprayed onto the pellets. All experimental diets including the control group, were coated using 1% gelatin. The control group was fed the basal diet without any added supplements. The fish were cultured with experimental diets for 12 weeks, during which the ration each day was 3% of their individual body weight, one quarter of the total meal at each time (6 a.m., 12 p.m., 6 p.m. and 12 a.m.) Biometric measurements were used to adjust the feed ration every two weeks to support sturgeon growth. The actual concentrations of AA and $CoCl_2$ in the experimental diets were determined by iodometric titration and atomic absorption spectrophotometry (AA-6800; Shimadzu, Kyoto, Japan), respectively. In the sample of Faradaneh diet, AA concentration was 690 mg/kg and $CoCl_2$ was 0.05 mg/kg by adding different experimental concentrations of these supplements.

Table 1: Description of the diet treatments used for Siberian sturgeon, *Acipenser baerii*.

Treatments	Details
Control	The standard feeding group consisted of a basal diet without any supplementation of $CoCl_2$ or AA, representing the standard feeding practice without the addition of the experimental additives.
2 $CoCl_2$	Cobalt chloride (2 mg/kg) not supplemented with AA
4 $CoCl_2$	Cobalt chloride (4 mg/kg) without AA
200 AA	Ascorbic acid (200 mg/kg) not supplemented with $CoCl_2$
800 AA	Ascorbic acid (800 mg/kg) without $CoCl_2$
200 AA+2 $CoCl_2$	Ascorbic acid (200 mg/kg) + Cobalt chloride (2 mg/kg)
800 AA+4 $CoCl_2$	Ascorbic acid (800 mg/kg) + Cobalt chloride (4 mg/kg)
800 AA+2 $CoCl_2$	Ascorbic acid (800 mg/kg) + Cobalt chloride (2 mg/kg)
200 AA+4 $CoCl_2$	Ascorbic acid (200 mg/kg) + Cobalt chloride (4 mg/kg)

In sample of Faradaneh food have AA is 690 mg/kg and $CoCl_2$ is 0.05 mg/kg.

Growth parameters

To assess growth parameters and feed conversion efficiency, the weight and total length of each group of 270 fish were measured biweekly throughout the experimental period. Prior to measurement, fish were anesthetized using clove powder at a concentration of 150 mg/L (Mohseni, 2015). Fish weight was measured using a DK-300 digital scale (ZhiHeng, Shanghai, China) with an accuracy of 1 g, and total length was measured using a graduated ruler with an accuracy of 1 mm (Falahatkar and Poursaeid, 2014):

Condition factor (CF = 100 × body weight (g) / total length (cm) ^{3})
Growth rate (%) (GR = 100 × (final weight - initial weight / days))
Weight gain (g) (WG = final weight (g) - initial weight (g))
Body weight increase (%) (BWI = 100 × [final body weight (g) - initial body weight (g)] / initial body weight (g))
Specific growth rate (%/day) (SGR = 100 × [ln final weight (g) - ln initial weight (g)] / rearing period (day))
Food conversion ratio (FCR = feed intake (g) / weight gain (g))
Protein efficiency rate (PER = wet weight gain (g) / protein intake (g))
Average daily growth (%) (ADG = 100 × final weight (g) - initial weight (g) / initial weight (g) × rearing period (day))
Survival rate (%) (SR = 100 × (number of fish at final / number of fish at start)) was assessed at the end of the experiment.

Biochemical indices

Fish were fasted for 24 hours prior to blood collection. Blood was collected from the caudal region using 2 mL syringes. After collecting 2 mL of blood (from 3 fish per treatment), 0.5 mL of blood was transferred to numbered 1 mL heparinized vials for complete blood count (CBC) analysis, and the remaining 1.5 mL was transferred to numbered 2 mL vials for serum preparation for blood biochemical analysis. Samples were transported to the laboratory in a cooler containing dry ice. Siberian sturgeon blood samples were centrifuged at room temperature at 3000 rpm for 10 minutes (Hestaran Teb, Tehran, Iran), and the serum was separated (Kazemi *et al.,* 2010).

The measurement of AA in blood samples (n=81 including 3 samples per each 3 replicates of 9 treatments) was conducted following a modified version of the Roe and Kuether (1942) method. This approach involved oxidizing AA by copper sulfate, forming a red-colored hydrazone in a robust acid solution containing 2,4-dinitrophenylhydrazine. This color's optical density (OD) was then read at a wavelength of 520 nm using a Biochrom spectrometer (Cambridge, England). Iron, protein, and albumin levels were assessed spectrophotometrically. Iron was determined at a wavelength of 600 nm using a colorimetric method (Higgine, 1981). Protein quantification was performed using the Biuret colorimetric method at 540 nm (Koller and Kaplan, 1984). Albumin concentrations were determined using the BCG assay with a Pars Azmun kit (Karaj, Iran), employing a photometric method at 546 nm. The difference between total protein and albumin values yielded the globulin levels. Lactate dehydrogenase (LDH) levels were measured using a quantitative photometric method (Pars Azmun, Karaj, Iran) at 340 nm (Hoseini *et al.,* 2011). Cortisol levels were determined using an ELISA kit (Monobind Inc., Lake Forest, California, USA) possessing inter-assay and intra-assay coefficients of variation of 3.22% and 5.96%. Glucose concentrations were determined using commercially available kits from Pars Azmun (Karaj, Iran) and a Unico UV/Vis 2100 spectrophotometer (Chicago, USA) at 546 nm (Bartonkova *et al.,* 2017).

Gene expressions

At the end of the experiment, 27 samples, including three fish from each nine treatments were randomly selected. Liver and brain tissues were collected under sterile conditions for gene expression analysis of *igf-1*, *p450, hsp70* and *gh*, respectively. Prior to tissue collection, fish were anesthetized by immersion in a 0.5 g/L clove powder solution and small sections of liver and brain tissues were excised and promptly transferred to sterile microtubes. The microtubes were then immediately cryopreserved in liquid nitrogen and subsequently stored at -80°C until RNA extraction (Safari *et al.,* 2016).

Total RNA was extracted from approximately 100 mg of homogenized tissue using the Biozol kit (Biozol-Bioflux-Bioer) according to the manufacturer's protocol. For RNA extraction, samples were pulverized to a fine powder using a pre-chilled mortar and pestle with liquid nitrogen. *beta-actin* gene was used as the reference gene for normalization of gene

expression data. Small sections of each tissue were promptly transferred to microtubes, immediately cryopreserved in liquid nitrogen and subsequently maintained at -80°C for subsequent processing. To assess the RNA both qualitative and quantitative methods were employed following extraction from the tissues. Qualitative assessment involved agarose gel electrophoresis (1%). Quantitative evaluation was conducted using a BioPhotometer, measuring the 260/280 absorbance ratio. cDNA synthesis was carried out using a commercial kit from Gent Bio (Gent Bio, Daejeon, Korea). For cDNA synthesis, 1 µL of oligo primer was admixture with 15 µL of the prepared RNA in new tubes, and the volume was brought to 10 µL with nuclease-free water. The mixture was deposited on a heat block at 65°C for 1 min, followed by immediate transfer to ice. Reverse transcription was performed by adding 10 µL of the master mix to each sample. The admixture incubated at 50°C for 60 minutes and 70 °C for 10 minutes. The resulting cDNA solution (120 µL) kept stored at -20°C. Standard PCR was carried out with 2 µL of the diluted cDNA sample (1:10 dilution), 1 µL of both forward and reverse primer, 3 µL of sterile, nuclease-free water, and 5 µL of PCR premix at 59°C. PCR was conducted in specialized tubes with 4 technical replicates for each treatment. Each tube contained a 20 µL reaction mixture consisting of 10 µL Cybergreen amplicon buffer, 1 µL of each Oligonucleotide primer (both forward and reverse) for the target and reference genes, 8 µL of water, 0.2 µL of tag enzyme, and 8 µL of the diluted cDNA. To optimize real-time PCR conditions, varying volumes (1.10, 1.20, and 1.50 µL) of the mixed cDNA samples from different treatments were prepared and amplified in quadruplicate with both target and reference primers at 59°C. Additionally, standard curves were generated for each primer to assess the efficiency of the assay, as described by Awad *et al*. (2011) and Safari *et al*. (2016). Gene expression levels were quantified using the -ΔΔCT method (ΔΔCT = ΔCT (target gene) – ΔCT (reference sample)) (Livak and Schmittgen, 2001) (Table 2).

Table 2: Oligonucleotide primers for quantitative PCR analysis of gene expression in Siberian sturgeon, *Acipenser baerii*.

