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SEASONAL VARIATION OF BLOOD BIOMARKERS IN HUCHEN, *HUCHO HUCHO* (ACTINOPTERYGII: SALMONIFORMES: SALMONIDAE) REARED IN CAPTIVITY

Andrada IHUŢ [1], Camelia RĂDUCU *[1], Daniel COCAN [2], Camelia MUNTEANU [3], Ionuț Traian LUCA [4], Paul UIUIU [2], Călin LAȚIU [2], Vasile RUS [5], and Vioara MIREŞAN [2*]

[1]*Department of Technological Sciences, Faculty of Animal Science and Biotechnologies, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania*

[2]*Department of Fundamental Sciences, Faculty of Animal Science and Biotechnologies, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania*

[3]*Department of Plant Culture, Faculty of Agriculture Sciences, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania*

[4]*Babes Bolyai University, Faculty of Business, Cluj-Napoca, Romania*

[5]*Department of Preclinical Sciences, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania*

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Background. Biomarker indices (protein, lipid, carbohydrate, mineral, enzymatic, and antioxidant enzymes) are used for the evaluation of health in fish blood. No studies conducted to date have provided standard values of various biomarkers in the blood plasma of *Hucho hucho* (Linnaeus, 1758). Therefore, we evaluated the physiological status and seasonal variation in metabolic profiles of this species. Significant seasonal changes were observed in all blood biomarkers, with the exception of red blood cells, hemoglobin, and mean corpuscular hemoglobin. Using these data, the physiological status and seasonal variation in the metabolic profiles of *H. hucho* were evaluated. Generally, the goal of this study was to characterize the variation in the blood biomarkers of huchen between different seasons.

Materials and methods. Fish were raised in captivity to obtain a breeding group to repopulate submontane rivers. Blood samples were collected by puncture of the caudal vein in a vacutainer with Li-Heparin to determine the seasonal values of hematological, biochemical parameters, and oxidative stress. Blood was collected from a random sample of 10 huchen in all four seasons.

Results. We found significant seasonal variation in several parameters: packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total protein (TP), albumin (ALB), γ-globulins (GG), urea (UREA), creatinine (CRN), triglycerides (TG), cholesterol (CHOL), glucose (GLC), calcium (Ca^{2+}), phosphorus (PO_4^{3-}), iron (Fe^{3+}), sodium (Na^{+}), potassium (K^{+}), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl aminotransferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK), α-Amylase (α-Amy), lipase (LIP), total bilirubin (TB), superoxide dismutase (SOD), and glutathione peroxidase (GPx).

Conclusion. This is the first study to provide standard values of proteins, lipids, carbohydrates, and minerals and the enzyme profile in huchen in all seasons, which may be a benchmark for future investigation that would favor the bioconservation and increase the spread of huchen.

Keywords: blood biomarkers reference, *Hucho hucho*, metabolic profiles, seasonal variation

* Correspondence: Professor C. Răducu, Departamentul de Științe Tehnologice Universitatea de Științe Agricole și Medicină Veterinară Cluj-Napoca, 3-5 Calea Mănăștur, 400372 Cluj-Napoca, Romania, phone: +40264596384, e-mail: (CR) *camelia.raducu@usamvcluj.ro*, (AI) *ihut.andrada@usamvcluj.ro*, (DC) *daniel.cocan@usamvcluj.ro*, (CM) *camelia.munteanu@usamvcluj.ro*, (ITL) *ionut.luca@tbs.ubbcluj.ro*, (PU) *paul.uiuiu@usamvcluj.ro*, (CL) *calin.latiu@usamvcluj.ro*, (VR) *vasile.rus@usamvcluj.ro*, (VM) *vioara.miresan@usamvcluj.ro*, ORCID: (CR) 0000-0003-1736-7450, (AI) 0000-0002-9793-1711, (DC) 0000-0001-7648-5997, (CM) 0000-0001-9322-7033, (TL) 0000-0002-0795-3643, (PU) 0000-0002-6835-3414, (CL) 0000-0002-0663-8086, (VR) 0000-0002-8380-6506, (VM) 0000-0002-9863-1577.