Gene	Accession sequence Number Abbreviation	Primers	Junction Temperature (°C)	Primer Efficiency (%)
igf-1	AB512770.1	F: GACACGCTTTGTGTGTGGAG R:ACTCGTTCACGATGCCCCTGTGGTG	59	95
gh	AB517597.1	F: TGTGGCTCTCATGAGGGAT R: CTGCATTTCATCACTTTCAGG	59	95
p450	JX013935.2	F: GTCATCTGTGCCATGTGCTT R: TCTTGTCGAAGGAGCGGTAG	59	95
hsp70	KF000408.1	F: CGCTGGCCTTAATGTTCTCC R: GCGCTTGAACTCTGCAATGA	59	95
beta-actin	AY878120.1	F: TTGCCATCCAGGCTGTGCT R: TCTCGGCTGTGGTGAA	59	95

Statistical analysis

The data distribution was assessed for normality using the Kolmogorov-Smirnov test, while Levene's test was used to evaluate the homogeneity of variances. To assess the mean differences, a two-factor ANOVA, including $CoCl_2$ and AA as factors, was conducted to evaluate the observed differences among the various treatments. Mean comparisons were conducted using Duncan's multiple-range test. Statistical analyses were performed using SPSS software (version 26, IBM, USA) with statistical significance set at $p<0.05$ (95% confidence interval).

Results

Growth performance

The highest final weight, final length, and WG were recorded for the diet containing 4 mg $CoCl_2$ per kg commercial diet ($p<0.05$). The analysis revealed no significant differences in BWI, FCR, SGR, GR, CF, ADG, and PER between the treatment groups and the control group ($p>0.05$). There was a significant interaction between $CoCl_2$ and AA, but only for final weight and length ($p<0.05$; Table 3).

Table 3: The growth performance of *Acipenser baerii* fed diets containing $CoCl_2$ and AA after 12 weeks (mean ± SE) (n= 81).

Treatments	Parameters												
	Initial weight, (g)	Final weight, (g)	Initial length, (cm)	Final length, (cm)	Weight gain, (g)	Body weight increase (%)	Condition factor	Growth rate, (%)	Food conversion ratio	Specific growth rate, (%/day)	Average daily growth (%)	Protein efficiency ratio	Survival rate (%)
Control	11.5 ± 0.0	97.0 ± 3.5^{d}	12.9 ± 0.3	30.4 ± 0.7^{bcd}	85.4 ± 3.4^{d}	742.3 ± 28.8	0.3 ± 0.0	101.7 ± 4.1^{d}	2.9 ± 0.0	2.5 ± 0.0	8.8 ± 0.3	0.7 ± 0.0	100
2 $CoCl_2$	11.5 ± 0.1	108.3 ± 1.5^{ab}	12.9 ± 0.3	31.6 ± 0.0^{ab}	96.8 ± 1.6^{ab}	841.8 ± 20.6	0.3 ± 0.0	115.2 ± 1.9^{ab}	2.8 ± 0.0	2.7 ± 0.0	10.0 ± 0.2	0.7 ± 0.0	100
4 $CoCl_2$	11.6 ± 0.3	110.9 ± 1.2^{a}	12.9 ± 0.1	32.4 ± 0.1^{a}	99.3 ± 1.0^{a}	857.4 ± 12.9	0.3 ± 0.0	118.3 ± 1.2^{a}	2.8 ± 0.0	2.7 ± 0.0	10.2 ± 0.2	0.7 ± 0.0	100
200 AA	11.5 ± 0.2	107.3 ± 4.8^{ab}	12.9 ± 0.2	30.1 ± 1.0^{cd}	95.7 ± 4.8^{ab}	830.0 ± 45.4	0.4 ± 0.0	114.0 ± 5.7^{ab}	2.8 ± 0.0	2.7 ± 0.1	9.9 ± 0.5	0.7 ± 0.0	100
800 AA	11.5 ± 0.6	106.2 ± 0.2^{abc}	12.9 ± 0.1	31.3 ± 0.1^{abc}	94.7 ± 0.6^{abc}	821.1 ± 48.1	0.3 ± 0.0	112.8 ± 0.7^{abc}	2.8 ± 0.0	2.6 ± 0.1	9.9 ± 0.6	0.7 ± 0.0	100

Table 3 (continued):

Treatments	Parameters											
	Initial weight, (g)	Final weight, (g)	Initial length, (cm)	Final length, (cm)	Weight gain, (g)	Body weight increase (%)	Condition factor	Growth rate, (%)	Food conversion ratio	Specific growth rate, (%/day)	Average daily growth (%)	Protein efficiency ratio	Survival rate (%)
2 $CoCl_2$ + 200 AA	11.4 ± 0.4	100.7 ± 1.1^{abcd}	12.9 ± 0.2	30.5 ± 0.3^{bcd}	89.2 ± 1.3^{bcd}	783.4 ± 34.2	0.4 ± 0.0	106.2 ± 1.6^{bcd}	2.8 ± 0.0	2.6 ± 0.0	9.3 ± 0.4	0.7 ± 0.0	100
4 $CoCl_2$ + 800 AA	11.5 ± 0.4	103.7 ± 1.8^{bcd}	12.9 ± 0.1	31.1 ± 0.1^{abcd}	92.2 ± 2.0^{abcd}	802.3 ± 41.4	0.3 ± 0.0	109.7 ± 2.4^{abcd}	2.8 ± 0.0	2.6 ± 0.1	9.6 ± 0.5	0.7 ± 0.0	100
2 $CoCl_2$ + 800 AA	11.4 ± 0.2	98.8 ± 2.3^{cd}	13.0 ± 0.0	29.7 ± 0.3^{d}	87.4 ± 2.5^{cd}	765.4 ± 33.3	0.4 ± 0.0	104.0 ± 2.4^{cd}	2.9 ± 0.0	2.6 ± 0.0	9.1 ± 0.4	0.7 ± 0.0	100
4 $CoCl_2$ + 200 AA	11.5 ± 0.5	108.4 ± 2.3^{ab}	13.0 ± 0.0	32.2 ± 0.3^{a}	96.9 ± 2.3^{ab}	844.1 ± 42.8	0.3 ± 0.0	115.4 ± 2.7^{ab}	2.8 ± 0.0	2.7 ± 0.1	10.0 ± 0.5	0.7 ± 0.0	100

	Two- Way ANOVA									
	Final weight	Final length	Weight gain	Body weight increase	Condition factor	Growth rate	Food conversion ratio	Specific growth rate	Average daily growth	Protein efficiency ratio
$CoCl_2$	0.038*	0.001*	0.600	0.379	0.057	0.045*	0.225	0.362	0.379	0.346
Ascorbic acid	0.642	0.039	0.112	0.940	0.098	0.659	0.060	0.935	0.940	0.929
Ascorbic acid + $CoCl_2$	0.002*	0.018*	0.946	0.141	0.234	0.002*	0.551	0.123	0.141	0.108

Non-identical symbols within a row indicate a significant differences (p<0.05) between treatments for that specific parameter.

An asterisk (*) denotes the presence of significant interaction effects between cobalt chloride and ascorbic acid at p<0.05.

Treatment Groups: Control: Basic diet without any added cobalt chloride or ascorbic acid. Treatment 1: 2 mg cobalt chloride per kg of diet. Treatment 2: 4 mg cobalt chloride per kg of diet. Treatment 3: 200 mg ascorbic acid per kg of diet. Treatment 4: 800 mg ascorbic acid per kg of diet. Treatment 5: 2 mg cobalt chloride + 200 mg ascorbic acid per kg of diet. Treatment 6: 4 mg cobalt chloride + 800 mg ascorbic acid per kg of diet. Treatment 7: 2 mg cobalt chloride + 800 mg ascorbic acid per kg of diet. Treatment 8: 4 mg cobalt chloride + 200 mg ascorbic acid per kg of diet.

Biochemical indices

Levels of vitamins C and B12 were lower in the control group than those of the treatment groups (p<0.05). The highest AA level was detected in *A. baerii*, fed 4 mg/kg $CoCl_2$ and 200 mg/kg AA (p<0.05), while the maximum vitamin B_{12} level was observed in those fed 2 mg $CoCl_2$ per kg diet (p<0.05). Regarding minerals levels, the treatment fed 800 mg AA per kg diet exhibited maximum iron concentrations (p<0.05). Glucose level was lower in the control group than those of treatments groups (p<0.05). Ultimately, the treatment had a significant impact on cortisol levels, with the peak level observed in fish fed 2 mg $CoCl_2$+200 mg AA per kg diet, whereas the control group exhibited the minimum level (p<0.05; Table 4).

Table 4: Biochemical indices of Siberian sturgeon, *Acipenser baerii*, following 12 weeks of dietary $CoCl_2$ and AA supplementation (mean ± SE) (n= 81).

Treatments	Parameters							
	Ascorbic acid (mg/mL)	Vitamin B_{12} (mg/mL)	Iron (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Glucose (mg/dL)	Cortisol (ng/mL)
Control	6.3 ± 0.1^{f}	81.7 ± 1.6^{h}	65.4 ± 1.8^{e}	1.3 ± 0.0^{c}	0.6 ± 0.0^{c}	0.7 ± 0.0^{b}	32.7 ± 0.6^{c}	91.6 ± 3.4^{e}
2 $CoCl_2$	6.5 ± 0.0^{e}	155.0 ± 2.6^{a}	61.6 ± 2.4^{e}	1.6 ± 0.1^{a}	0.7 ± 0.0^{b}	0.9 ± 0.1^{a}	38.7 ± 0.8^{b}	131.0 ± 0.4^{b}
4 $CoCl_2$	6.6 ± 0.0^{d}	135.0 ± 2.7^{dc}	67.8 ± 0.6^{cd}	1.5 ± 0.0^{a}	0.7 ± 0.0^{b}	0.8 ± 0.0^{a}	40.7 ± 0.6^{b}	126.0 ± 2.3^{b}
200 AA	7.2 ± 0.0^{c}	103.0 ± 2.0^{g}	73.2 ± 0.5^{ab}	1.4 ± 0.0^{b}	0.6 ± 0.0^{c}	0.8 ± 0.0^{a}	42.7 ± 0.2^{b}	98.0 ± 1.1^{d}
800 AA	7.3 ± 0.0^{b}	112.7 ± 1.2^{f}	74.0 ± 0.9^{a}	1.3 ± 0.0^{c}	0.6 ± 0.0^{c}	0.7 ± 0.0^{b}	42.0 ± 0.2^{b}	93.3 ± 0.6^{de}
2 $CoCl_2$ + 200 AA	7.2 ± 0.0^{c}	142.7 ± 0.8^{bc}	64.9 ± 1.1^{e}	1.6 ± 0.0^{a}	0.8 ± 0.0^{a}	0.8 ± 0.0^{a}	54.7 ± 0.6^{a}	147.0 ± 0.8^{c}
4 $CoCl_2$ + 800 AA	7.4 ± 0.0^{b}	130.3 ± 0.6^{e}	69.7 ± 0.5^{bc}	1.5 ± 0.0^{a}	0.9 ± 0.0^{a}	0.6 ± 0.0^{c}	55.3 ± 0.4^{a}	116.3 ± 0.6^{c}

Table 4 (continued):

Treatments	Parameters							
	Ascorbic acid (mg/mL)	Vitamin B_{12} (mg/mL)	Iron (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Glucose (mg/dL)	Cortisol (ng/mL)
2 $CoCl_2$ + 800 AA	7.4 ± 0.1^{b}	145.7 ± 2.1^{b}	66.6 ± 1.0^{cd}	1.5 ± 0.0^{a}	0.9 ± 0.0^{a}	0.6 ± 0.0^{c}	53.0 ± 0.4^{a}	97.7 ± 1.0^{d}
4 $CoCl_2$ + 200 AA	7.9 ± 0.1^{a}	139 ± 1.6^{cd}	69.2 ± 0.7^{c}	1.6 ± 0.0^{a}	0.8 ± 0.0^{a}	0.6 ± 0.0^{c}	53.7 ± 0.8^{a}	93.0 ± 2.4^{dc}

Two-Way ANOVA

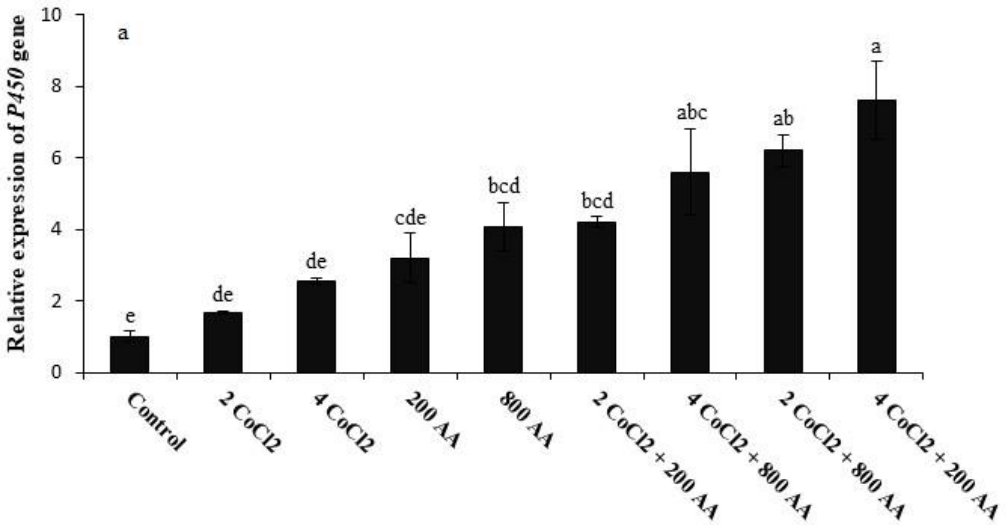
	Ascorbic acid	Vitamin B_{12}	Iron	Total protein	Albumin	Globulin	Glucose	Cortisol
$CoCl_2$	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}
Ascorbic acid	0.000^{*}	0.003^{*}	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}	0.000^{*}
Ascorbic acid + $CoCl_2$	0.000^{*}	0.060^{*}	0.060^{*}	0.000^{*}	0.000^{*}	0.000^{*}	0.196^{*}	0.000^{*}

Non-synonymous letters in the column indicate a significant difference (p<0.05).
The asterisk (*) indicates the presence of significance and interaction effects at the level of p<0.05.

Gene expression

The highest upregulation of *hsp70* and *p450* mRNA levels was observed in the group fed 4 mg/kg $CoCl_2$ with 200 mg/kg AA, which were significantly higher than those of the control group (p<0.05).

Additionally, peak expression levels of *GH* and *igf-1* were seen in the fish receiving 4 mg/kg $CoCl_2$ and 200 mg/kg AA in their diet, while the lowest expression was found in the control group (p<0.05; Fig. 1).



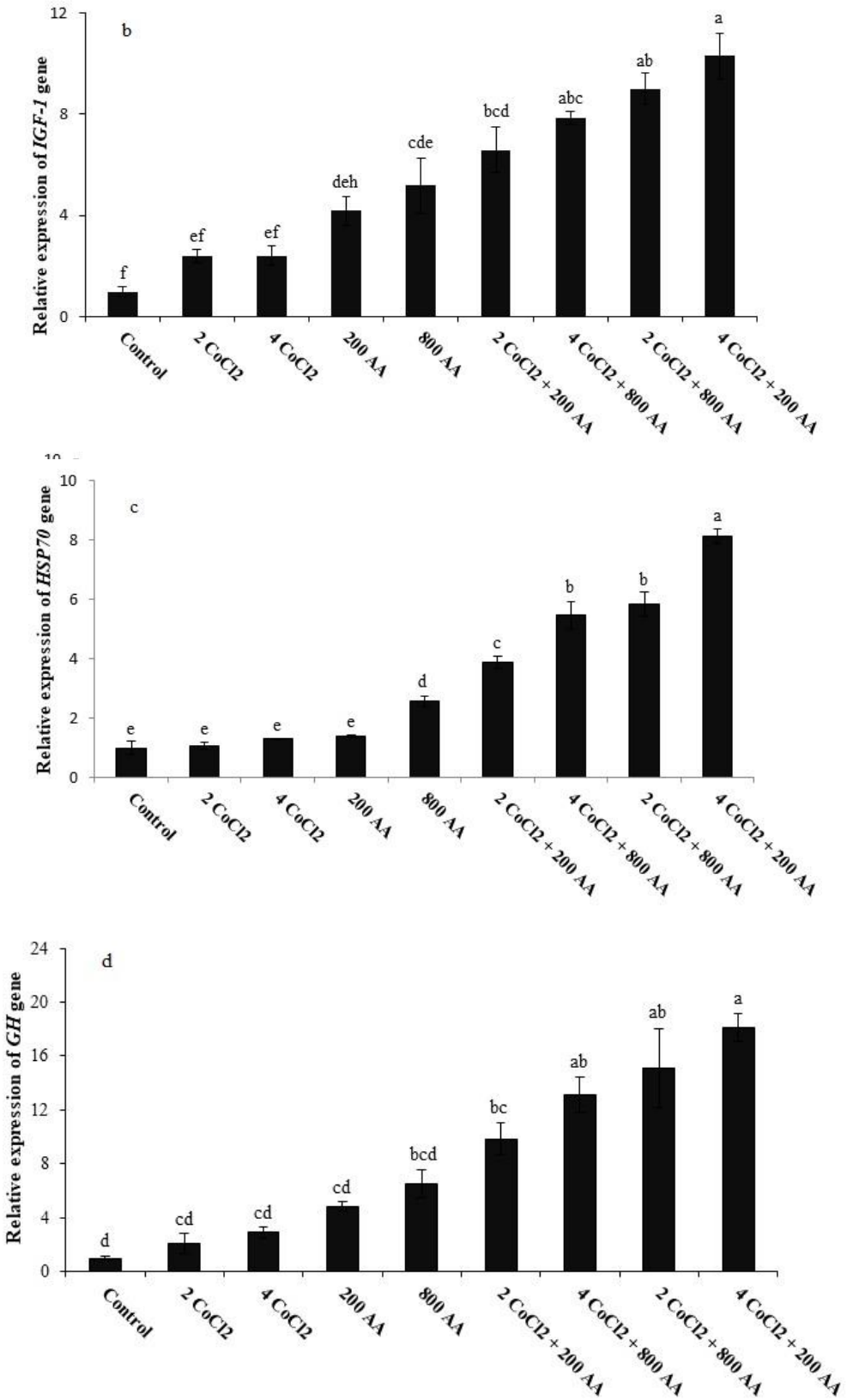


Figure 1: The effect of treatment uses of AA and $CoCl_2$ on relative expression of *p450* (a), *igf-1* (b), *hsp70* (c) and *GH* (d) after 12 weeks of feeding. Non-synonymous letters in the column indicate a significant difference (p<0.05) (n= 27).