INTRODUCTION

Huchen, *Hucho hucho* (Linnaeus, 1758), also known as Danube salmon (Holčík et al. 1988), is the largest species in the family Salmonidae in Europe (Bănărescu 1964). Unfortunately, habitat destruction and intense poaching have led to a drastic decrease in huchen populations in Europe (Ihuț et al. 2014). This species is listed as endangered by the IUCN Red List (Freyhof and Kottelat 2008). The limited habitat of huchen populations has led to a reduction in their abundances and consequently to genetic drift within populations (Weiss and Schenekar 2016).

Huchen is usually co-occurring with grayling, *Thymallus thymallus* (Linnaeus, 1758), at elevations of 250–450 m (Bănărescu 1964, Zitek and Schmutz 2004). If the habitats in which huchen reproduce are not disturbed (by, for example, micro-hydropower plants, dams, reservoirs, ballast exploitation), juvenile specimens can occur at altitudes as high as 600 m (Cocan et al. 2019). The importance of this species is also related to its relatively limited habitat as an endemic species in the Danube basin (Witkowski et al. 2013).

According to Bănărescu (1964), the distribution of huchen in Romania included the Tisa (Vişeu River, Vaser River, Ruscova River), Siret (Bistriţa Moldovenească River, Dorna River), Mureş (Mureş River, Deda—Răstoliţa—Topliţa Gorge), Someş (Someşul Rece River), Criş (Drăgan River), Cerna, and Olt catchments. By comparison, the current distribution of huchen is significantly reduced and is only known from isolated populations in the Tisa (Curtean-Bănăduc et al. 2019), Mureş, Siret, and Cerna catchments (Cristea 2007).

Rehabilitation and conservation of endangered species require sustainable aquatic ecosystem management programs (Ihuț et al. 2014, Kucinski et al. 2015). To implement the protection and conservation measures, a delimitation of fishing areas from anthropogenic activities is needed. When habitat degradation is irreversible, other conservation measures are required. One of these measures involves the repopulation of habitats with specimens obtained by artificial reproduction (Sousa-Santos et al. 2014), albeit the success rate of these procedures is often low (Holčík 1990).

In huchen, artificial reproduction is difficult, as many details of the biology of this species remain unclear (Zingraff-Hamed et al. 2018), especially under captive conditions (Jungwirth 1978). Indeed, there have been few studies of huchen growth in captivity, and the majority of them have only examined a few stages of development (Andreji and Stráňai 2013, Bajić et al. 2015).

To obtain a satisfactory rate of huchen growth and reproduction in captivity before technology is applied, fundamental aspects of the biology of this species need to be characterized. However, few references in this field are available (Jungwirth 1979, Lahnsteiner et al. 1995).

Blood biomarkers in fish are essential for assessing physiological status (Jamalzadeh and Ghomi 2009) and establishing baseline values for reference (Ahmed et al. 2020). Seasonal variation in blood biomarkers may occur because of endogenous factors (e.g., age, sex, sexual maturity, species) and exogenous factors related to the response of fish to the living environment (Lusková 1998). Temperature is known to be one of the most important medial parameters affecting seasonal variation in blood biomarkers (Martinez et al. 1994). The metabolic rate of fish is closely correlated to water temperature in species living in warm waters. The metabolic rate of fish species living in cold waters such as salmonids may continue at low temperatures; however, at temperatures above 20°C, the metabolic rate decreases, and fish are less active and no longer feed (Svobodová et al. 1993).

Hematological parameters are important for assessing both the health of fish (Fazio 2019) and their aquatic environment (Svobodová et al. 1993). The number of blood cells is an important and powerful diagnostic tool as well as a component of a minimum database. It can be used to monitor the state of health of fish as well as responses to changes in nutrition, water quality, disease, and even therapies (Fazio 2019).

Proteins are key factors for the normal functioning of fish blood cells. The expression, location, and activity of proteins differ depending on several conditions. Therefore, the study of protein expression in several cell types or different circumstances is important for identifying and understanding their biological functions (Amiri-Dashatan et al. 2018).

Lipids are essential components of cells and are used as energy substrates for cellular processes. They are also used to signal intermediate pathways and are important parts of biological membranes. Lipids are constantly recycled and redistributed inside cells (Jaishy and Abel 2016).

Carbohydrates play an important role in metabolic processes. They act as an energy source, help control blood glucose and insulin metabolism, and participate in cholesterol and triglyceride metabolism. An increase or decrease in carbohydrates beyond appropriate amounts can affect both physiological and metabolic processes (Mantantzis et al. 2019).

The prevalence of each mineral element in body tissues is closely related to its functional role. As constituents of bones and teeth, minerals provide strength and rigidity to skeletal structures. In their ionic states in body fluids, they are indispensable for maintaining the acid-base balance and the osmotic relation with the aquatic environment as well as for integrating activities involving the nervous and endocrine systems. As components of pigments, enzymes, and organic compounds in tissues and organs, they are indispensable for essential metabolic processes involving the exchange of gas and energy (Chow and Schell 1980).

Alanine aminotransferase (ALT) and aspartate aminotransaminase (AST) are two of the most important amino acid metabolizing enzymes and reflect the intensity of amino acid metabolism in fish (Ballantyne 2001). Intestinal ALP, Na^+, K^+, GGT, and CK are also important enzymes that play a role in regulating nutrient absorption in fish (Zhao et al. 2015).

The antioxidant defense system includes antioxidant enzymes that fight reactive oxygen species. These free radical

scavengers can remove electrophilic chemicals or metabolites and reduce organic peroxides (Aladesanmi et al. 2017).

As poikilothermic organisms, fish show changes in physiological parameters depending on medial factors, especially seasonal changes (Morgan et al. 2008). Romania has a temperate continental climate with four seasons. In addition to this temperate continental climate, the Scandinavian–Baltic environment also has an influence on the distribution of huchen in Romania (with lower temperatures than the rest of the country and accentuated air currents). Therefore, to evaluate the physiological status of huchen, seasonal variation in blood biomarkers needs to be monitored. Once normal values and seasonal variation are established after the analysis of hematological parameters, these parameters can be monitored and can provide important information for the diagnosis and treatment of possible metabolic and nutritional imbalances that may occur seasonally (Sandnes et al. 1988, Pedro et al. 2005).

The purpose of this study is to characterize seasonal variation in the hematological, biochemical, and oxidative stress parameters in *H. hucho*.

MATERIALS AND METHODS

Fish, diet, and rearing technology. This research was conducted on *H. hucho* obtained from a salmonid reproduction center in Transylvania. The fish were raised in captivity under standard salmonid conditions (Jungwirth 1978, Ihuț et al. 2014) to obtain a breeding group to repopulate the submontane rivers. Huchen were grown in concrete basins. The feed was Aqua Garant with varying granulation depending on the weight of fish and was administered ad libitum with an automatic feeder. The analyzed physicochemical parameters of water (Table 1) were the following: temperature with a digital thermometer type TEMP 102C–50 ℃+150℃; pH and dissolved oxygen (DO) with multi-parameter type 340i WTW RO 241-98; nitrite (NO_2^-) with spectrophotometer type Cecil CE 2011 122·230; nitrate (NO_2^-) with spectrophotometer Jenway 6100; and turbidity with turbid meter type TurbiDirect Lovibond.

Ethical approval. The Committee of the UASVM Cluj-Napoca approved this research on animals (No. 117·2018) according to the national legislation (Law 43·2014) and European Directive 2010·63·EU.