Discussion

This research showed that supplementation with both AA and $CoCl_2$ positively affected the growth parameters of Siberian sturgeon. Notably, the diet that enriched with 4 mg $CoCl_2$ per kg demonstrated the most significant improvements in final weight and length, WG, and GR. This enhancement might be attributed to the synergistic actions of AA and $CoCl_2$ when it comes to promoting intestinal health and nutrient absorption. The diet containing 4 mg/kg $CoCl_2$ and 200 mg/kg AA significantly improved several biochemical indices, including the highest levels of ascorbic acid, total protein, albumin, and glucose, while also reducing cortisol levels in Siberian sturgeon (*A. baerii*). These findings are consistent with earlier research on Siberian sturgeon, including a study by Pourgholam *et al*. (2016), which demonstrated that dietary micronutrient with 800 mg/kg of AA for 12 weeks significantly improved growth parameters. It is important to consider that some research on two other species has shown different results. For example, Moreau and Dabrowski, (2000) and Gy. Papp *et al*. (1999) reported good growth performance in hybrid *A. ruthenus* × *A. baerii* even without AA supplementation. Furthermore, a study by Desimira *et al*. (2013) showed a negative correlation between SGR and AA levels in stellate sturgeon (*A. stellatus*), where the lowest SGR values were observed in both the control and the group receiving 800 mg/kg AA. These findings suggest that lower AA concentrations may be more beneficial for growth in certain juvenile sturgeon species. In contrast, dietary AA has consistently shown positive effects on final body weight, SGR, and WG across different fish species, e.g., Nile tilapia (*O. niloticus*) (Ibrahim *et al*., 2020). Aboseif *et al*. (2022) also demonstrated that 200 mg/kg AA of feed enhanced growth factors and intestinal mucosal epithelium structure in common carp (*Cyprinus carpio*), supporting our findings. The significant growth enhancement observed in Siberian sturgeon fed a diet containing AA due to its potential to elevate circulating GH levels, induce beneficial alterations in intestinal morphology, and enhance the gut's ability to absorb nutrients (Rahman *et al*., 2018). The other reason is that stimulates the production of proteins could also contribute to the observed increase in WG and faster growth following supplementation (Chagas and Val, 2003).

Dietary supplementation with $CoCl_2$ and AA has shown promise in enhancing growth performance across various fish species. For instance, Anadu *et al*. (1990) demonstrated that *Tilapia zilli*, which received diets AA, exhibited superior growth in comparison to those receiving $CoCl_2$ or the control diet. Similarly, $CoCl_2$ supplementation has been linked to increased total length and weight in rainbow trout (*Oncorhynchus mykiss*), *C. carpio*, royal carp (*Astronotus ocellatus*), grouper (*E. malabaricus*), red-bellied tilapia (*Coptodon zillii*), Nile tilapia (*O. niloticus*), and Golden mahseer, *Tor putitora* (Yada *et al*., 2002; Mukherjee and Kaviraj, 2009; Younus *et al*., 2020). To ensure optimal growth and survival in economically important fish species like *O. spilurus* (Al-Amoudi *et al*. 1992), redlip croaker (*Larymichthys polyactis*) (Ai *et al*., 2006), and parrotfish (*O. fasciatus*) (Wang

et al., 2003), adequate AA levels in fish feeds are essential. Ibrahim *et al*. (2020) further emphasized the positive influence of AA on *O. niloticus* growth, attributing it to the vitamin's role in elevating serum growth hormone levels, improving intestinal morphology, and enhancing nutrient absorption. In fish, *GH* and *igf-1* play crucial roles in regulating metabolic homeostasis and growth. Hepatic *igf-1* levels are often dependent on nutritional status. A considerable body of research has demonstrated the beneficial effects of AA and $CoCl_2$ on growth performance in a range of bony fish species (Dabrowski, 2000; Harsij *et al*., 2020; Singh *et al*., 2021; Delavari *et al*., 2022; Ghafarifarsani *et al*., 2022; Xu *et al*., 2022). Growth enhancement was most significant with 4 mg $CoCl_2$ per kg; however, no significant differences were observed in SGR, ADG, PER, CF, BWI, FCR, and SR across the treatment groups. This lack of pronounced differences in growth parameters, despite varying AA supplementation levels in *A. baerii*, is potentially attributed to the presence of L-gluconolactone oxidase. This enzyme enables the de novo synthesis of L-ascorbic acid from D-glucose or D-galactose, potentially fulfilling the fish's ascorbic acid requirements for growth, immunity, and health even in the absence of dietary supplementation (Dabrowski, 2000; Moreau and Dabrowski, 2000). Consistent with our findings, many studies have demonstrated the beneficial effects of cobalt supplementation on growth, survival, protein synthesis, and glucose homeostasis in a range of fish species, including *O. mykiss* (Blust, 2012), and *C. carpio* (Wilson, 1991). Notably, Tonye and Sikoki, (2014) observed significant growth enhancement in Nile tilapia fed a $CoCl_2$-supplemented diet, even though FCR and carcass composition remained unchanged. These results suggest that $CoCl_2$, even at a 0.1% inclusion level, possesses growth-promoting properties. In the current study, supplementing the diet with 4 mg/kg $CoCl_2$ alongside 200 mg/kg AA had the most pronounced positive impact on growth parameters in Siberian sturgeon. We observed a significant increase in WG with 4 mg/kg dietary $CoCl_2$, with this group exhibiting the highest percentage of WG. Cobalt chloride is crucial for fish metabolism by enhancing muscular protein synthesis and nitrogen assimilation, contributing to overall growth and development. Additionally, the chlorine ions present in $CoCl_2$ activate digestive enzymes, potentially improving nutrient digestion and absorption.

Therefore, studying hematological and biochemical parameters is crucial for assessing fish growth and health status. Our findings indicate that serum biochemical indices, such as serum protein, albumin, and globulin concentrations, were significantly elevated in the groups supplemented with $CoCl_2$ and AA, compared to the control group. Total protein, which is synthesized by liver parenchymal cells, serves as an important clinical indicator of overall health and well-being, including nutritional status, immune function, and stress response in fish (Safari *et al.,* 2019). Elevated levels of total protein, albumin, and globulin may indicate an enhanced innate immune response (Wiegertjes *et al.,* 1996) and could be attributed to non-specific immune reactions

stimulated by AA and $CoCl_2$ supplementation. Furthermore, in contrast to the treatments, the control group had significantly lower iron levels. Siberian sturgeon groups that received diets with 200 mg and 800 mg of AA exhibited the highest iron levels, highlighting AA crucial role in iron metabolism. Ascorbic acid is necessary for releasing iron bound to ferritin in the liver, making it available for erythropoiesis (red blood cell formation). These findings underscore the complex interplay of various factors influencing blood indices and overall fish health, emphasizing the multifaceted effects of nutritional interventions on fish physiology. Ascorbic acid is also crucial for iron uptake in some fish species. Iron deficiency may be attributed to reduced dietary absorption and impaired iron metabolism due to a lack of AA. Without AA, ferric hemoglobin (Fe^{3+}) is converted to ferrous hemoglobin (Fe^{2+}), hindering plasma transport and cellular iron uptake. Ascorbic acid improves the absorption of iron from dietary sources, and a deficiency in AA can cause damage to iron mobilized from reticuloendothelial reserves (Elbaraasi *et al*., 2004).

Cortisol, a principal stress hormone in teleost fish (Hsieh *et al*., 2003), serves as a well-established indicator of the physiological response to stress and is crucial in regulating glucose metabolism, particularly in stress-induced hyperglycemia (Vijayan *et al*., 1997). Stress initiates a cascade of physiological responses in teleosts, notably activating the hypothalamic-pituitary-interrenal (HPI) axis, leading to heightened catecholamine secretion and elevated serum cortisol levels. Our results, consistent with those of Montero *et al*. (1999), demonstrate that higher concentrations of AA can effectively decrease cortisol levels, suggesting that AA may have anti-stress properties. The observed reduction in cortisol levels resulting from AA supplementation may be explained by its ability to inhibit steroidogenesis. Through its regulation of unsaturated fatty acids peroxidation, AA may limit the availability of cholesterol, an essential precursor for cortisol synthesis, ultimately reducing cortisol production (Ming *et al*., 2012). Functionality is consistent with the results reported by Zhang *et al*. (2022), who found that dietary AA in *Pampus argenteus* enhanced immunity and mitigated stress responses. This investigation revealed that glucose concentrations were markedly lower for the control group compared to those receiving dietary treatments. Interestingly, among the various dietary treatments, the one featuring 2 mg/kg $CoCl_2$ and 200 mg/kg AA showed the peak cortisol level. This seemingly contradictory finding warrants further investigation to elucidate the complex interactions between AA, $CoCl_2$, cortisol, and glucose metabolism in *A. baerii*. However, it is worth noting that the overall trend of reduced cortisol levels with AA supplementation supports its potential anti-stress properties. The results showed increased cortisol and glucose levels in the non-supplemented group when exposed to stress, followed by a reduction in these levels upon supplementing the diet with AA, which agrees with the findings of Mustafa *et al*. (2013) in Nile tilapia (*O. niloticus*). This further strengthens the evidence for the stress-mitigating and

adaptive benefits of ascorbic acid in fish diets.