Blood sample harvesting. The blood samples were collected from July 2014 to April 2015 in all four seasons characteristic to the continental temperate climate in Romania. The sampling was performed once for each season in the following months: July (summer), October (autumn), January (winter), and April (spring). Blood samples were collected randomly from 10 huchen specimens in each season from the same basin. As the fish were immature, differences between the sexes could not be distinguished. Fish mean body weight (BW) at the beginning of the study was 135.74 ± 9.32 g and 251.39 ± 11.83 g at the end. Blood was collected by puncture of the caudal vein and transferred to vacutainers with Li-Heparin following anesthesia with clove oil at a dose of 30 mg · L^{-1} (Javahery et al. 2012). The blood samples were transported under refrigerated conditions (2–4°C) to the hematology and biochemistry laboratory at UASVM Cluj-Napoca. After the blood samples were collected, the fish were handled gently, transferred to well-oxygenated water and monitored to facilitate recovery from anesthesia.

Hematological analysis. The hematological profile was characterized by counting the RBC, PCV, and Hb from whole blood. RBC counting was made with Gowers reagent through a turbidimetric reaction in the visible range at a wavelength λ = 546 nm and at 37°C. The hemoglobin concentration was determined within a maximum of 6 h after harvesting by a colorimetric reaction of END-POINT type, with VIS reading at λ = 546 nm and 37°C. The PCV was determined from whole blood by centrifugation in capillary tubes at 12 000 rotations per minute for 3 min. The RBC indices MCV, MCH, and MCHC were calculated according to standard formulas (Sarma 1990)

Plasma biochemical analysis. All analyses were performed per the instructions of commercial test kits available for each parameter with the UV-VIS Screen Master Touch spectrophotometer in the 340–620 nm wavelength range. The protein profile parameters determined were the following: TP concentration, ALB, GG, UREA, and CRN. TP was determined at λ = 546 nm in the VIS domain and ALB at λ = 630 nm by the END-POINT type method. The GG concentration was determined turbidimetrically at

Table 1

Sessions of blood sampling, the physicochemical parameter of water, fish body weight, and total length

Parameter	Season			
	Summer	Autumn	Winter	Spring
Water temperature [°C]	18.6	12.6	4.6	10.5
pH	7.42	7.65	7.58	7.86
DO [mg · L^{-1}]	8.20	8.80	10.10	9.40
NO_3^- [mg · L^{-1}]	1.90	2.10	2.33	2.06
NO_2^- [mg · L^{-1}]	0.02	0.01	0.01	0.01
Turbidity [NTU]	19.5	7.3	4.1	2.89
BW [g] (Mean ± SD)	135.74 ± 9.32	198.71 ± 7.86	222.85 ± 12.61	251.39 ± 11.83
TL [mm] (Mean ± SD)	237.93 ± 9.18	288.43 ± 3.43	298.52 ± 5.31	302.72 ± 1.45

DO = dissolved oxygen; NO_2^- = nitrite; NO_3^- = nitrate; BW = body weight; TL = total length

λ = 546 nm in the VIS domain and UREA at λ = 340 with the kinetic enzymatic method. CRN was determined by a fixed-time colorimetric method (FXT) with VIS reading at λ = 510 nm. To analyze the lipid profile, the concentrations of TG and CHOL were determined through END-POINT-type colorimetric reaction at λ = 546 nm (TG) and λ = 510 nm (CHOL). GLC was analyzed by the END-POINT colorimetric enzymatic method, and the reading was completed at λ = 500 nm in the VIS domain. The following enzymes were determined for the enzymatic profile: ALT, AST, GGT, ALP, LDH, CK, α-Amy, LIP, and TB. ALT, AST, CK, and LDH were determined at the wavelength λ = 340 nm by the kinetic method. ALP, GGT, and α-Amy were determined at the wavelength λ = 405 nm. LIP was analyzed at the wavelength λ = 580 nm and TB at the wavelength λ = 540 nm. The following parameters were analyzed to determine the mineral profile: Ca^{2+}, PO_4^{3}, Fe^{3+}, Na^{+}, and K^{+}. The Ca^{2+}, PO_4^{3}, and Fe^{3+} determinations were made using an END-POINT-type colorimetric reaction at λ = 650 nm (Ca^{2+}), λ = 340 nm (PO_4^{3}), and λ = 405 nm (Fe^{3+}). Na^{+} was made using the colorimetric enzyme type method. K^{+} was determined by the kinetic method at λ = 380 nm. Oxidative stress was analyzed through the activity of the antioxidant enzymes SOD (superoxide dismutase) according to Suttle (1986) and GPx (glutathione peroxidase) according to Kraus and Ganther (1980).

Statistical analysis. We generated box-and-whisker plots to analyze the distribution of the samples (The distribution was normal). Blood samples were collected in all four seasons of the year. To identify whether there were significant differences between seasons, we performed an ANOVA test followed by a post hoc test Bonferroni multiple range test for multiple group comparisons. We tested two hypotheses: the mean values (calculated for the entire population in each season) were equal (null) and the mean values (calculated for the entire population in each season) were unequal. When the null hypothesis was rejected, a comparison test for the difference of means was performed to identify seasons with significant differences between mean values and seasons where the mean values did not differ significantly. The confidence level was 95% for all tests.

RESULTS

During the four seasons characteristic of the temperate continental climate of Romania, huchen BW increased from 135.74 ± 9.32 g to 251.39 ± 11.83 g. Fish behaved normally and did not show any signs of disease or infection. The blood sampling events are shown in Table 1.

Hematological parameters and erythrocyte indices. Table 2 shows seasonal variation in hematological parameters and statistical results. There was significant seasonal variation in the hematological parameters of PCV, MCV, and MCHC. For nearly all of these parameters, values in the summer and spring were significantly greater than those in the autumn and winter.

Protein profile. Table 3 shows seasonal variations in biochemical parameters and statistical results. TP concentrations increased significantly in the spring and decreased in the summer. Similar to TP, ALB concentrations increased significantly in the spring and decreased in the summer. The value of GG decreased significantly in the winter and increased in the spring and autumn. Among parameters associated with the protein profile, UREA showed significant variation among all seasons. CRN decreased in the summer and autumn and increased significantly in the winter and spring.

Lipid profile. For both CHOL and TG, the lowest values were observed in the spring (Table 3).

Carbohydrate profile. The plasma GLC concentration increased significantly across seasons (Table 3); GLC levels were lowest in the summer and highest in the winter and spring.

Mineral profile. Seasonal variation in almost all parameters of the analyzed mineral profile was similar in the autumn and winter among parameters that significantly varied among seasons (Table 3). Plasma Ca^{2+} was lowest in the spring and highest in the winter. PO_4^{3-} was highest in the summer and lowest in the autumn and winter, and these differences were significant. Plasma Fe^{3+} was lowest in the summer and highest in the autumn and winter. Plasma Na^{+} was lowest in the summer and highest in the spring. Plasma K^{+} values increased significantly in the summer and autumn and decreased in the winter and spring.

Enzymatic profile. Among all parameters analyzed for the enzymatic profile (Table 3), AST, and LDH significantly

Table 2

Seasonal variation of hematological profile and erythrocyte indices in huchen, *Hucho hucho*

Blood indices	Abbr.	Season (Mean ± SD)				
		Summer	Autumn	Winter	Spring	*P*-value
Red Blood Cells [$\times 10^{12} \cdot L$]	RBC	$3.89^{a} \pm 0.35$	$3.54^{a} \pm 0.64$	$3.68^{a} \pm 0.34$	$3.39^{a} \pm 0.66$	0.200
Haematocrit [%]	PCV	$40.60^{a} \pm 2.46$	$34.90^{b} \pm 2.72$	$34.60^{b} \pm 4.00$	$40.70^{a} \pm 3.80$	0.000
Haemoglobin [$g \cdot dL^{-1}$]	Hb	$13.68^{a} \pm 0.79$	$13.69^{a} \pm 0.77$	$13.93^{a} \pm 1.12$	$13.73^{a} \pm 1.49$	0.952
Mean corpuscular volume [fL]	MCV	$105.40^{ab} \pm 14.21$	$95.48^{a} \pm 11.53$	$95.04^{a} \pm 17.09$	$121.97^{b} \pm 12.93$	0.000
Mean corpuscular haemoglobin [$pg \cdot 10^{-5}$]	MCH	$35.49^{a} \pm 4.47$	$37.64^{a} \pm 6.47$	$38.24^{a} \pm 6.05$	$41.05^{a} \pm 3.94$	0.154
Mean corpuscular haemoglobin concentration [$g \cdot dL^{-1}$]	MCHC	$33.62^{a} \pm 0.83$	$39.31^{b} \pm 4.80$	$40.49^{b} \pm 2.90$	$33.98^{a} \pm 2.22$	0.006