$CoCl_2$ induces hypoxia response genes, including *hifα*, by mimicking hypoxia (Ji *et al*., 2012). Our findings indicate that the levels of vitamin B_{12} were elevated in the treatments that included $CoCl_2$ supplementation showing a significant increase relative those in the control group as well as the groups receiving other dietary regimens. In addition, among Siberian sturgeon fed various diets, those receiving a diet with 4 mg/kg of $CoCl_2$ and 200 mg/kg of AA displayed maximum expression of *hsp70* and *p450* genes. Peak gene expression levels of *GH* and *igf-1* were observed in the group fed the combined diet, while the control group showed the lowest expression these genes Growth hormone, a polypeptide hormone, is synthesized by the pituitary gland and is essential for a range of physiological processes, such as growth, carbohydrate, and protein metabolism, and energy balance. Growth hormone secretion stimulates *igf-1* production, which then carries out cell division processes. Thus, the production of *igf-1* depends on both the production and release of *gh*, ultimately regulated by the neuroendocrine *hpi* axis and nutritional factors, including food intake and nutrient absorption. Cytochrome *p450*, a type of hemoprotein, is highly present in the liver and involved in the first phase of xenobiotic substance metabolism (Parhar *et al*., 2003). The findings of this study indicated substantial differences in the expression of genes among the different treatment groups and the control group. Dietary supplementation with both AA and $CoCl_2$ led to increased levels of *gh*, *igf-1*, *hsp70*, and *p450* relative to the groups receiving only AA or $CoCl_2$ as well as the control group, with peak levels observed for the 4 $CoCl_2$+200 AA treatment. The heat shock response involves the upregulation of several *hsps*, including *hsp70*, *hsp90*, and *hsp60*, with *hsp70* playing a particularly crucial role in mitigating the effects of cellular stress (Zheng *et al*., 2010). However, fish immunostimulants can reduce *hsp70* expression (Ahmadi *et al*., 2014). Research by Ming *et al*. (2012) showed that dietary AA resulted in increased levels of *hsp70*, including its *mRNA*, in fish exposed to heat stress that were given ascorbic acid. The *p450* family comprises numerous enzymes that play a crucial role in detoxifying and metabolizing an extensive range of substances, both those produced naturally within the body and those foreign to it. (Miandare *et al*., 2016). Downregulation of *p450*, *rpl6*, and *hsp70* gene expression was reported in treated *A. baerii* using barberry fruit extract, indicating increased tolerance against potential stressors in fish husbandry (Shekarabi *et al*., 2022). Similar results were observed in the reduction of cytochrome C gene expression in *Ctenopharyngodon idella* using berberine (Yang *et al*., 2019). The results of studies showed *igf-I* exhibits the greatest resistance in rainbow trout, while GH is the most sensitive component to downregulation of gene expression when exposed to cobalt and zinc. Ekinci *et al*. (2011) reported that *igf-I* exhibited the most resistance and *GH* the most sensitivity to decreased gene expression when exposed to cobalt and zinc in *O. mykiss*. In this investigation, the combined supplementation AA and $CoCl_2$

resulted peak expression levels of these genes. This suggests a potential synergistic effect of these nutrients on gene expression. Ascorbic acid and $CoCl_2$ may reduce *hsp70* gene expression by mitigating cellular stress, as demonstrated by Ming *et al*. (2010). According to Wan *et al*. (2014) demonstrated that dietary inclusion of AA (133.7-251.5 mg/kg) upregulated the *mRNA* expression of three *hsps* in *Megalobrama amblycephala* (juvenile blunt snout bream). In conclusion, the combined effects of $CoCl_2$ and AA promote growth and immune function in fish and have a positive impact on key physiological markers, suggesting improved resilience against stressors. These findings highlight the crucial role of dietary supplementation in aquaculture practices to optimize fish health and performance.

Cobalt chloride mimics hypoxic conditions by stabilizing the transcription factor *hifα*, a crucial regulator of numerous genes involved in growth and metabolism, notably those encoding *GH* and *igf-1*. Therefore, $CoCl_2$-induced *hifα* activation likely contributes to the increased *GH* and *igf-1* gene expression. Furthermore, AA has immunomodulatory effects, and a robust immune system indirectly promotes growth. While $CoCl_2$ mimics hypoxia, AA is a cofactor in various enzymatic reactions. Their supplementation may improve overall nutritional status and metabolic homeostasis, creating a conducive environment for *GH* and *igf-1* gene expression and protein synthesis.

Conclusions

The data presented here show that supplementing the diet with AA and $CoCl_2$, especially when administered together at levels of 200 mg/kg AA and 4 mg/kg $CoCl_2$, can enhance growth performance, hematological and biochemical indices, and the modulation of genes associated with growth, stress response, and immunity in Siberian sturgeon. Ascorbic acid exhibited a more substantial impact on these parameters compared to $CoCl_2$. Thus, we propose supplementing the feed with this specific combination of AA and $CoCl_2$ to improve overall health, growth, and potential disease resistance in Siberian sturgeon. However, further studies must examine the effects over an extended period and possible interactions of AA and $CoCl_2$ with other dietary components in Siberian sturgeon and other commercially important fish species.

Acknowledgements

The authors would like to acknowledge the Lahijan branch Islamic Azad University's support in conducting this study, Aria Chemistry Sharif Co, ViroMed Laboratory and Gorgan University of Agricultural Sciences and natural resources for feed analysis and analyzed of the hematological parameters and genetic tests.

Conflicts of Interest

The authors declare no competing interests.

References

Abbas, S. and Javed, M., 2016. Growth performance of *Labeo rohita* under chronic dual exposure of water-borne and dietary cobalt. *Pakistan Journal of Zoology*, 48(**1**), 257-264.

Aboseif, A.M., Flefil, N.S., Taha, M.K., Tahoun, U.M., Mola, H.R.A., El-Haroun, E., Van Doan, H. and **Goda, A.M.A., 2022.** Influence of dietary C: N: P ratios on Nile tilapia *Oreochromis niloticus* growth performance and formation of water biotic communities within a biofloc system containment. *Aquaculture Reports*, 24, 101136. DOI:10.1016/j.aqrep.2022.101136

Adel, A. and Khara, H., 2016. The effects of different dietary vitamin C and iron levels on the growth, hematological and immunological parameters of rainbow trout, Oncorhynchus mykiss fingerlings. *Iranian Journal of Fisheries Sciences*, 15(**2**), 886-897.

Ahmadi, P.Y., Farahmand, H., Miandare, H.K., Mirvaghefi, A. and Hoseinifar, S.H., 2014. The effects of dietary Immunogen® on innate immune response, immune related genes expression and disease resistance of rainbow trout (*Oncorhynchus mykiss*). *Fish and Shellfish Immunology*, 37(**2**), 209-214. DOI:10.1016/j.fsi.2014.02.006

Ahmadi-Noorbakhsh, S., Mirabzadeh Ardakani, E., Sadighi, J., Aldavood, S. J., Farajli Abbasi, M., Farzad-Mohajeri, S., Shamsi Gooshki, E., 2021. Guideline for the care and use of laboratory animals in Iran. *Lab animal*, 50(**11**), 303-305. DOI:10.1038/s41684-021-00871-3

Ai, Q., Mai, K., Tan, B., Xu, W., Zhang, W., Ma, H. and Liufu, Z., 2006. Effects of dietary vitamin C on survival, growth, and immunity of large yellow croaker, Pseudosciaena crocea. *Aquaculture*, 261(**1**), 327-336. DOI:10.1016/j.aquaculture.2006.07.027

Akdemir, F., Orhan, C., Tuzcu, M., Sahin, N., Juturu, V. and Sahin, K., 2017. The efficacy of dietary curcumin on growth performance, lipid peroxidation and hepatic transcription factors in rainbow trout *Oncorhynchus mykiss* (Walbaum) reared under different stocking densities. *Aquaculture Research*, 48(**8**), 4012-4021. DOI:10.1111/are.13223

Al-Amoudi, M.M., El-Nakkadi, A.M.N. and El-Nouman, B.M., 1992. Evaluation of optimum dietary requirement of vitamin C for the growth of *Oreochromis spilurus* fingerlings in water from the Red Sea. *Aquaculture*, 105(**2**), 165-173. DOI:10.1016/0044-8486(92)90128-8

Anadu, D.I., Anozie, O.C. and Anthony, A.D., 1990. Growth responses of *Tilapia zillii* fed diets containing various levels of ascorbic acid and cobalt chloride. *Aquaculture*, 88(**3-4**), 329-336.

Arukwe, A. and Goksøyr, A., 2003. Eggshell and egg yolk proteins in fish: hepatic proteins for the next generation: oogenetic, population, and evolutionary implications of endocrine disruption. *Comparative Hepatology*, 2, 1-21. DOI:10.1186/1476-5926-2-4

Awad, H., Antunes, S., Galindo, RC., DO Rosario, VE., DE Lafuente, J., Domingos, A., Hussein, AM., 2011. Prevalence and genetic diversity of Babesia and Anaplasma species in cattle in Sudan. *Veterinary Parasitology*, 181, 146-1452. DOI:10.1016/j.vetpar.2011.04.007

Bartonkova, J., Hyrsl, P. and Vojtek, L., 2017. Glucose determination in fish plasma by two different moderate methods. *Acta Veterinaria Brno*, 85(**4**),

349-353. DOI:10.2754/avb201685040349

Basu, N., Todgham, A.E., Ackerman, P.A., Bibeau, M.R., Nakano, K., Schulte, P.M. and Iwama, G.K., 2002. Heat shock protein genes and their functional significance in fish. *Gene*, 295(**2**), 173-183. DOI:10.1016/S0378-1119(02)00687-X

Biesalski, H.K. and Jana, T., 2018. Micronutrients in the life cycle: requirements and sufficient supply. *Nutrition and Food Science Journal*, 11, 1-11. DOI:10.1016/j.nfs.2018.03.001

Blust, R., 2012. Cobalt. *Fish Physiology*, 31, 291-326. DOI:10.1016/S1546-5098(11)31006-0

Boachie, J., Adaikalakoteswari, A., Samavat, J. and Saravanan, P., 2020. Low vitamin B_{12} and lipid metabolism: evidence from pre-clinical and clinical studies. *Nutrients*, 12(**7**), 1925. DOI:10.3390/nu12071925

Brunt, J. and Austin, B., 2005. Use of a probiotic to control lactococcosis and streptococcosis in Rainbow trout, *Oncorhynchus mykiss*. *Journal of Fish Diseases*, 28, 693-701. DOI:10.1111/j.1365-2761.2005.00672.x

Chagas, E.C. and Val, A.L., 2003. Effect of vitamin C on weight and hematology of tambaqui. *Pesquisa agropecuaria brasileira*, 38, 397-402. DOI:10.1590/S0100-204X2003000300009

Cheng, A.C., Chen, C.Y., Liou, C.H. and Chang, C.F., 2006. Effects of dietary protein and lipids on blood parameters and superoxide anion production in the Grouper, *Epinephelus coioides*. *Zoological Studies-Taipei*, 45, 492-502. DOI:10.29822/JFST.201509_42(3).0004

Dabrowski, K., 2000. Ascorbic acid in aquatic organisms: status and perspectives. *CRC Press, Boca Raton, FL, USA.* 287 P.

Dawood, M.A. and Koshio, S., 2018. Vitamin C supplementation to optimize growth, health and stress resistance in aquatic animals. *Reviews in Aquaculture,* 10(**2**), 334-350. DOI:10.1111/raq.12163

Delavari, N.M., Gharaei, A., Mirdar, H.J., Davari, A. and Rastiannasab, A., 2022. Modulatory effect of dietary copper nanoparticles and vitamin C supplementations on growth performance, hematological and immune parameters, oxidative status, histology, and disease resistance against *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry*, 1-19. DOI: 10.1007/s10695-021-01036-2

Desimira, D. S. M., Victor, C., Catalina, M. C., Săndiţa, P., Mihai Ştefan, P. and Tiberiu, C. M., 2013. Effects of different levels of dietary vitamins C on growth performance of stellate sturgeon (*Acipenser stellatus*, Pallas, 1771). *Scientific Papers: Animal Science and Biotechnologies/Lucrari Stiintifice: Zootehnie si Biotehnologii,* 46(**2**).

Djissou, A.S.M., Tossavi, C.E., Odjo, I.N., Koshio, S. and Fiogbe, E.D., 2019. Use of *Moringa oleifera* leaves and maggots as protein sources in complete replacement for fish meal in Nile tilapia (*Oreochromis niloticus*) diets. *Turkish Journal of Fisheries and Aquatic Sciences*, 20(**3**), 177-183. DOI:10.4194/1303-2712-v20-3-02

Duan, C., Ren, H. and Gao, S., 2010. Insulin-like growth factors (*IGFs*), *IGF* receptors, and IGF-binding proteins: Roles in skeletal muscle growth and

differentiation. *General and Comparative Endocrinology*, 167(**3**), 344-351. DOI:10.1016/j.ygcen.2010.04.009

Ekinci, D., Ceyhun, S. B., Aksakal, E. and Erdoğan, O., 2011. *IGF* and *GH* mRNA levels are suppressed upon exposure to micromolar concentrations of cobalt and zinc in rainbow trout white muscle. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 153(**3**), 336-341. DOI:10.1016/j.cbpc.2010.12.004

Elbaraasi, H., Mézes, M., Balogh, K., Horváth, L. and Csengeri, I., 2004. Effects of dietary ascorbic acid/iron ratio on some production traits, lipid peroxide state and amount/activity of the glutathione redox system in African catfish *Clarias gariepinus* (Burchell) fingerlings. *Aquaculture Research*, 35(**3**), 256-262. DOI:10.1111/j.1365-2109.2004.01004.x

Eo, J. and Lee, K.J., 2008. Effect of dietary ascorbic acid on growth and non-specific immune responses of tiger puffer, *Takifugu rubripes*. *Fish & Shellfish Immunology*, 25(**5**), 611-616. DOI:10.1016/j.fsi.2008.08.009

Eslamloo, K., Falahatkar, B. and Yokoyama, S., 2012. Effects of dietary bovine lactoferrin on growth, physiological performance, iron metabolism and non-specific immune responses of Siberian sturgeon, *Acipenser baerii*. *Fish and Shellfish Immunology*, 32(**6**), 976-985. DOI:10.1016/j.fsi.2012.02.007

Falahatkar, B., Soltani, M., Abtahi, B., Kalbassi, M.R. and Pourkazemi, M., 2006. Effects of dietary vitamin C supplementation on performance, tissue chemical composition and alkaline phosphatase activity in great sturgeon (*Huso huso*). *Journal of Applied Ichthyology*, 22, 283-286. DOI:0.1111/j.1439-0426.2007.00969.x

Falahatkar, B. and Poursaeid, S., 2014. Effects of hormonal manipulation on stress responses in male and female broodstocks of pikeperch Sander lucioperca. *Aquaculture international*, 22, 235-244. DOI:10.1007/s10499-013-9678-x

Falahatkar, B., 2018. Nutritional requirements of the Siberian sturgeon: an updated synthesis. In: Williot, P., Nonnotte, G., Chebanov, M., and Kasimov, R. (eds), *The Siberian Sturgeon (Acipenser baerii, Brandt, 1869), Volume 1 – Biology*. Springer, Cham, Switzerland. 207–228.

Fontagné, S., Bazin, D., Brèque, J., Vachot, C., Bernarde, C., Rouault, T. and Bergot, P., 2006. Effects of dietary oxidized lipid and vitamin A on the early development and antioxidant status of Siberian sturgeon (*Acipenser baerii*) larvae. *Aquaculture*, 257(**1-4**), 400-411. DOI:10.1016/j.aquaculture.2006.01.025

Ghafarifarsani, H., Hoseinifar, S.H., Javahery, S., Yazici, M. and Van Doan, H., 2022. Growth performance, biochemical parameters, and digestive enzymes in common carp (*Cyprinus carpio*) fed experimental diets supplemented with vitamin C, thyme essential oil, and quercetin. *Italian Journal of Animal Science*, 21(**1**), 291-302. DOI:10.1080/1828051X.2021.1965923

González-Montaña, J. R., Valente, F., Alonso, A., Lomillos, J., Robles, R., Alonso, M., 2020. Relationship between Vitamin B12 and Cobalt metabolism in domestic ruminant. *An update. Animal*,

10(**10**).1858. DOI:10.3390/ani10101855

Gouda, A., Amer, S.A., Gabr, S. and Tolba, S.A., 2020. Effect of dietary supplemental ascorbic acid and folic acid on the growth performance, redox status, and immune status of broiler chickens under heat stress. *Tropical Animal Health and Production*, 52, 2987-2996. DOI:10.1007/s11250-020-02316-4

Guo, X., Liang, X. F., Fang, L., Yuan, X., Zhou, Y., Zhang, J. and Li, B., 2015. Effects of dietary non-protein energy source levels on growth performance, body composition and lipid metabolism in herbivorous grass carp (*Ctenopharyngodon idella* Val.). *Aquaculture Research*, 46(**5**), 1197-1208. DOI:10.1111/are.12275

Gy. Papp, Z., Saroglia, M., Jeney, Z., Jeney, G. and Terova, G., 1999. Effects of dietary vitamin C on tissue ascorbate and collagen status in sturgeon hybrids (*Acipenser ruthenus* L. × *Acipenser baerii* Brandt). *Journal of Applied Ichthyology*, 15(**4-5**), 258-260. DOI:10.1111/j.1439-0426.1999.tb00246.x

Hall, J.E. and Hall, M.E., 2020. Guyton and Hall textbook of medical physiology. *14th ed. Elsevier, Philadelphia, PA, USA.* 1099 P.

Harsij, M., Kanani, H.G. and Adineh, H., 2020. Effects of antioxidant supplementation (nano-selenium, vitamin C and E) on growth performance, blood biochemistry, immune status and body composition of rainbow trout (*Oncorhynchus mykiss*) under sub-lethal ammonia exposure. *Aquaculture*, 521, 734942. DOI:10.1016/j.aquaculture.2020.734942

Hernandez, A. J., Satoh, S. and Kiron, V., 2012. Supplementation of citric acid and amino acid chelated trace elements in low-fish meal diet for rainbow trout affect growth and phosphorus utilization. *Journal of the World Aquaculture Society*, 43(**5**), 688-696. DOI:10.1111/j.1749-7345.2012.00589.x

Higgine, T., 1981. Novel Chromogen for serum iron determination. *Clinical Chemistry*, 27, 16-19.