Presented values are mean ± standard deviation (n = 10); SD = standard deviation; values with different letters marking significant differences.

varied among seasons. ALT plasma concentration was highest in the winter. Significant differences in the GGT level were observed between summer and spring; concentrations of GGT were highest in the summer and lowest in the spring. ALP plasma concentration was highest in the spring. The α-Amy level was significantly higher in the spring and summer compared with autumn and winter. The LIP plasma concentration was highest in the summer when it differed significantly from that in the other seasons, and the LIP plasma concentration linearly decreased after autumn. CK decreased significantly in spring and increased in autumn. TB concentration was lowest in the summer.

Oxidative stress. Table 4 shows seasonal variation in the main factors determining oxidative stress markers as well as the statistical results. The plasma concentration of GPx was significantly decreased in the autumn compared with the other seasons, while SOD increased significantly in the spring.

DISCUSSION

The analyzed parameters were determined following the methods of Kraft and Dürr (2005). Seasonal differences in blood biomarkers can arise from changes in the medial parameters of water, such as differences in temperature, dissolved oxygen concentration, and rainfall. In addition,

Table 3

Seasonal variation of blood biomarkers in huchen, *Hucho hucho*

Blood index	Abbr.	Season				*P*-value
		Summer	Autumn	Winter	Spring	
Total protein [g ·dL^{-1}]	TP	$3.07^{a} \pm 0.07$	$3.81^{b} \pm 0.29$	$3.82^{b} \pm 0.28$	$4.53^{c} \pm 0.36$	0.000
Albumin [g ·dL^{-1}]	ALB	$1.91^{a} \pm 0.04$	$2.17^{bc} \pm 0.17$	$2.08^{b} \pm 0.08$	$2.29^{c} \pm 0.16$	0.000
ɣ-globulin [g ·dL^{-1}]	GG	$0.41^{a} \pm 0.02$	$0.54^{b} \pm 0.08$	$0.32^{c} \pm 0.04$	$0.59^{b} \pm 0.11$	0.000
Urea [mmol ·L^{-1}]	UREA	$4.12^{a} \pm 0.31$	$2.96^{b} \pm 0.49$	$2.47^{c} \pm 0.39$	$1.95^{d} \pm 0.22$	0.000
Creatinine [µmol ·L^{-1}]	CRN	$19.26^{a} \pm 4.14$	$21.92^{a} \pm 2.69$	$48.26^{b} \pm 8.26$	$43.13^{b} \pm 7.80$	0.000
Lipid profile						
Cholesterol [mmol ·L^{-1}]	CHOL	$4.17^{a} \pm 0.23$	$4.79^{b} \pm 0.72$	$4.31^{ab} \pm 0.21$	$2.47^{c} \pm 0.49$	0.000
Triglycerides [mmol ·L^{-1}]	TG	$3.26^{a} \pm 0.17$	$2.96^{b} \pm 0.21$	$3.30^{a} \pm 0.23$	$2.21^{c} \pm 0.12$	0.000