Hoseini, S.M., Hosseini, S.A. and Nodeh, A.J., 2011. Serum biochemical characteristics of Beluga, *Huso huso* (L.), in response to blood sampling after clove powder solution exposure. *Fish Physiology and Biochemistry*, 37, 567-572. DOI:10.1007/s10695-010-9458-8.

Hossain, K.S., Amarasena, S. and Mayengbam, S., 2022. B vitamins and their roles in gut health. *Microorganisms*, 10(**6**), 1168. DOI:10.3390/microorganisms10061168

Hosseini, S.H., Sourinejad, I. and Ashori, S., 2015. Effect of dietary supplementation of Vitamin C on growth performance, survival rate and some hematological parameters in red pacu (*Piaractus brachypomus*). *Journal of Animal Environment*, 7(**2**), 197-204. (In Persian).

Hsieh, S.L., Chen, Y.N. and Kuo, C.M., 2003. Physiological responses, desaturase activity, and fatty acid composition in milkfish (*Chanos chanos*) under cold acclimation. *Aquaculture*, 220, 903-918. DOI:10.1016/S0044-8486(02)00579-3

Ibiyo, L.M.O., Atteh, J.O., Omotosho, J.S. and Madu, C.T., 2007. Vitamin C (ascorbic acid) requirements of *Heterobranchus longifilis*

fingerlings. *African Journal of Biotechnology*, 6(**13**).

Ibrahim, R.E., Ahmed, S.A., Amer, S.A., Al-Gabri, N.A., Ahmed, A.I., Abdel-Warith, A.W.A., Younis, E.S.M. and Metwally, A.E., 2020. Influence of vitamin C feed supplementation on the growth, antioxidant activity, immune status, tissue histomorphology, and disease resistance in Nile tilapia, *Oreochromis niloticus*. *Aquaculture Reports*, 18, 100545. DOI:10.1016/j.aqrep.2020.100545

Ji, Q., Yang, L., Zhou, J., Lin, R., Zhang, J., Lin, Q., Wang, W. and Zhang, K., 2012. Protective effects of paeoniflorin against cobalt chloride-induced apoptosis of endothelial cells via HIF-1α pathway. *Toxicology in Vitro*, 26(**3**), 455-461. DOI:10.1016/j.tiv.2012.01.016

Kazemi, R., Yousefi Joudehi, A., Pourdehghani, M., Yarmohammadi, M., Nasri Tajan, M., 2010. Cardiovascular system physiology of aquatic animals and applied techniques of fish haematology. *1st ed. Bazargan Co.,* Rasht, Iran. 194 P. (In Persian).

Khan, K. U., Zuberi, A. and Ullah, I., 2015. Effects of Graded Level of Dietary L-Ascorbyl-2-Polyphosphate on Growth Performance and Some Hematological Indices of Juvenile Mahseer (*Tor putitora*). *International Journal of Agriculture and Biology*, 17(**4**). DOI:1017957/IJAB/14.0023.

Khanjani, M. H. and Sharifinia, M., 2020. Biofloc technology as a promising tool to improve aquaculture production. *Reviews in Aquaculture*, 12(**3**), 1836-1850. DOI:10.1111/raq.12412

Koller, A. and Kaplan, L.A., 1984. Total serum protein. In: Kaplan, L.A. and Pesce, A.J. (eds), *Clinical Chemistry: Theory, Analysis, Correlation*. C.V. Mosby Company, St. Louis, MO, USA. 1316–1324.

Lall, S.P. and Kaushik, S.J., 2021. Nutrition and metabolism of minerals in fish. *Animals*, 11(**09**), 2711. DOI:10.3390/ani11092711.

Larsen, D.A., Beckman, B.R. and Dickhoff, W.W., 2001. The effect of low temperature and fasting during the winter on metabolic stores and endocrine physiology (insulin, insulin-like growth factor-I, and thyroxine) of coho salmon, *Oncorhynchus kisutch*. *General and Comparative Endocrinology*, 123(**3**), 308-323. DOI:10.1006/gcen.2001.7677

Li, M., Liang, H., Xie, J., Chao, W., Zou, F., Ge, X. and Ren, M., 2021. Diet supplemented with a novel Clostridium autoethanogenum protein have a positive effect on the growth performance, antioxidant status and immunity in juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquaculture Reports*, 19, 100572. DOI:10.1016/j.aqrep.2020.100572

Li, X., Lin, H., Zhu, Z., Watson Ray, G., Zhou, S., Yang, Q. and Tan, B., 2022. Effects of cobalt sources and levels on growth performance, serum biochemistry, metabolic activities, and cobalt contents in the tissue of juvenile *litopenaeus vannamei*. *North American Journal of Aquaculture*, 84(**3**), 336-344. DOI:10.1002/naaq.10243

Lin, M.F. and Shiau, S.Y., 2005. Requirements of vitamin C (l-ascorbyl-2-sulphate and l-ascorbyl-2-polyphosphate) and its effects on non-specific immune responses of grouper, *Epinephelus malabaricus. Aquaculture Nutrition*, 11(**3**), 183-189.

Livak, K.J. and Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, 25(**4**), 402-408.

Miandare, H.K., Niknejad, M., Shabani, A. and Safari, R., 2016. Exposure of Persian sturgeon (*Acipenser persicus*) to cadmium results in biochemical, histological and transcriptional alterations. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 181, 1-8. DOI:10.1016/j.cbpc.2015.12.004

Ming, J., Xie, J., Xu, P., Liu, W., Ge, X., Liu, B., He, Y., Cheng, Y., Zhou, Q. and Pan, L., 2010. Molecular cloning and expression of two *HSP70* genes in the Wuchang bream (*Megalobrama amblycephala* Yih). *Fish and Shellfish Immunology*, 28(**3**), 407-418. DOI:10.1016/j.fsi.2009.11.018

Ming, J., Xie, J., Xu, P., Ge, X.P., Liu, W.B. and Ye, J.Y., 2012. Effects of emodin and vitamin C on growth performance, biochemical parameters and two *HSP70s* mRNA expression of Wuchang bream (*Megalobrama amblycephala* Yih) under high temperature stress. *Fish and Shellfish Immunology*, 32, 651-661. DOI:10.1016/j.fsi.2012.01.008

Mohseni, M., 2015. Effects of vitamin E deficiency on dietary vitamin C requirement in Siberain Sturgeon (*Acipenser baerii*). *Iranian Scientific Fisheries Journal*, 24(**3**), 45-57. DOI:10.22092/isfj.2017.11019 (In Persian)

Montero, D., Marrero, M., Izquierdo, M.S., Robaina, L., Vergara, J.M. and Tort, L., 1999. Effect of vitamin E and C dietary supplementation on some immune parameters of gilthead seabream (*Sparus aurata* L.) phagocytes. *Veterinary Immunological Immunopathology*, 66, 185-199. DOI:10.1016/S0044-8486(98)00387-1

Moreau, R. and Dabrowski, K., 2000. Biosynthesis of ascorbic acid by extant actinopterygians. *Journal of Fish Biology*, 57(**3**), 733-745. DOI:10.1111/j.1095-8649.2000.tb00271.x

Mukherjee, S. and Kaviraj, A., 2009. Evaluation of growth and bioaccumulation of cobalt in different tissues of common carp, *Cyprinus carpio* (Actinopterygii: Cypriniformes: Cyprinidae), fed cobalt-supplemented diets. *Acta Ichthyologica et Piscatoria*, 39, 87-93.

Mustafa, A., Hayat, S.A. and Quarrar, P., 2013. Stress Modulated Physiological Responses in Nile Tilapia, Oreochromisniloticus, Treated with Non-Ascorbic Acid Supplemented Feed. *Advances in Zoology and Botany*, 1, 39-45. DOI:10.13189/azb.2013.010204

National Research Council (NRC), 2011. Nutrient Requirements of Fish and Shrimp. *The National Academies Press,* Washington, DC, USA. 376 P.

Nsonga, A., Kang'Ombe, J., Mfitilodze, W., Soko, C. and Mtethiwa, A., 2009. Effect of varying levels of dietary vitamin C (ascorbic acid) on growth, survival and hematology of juvenile tilapia, (*Oreochromis karongae*) (Trewavas 1941) reared in aquaria. *Brazilian Journal of Aquatic Science and Technology*, 13(**2**), 17-23.

Parhar, I.S., Sato, H. and Sakuma, Y., 2003. Ghrelin gene in cichlid fish is modulated by sex and development. *Biochemical and Biophysical Research*

Communications, 305(**1**), 169-175. DOI:10.1016/S0006-291X(03)00729-0

Percin, F. and Konyalioglu, S., 2008. Serum biochemical profiles of captive and wild northern bluefin tuna (*Thunnus thynnus* L. 1758) in the Eastern Mediterranean. *Aquaculture Research*, 39(**9**), 945-953. DOI:10.1111/j.1365-2109.2008.01954.x

Percin, F., Konyalioglu, S., Firat, K. and Saka, S., 2010. Serum electrolytes of wild and captive Bluefin Tuna (*Thunnus thynnus* L.) in Turkish seas. *Journal of Animal and Veterinary Advances*, 9, 2207-2213. DOI:10.3923/javaa.2010.2207.2213

Pourgholam, M.A., Khara, H., Safari, R., Sadati, M.A.Y. and Aramli, M.S., 2016. Dietary administration of *Lactobacillus plantarum* enhanced growth performance and innate immune response of Siberian sturgeon, *Acipenser baerii*. *Probiotics and Antimicrobial Proteins*, 8, 1-7. DOI:10.1007/s12602-015-9205-7

Rahimi, M., Sudagar, M., Ouraji, H., Hosseini, S.A. and Taghizadeh, V., 2012. The effect of vitamin C on growth performance, survival rate, hematological parameters and response to heat stress in rainbow trout (*Oncorhynchus mykiss*). *Journal of Veterinary Research*, 67, 373-380. DOI:full/10.5555/20133049860

Rahman, A.N.A., Khalil, A. A., Abdallah, H. M. and El-Hady, M., 2018. The effects of the dietary supplementation of *Echinacea purpurea* extract and/or vitamin C on the intestinal histomorphology, phagocytic activity, and gene expression of the Nile tilapia. *Fish and Shellfish Immunology*, 82, 312-318. DOI:10.1016/j.fsi.2018.08.024

Rahman, A.N.A., El-Hady, M. and Shalaby, S.I., 2019. Efficacy of the dehydrated lemon peels on the immunity, enzymatic antioxidant capacity and growth of Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*). *Aquaculture*, 505, 92-97. DOI:10.1016/j.aquaculture.2019.02.051

Rahmati, F., 2020. Microencapsulation of *Lactobacillus acidophilus* and *Lactobacillus plantarum* in Eudragit S100 and alginate chitosan under gastrointestinal and normal conditions. *Applied Nanoscience*, 10(**2**), 391-399. DOI:10.1007/s13204-019-01174-3

Roche, H. and Bogé, G., 1996. Fish blood parameters as a potential tool for identification of stress caused by environmental factors and chemical intoxication. *Marine Environmental Research*, 41(**1**), 27-43.

Roe, J.H. and Kuether, C.A., 1942. The determination of ascorbic acid in whole blood and urine through the 2,4 - dinitrophenylhydrazine derivative of dehydroascorbic acid. *Journal of Biological Chemistry*, 147, 399-407.

Safari, O., Sarkheil, M. and Paolucci, M., 2019. Dietary administration of Ferula, *Ferula asafoetida* powder as a feed additive in diet of Koi carp, *Cyprinus carpio* koi: effects on hemato-immunological parameters, mucosal antibacterial activity, digestive enzymes, and growth performance. *Fish Physiology and Biochemistry*, 45, 1277-1288. DOI:10.1007/s10695-019-00674-x

Safari, R., Hoseinifar, S. H., Nejadmoghadam, S. and Jafar, A.,

2016. Transciptomic study of mucosal immune, antioxidant and growth related genes and non-specific immune response of common carp (*Cyprinus carpio*) fed dietary Ferula (*Ferula assafoetida*). *Fish and Shellfish Immunology*, *55*, 242-248. DOI:10.1016/j.fsi.2016.05.038

Shekarabi, S.P.H., Mehrgan, M.S., Ramezani, F., Dawood, M.A., Van Doan, H., Moonmanee, T. and Kari, Z.A., 2022. Effect of dietary barberry fruit (*Berberis vulgaris*) extract on immune function, antioxidant capacity, antibacterial activity, and stress-related gene expression of Siberian sturgeon (*Acipenser baerii*). *Aquaculture Reports*, 23, 101041. DOI: 10.1016/j.aqrep.2022.101041

Singh, G., Khati, A. and Chauhan, R.S., 2021. Evaluation of probiotic and vitamin c as growth promoters for freshwater major carp, *Cyprinus carpio*. *Journal of Experimental Zoology India*, 24(**1**). DOI:full/10.5555/20210040601

Stegeman, J.J., 1994. Biochemistry and molecular biology of monooxygenases: current perspectives on forms, functions, and regulation of cytochrome P450 in aquatic species. In: Malins, D.C. and Ostrander, G.K. (eds), *Aquatic Toxicology: Molecular, Biochemical, and Cellular Perspectives*. CRC Press, Boca Raton, FL, USA. pp. 87–206

Stoica, A.I., Peltea, M., Baiulescu, G.E. and Ionica, M., 2004. Determination of cobalt in pharmaceutical products. *Journal of pharmaceutical and biomedical analysis*, 36(**3**), 653-656. DOI:10.1016/j.jpba.2004.07.030

Tonye, I.A. and Sikoki, F.D., 2014. Growth Performance and Proximate Composition of *Oreochromis Niloticus* (Trewavas) Fed Cobalt Chloride Incorporated Diet. *Growth*, 4(**5**), 106-125. DOI:full/10.5555/20143148652

Trichet, V.V., Santigosa, E., Cochin, E. and Gabaudan, J., 2015. The effect of vitamin C on fish health. In: Davis, D.A. (ed), *Dietary Nutrients, Additives, and Fish Health*. Wiley-Blackwell, Hoboken, NJ, USA. 151-171. DOI:10.1002/9781119005568.ch7

Van der Oost, R., Beyer, J. and Vermeulen, N.P., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology*, 13(**2**), 57-149. DOI:10.1016/S1382-6689(02)00126-6

Vijayan, M.M., Pereira, C., Graul, E.G. and Lwama, G.K., 1997. Metabolic responses to confine-ment stress in tilapia: the role of cortisol. *Comparative Biochemistry and Physiology*, 116C, 89-95. DOI: 10.1079/9781845935535.0182

Wan, J., Ge, X., Liu, B., Xie, J., Cui, S., Zhou, M., Xia, S. and Chen, R., 2014. Effect of dietary vitamin C on non-specific immunity and mRNA expression of three heat shock proteins (HSPs) in juvenile Megalobrama amblycephala under pH stress. *Aquaculture*, 434, 325-333. DOI:10.1016/j.aquaculture.2014.08.043

Wang, X., Kim, K.W., Bai, S.C., Huh, M.D. and Cho, B.Y., 2003. *Effects of the different levels of dietary vitamin C on growth and tissue ascorbic acid changes in parrot fish (Oplegnathus fasciatus). Aquaculture,* 215(**1-4**), 203-211. DOI:10.1016/S0044-8486(02)00042-X

Wiegertjes, G.F., Stet, R.J.M., Parmentier, H.K. and Van Muiswinkel, W.B., 1996. Immunogenetics of disease resistance in

fish: a comparable approach, *Developmental and Comparative Immunology*, 20, 365-381.

Wilson, R.P., 1991. Handbook of nutrient requirements of finfish. *CRC Press,* Boca Raton, FL, USA. 176 P.

Xu, C.M., Yu, H.R., Li, L.Y., Li, M., Qiu, X.Y., Fan, X.Q., Fan, Y.I. and Shan, L.I., 2022. Effects of dietary vitamin C on the growth performance, biochemical parameters, and antioxidant activity of Coho Salmon (*Oncorhynchus kisutch*, Walbaum, 1792) Postsmolts. *Aquaculture Nutrition,* Article ID 6866578, 12 P. DOI:10.1155/2022/6866578

Yada, T., Moriyama, S., Suzuki, Y., Azuma, T., Takahashi, A., Hirose, S. and Naito, N., 2002. Relationships between obesity and metabolic hormones in the "cobalt" variant of rainbow trout. *General and Comparative Endocrinology,* 128(**1**), 36-43. DOI:10.1016/S0016-6480(02)00047-3

Yang, S.S., Yu, C.B., Luo, Z., Luo, W. L., Zhang, J., Xu, J. X. and Xu, W.N., 2019. Berberine attenuates sodium palmitate-induced lipid accumulation, oxidative stress and apoptosis in grass carp (*Ctenopharyngodon idella*) hepatocyte in vitro. *Fish and Shellfish Immunology*, 88, 518-527. DOI:10.1016/j.fsi.2019.02.055

Younus, N., Zuberi, A., Rashidpour, A. and Metón, I., 2020. Dietary cobalt supplementation improves growth and body composition and induces the expression of growth and stress response genes in Tor putitora. *Fish Physiology and Biochemistry,* 46, 371-381. DOI:10.1007/s10695-019-00723-5

Zhang, M., Kuang, S., Sun, Y., Sun, J., Tian, X., Hu, Y., Y., Hu, J., Wang, Y., Xu, S.L., Xu, W. and Zhang, D., 2022. Effects of dietary vitamin C on growth, antioxidant enzyme activity and immune-related gene expression of Pampus argenteus. *Aquaculture Research,* 53(**15**), 5342-5353. DOI:10.1111/are.16017

Zheng, H., Nagaraja, G. M., Kaur, P., Asea, E. E. and Asea, A., 2010. Chaperokine function of recombinant Hsp72 produced in insect cells using a baculovirus expression system is retained. *Journal of Biological Chemistry*, 285(**1**), 349-356. DOI:10.1074/jbc.M109.024612