Carbohydrate profile						
Glucose [mmol ·L^{-1}]	GLC	$2.31^{a} \pm 0.95$	$4.36^{b} \pm 0.72$	$6.24^{c} \pm 0.35$	$6.73^{c} \pm 0.95$	0.000
Mineral profile						
Calcium [mmol ·L^{-1}]	Ca^{2+}	$2.93^{a} \pm 0.06$	$3.03^{ab} \pm 0.17$	$3.17^{b} \pm 0.21$	$2.68^{c} \pm 0.21$	0.000
Phosphorus [mmol ·L^{-1}]	PO_4^{3-}	$3.78^{a} \pm 0.07$	$2.11^{b} \pm 0.14$	$2.29^{b} \pm 0.07$	$2.90^{c} \pm 0.28$	0.000
Iron [µmol ·L^{-1}]	Fe^{3+}	$17.70^{a} \pm 0.31$	$26.33^{b} \pm 3.38$	$25.13^{b} \pm 3.08$	$22.08^{c} \pm 0.88$	0.000
Sodium [mmol ·L^{-1}]	Na^{+}	$127.57^{a} \pm 1.88$	$145.52^{b} \pm 10.10$	$139.35^{b} \pm 4.64$	$158.00^{c} \pm 7.54$	0.000
Potassium [mmol ·L^{-1}]	K^{+}	$4.03^{a} \pm 0.14$	$3.87^{a} \pm 0.42$	$3.45^{b} \pm 0.30$	$3.14^{b} \pm 0.12$	0.000
Enzyme profile						
Alanine aminotransaminase [U ·L^{-1}]	ALT	$7.12^{a} \pm 0.62$	$6.53^{a} \pm 2.08$	$10.44^{b} \pm 1.13$	$6.59^{a} \pm 1.49$	0.000
Aspartate aminotransaminase [U ·L^{-1}]	AST	$76.01^{a} \pm 9.15$	$35.32^{d} \pm 7.71$	$52.61^{c} \pm 8.40$	$63.35^{b} \pm 5.37$	0.000
Gama-glutamyl transferase [U ·L^{-1}]	GGT	$5.54^{a} \pm 1.23$	$4.75^{ab} \pm 1.27$	$4.85^{ab} \pm 0.68$	$4.02^{b} \pm 1.12$	0.034
Alkaline phosphatase [U ·L^{-1}]	ALP	$183.35^{a} \pm 17.97$	$185.28^{a} \pm 29.77$	$176.61^{a} \pm 16.51$	$345.28^{b} \pm 43.99$	0.000
α-Amylase [U ·L^{-1}]	α-Amy	$31.01^{a} \pm 5.82$	$19.84^{b} \pm 6.17$	$17.35^{b} \pm 3.89$	$31.36^{a} \pm 9.50$	0.000
Lipase [U ·L^{-1}]	LIP	$7.41^{a} \pm 0.74$	$6.31^{ab} \pm 1.86$	$5.13^{bc} \pm 0.62$	$4.87^{c} \pm 0.73$	0.000
Lactate dehydrogenase [U ·L^{-1}]	LDH	$617.87^{a} \pm 22.74$	$818.64^{b} \pm 121.54$	$1034.92^{c} \pm 125.18$	$1250.86^{d} \pm 189.02$	0.000
Creatine Kinase[U ·L^{-1}]	CK	$247.69^{a} \pm 40.29$	$332.47^{b} \pm 46.11$	$230.68^{a} \pm 50.69$	$61.62^{c} \pm 8.13$	0.000
Total Bilirubin [µmol ·L^{-1}]	TB	$0.23^{a} \pm 0.11$	$1.20^{b} \pm 0.79$	$1.13^{b} \pm 0.50$	$1.35^{b} \pm 0.40$	0.000

Presented values are means ± standard deviation (n = 10); SD – standard deviation; values with different letters marking significant differences.

Table 4

Seasonal variation of antioxidative enzymes in huchen, *Hucho hucho*

Antioxidative enzyme	Abbr.	Season				*P*-value
		Summer	Autumn	Winter	Spring	
Glutathione peroxidase [U · g^{-1} Hb]	GPx	$95.56^{a} \pm 4.96$	$75.02^{b} \pm 4.83$	$87.90^{a} \pm 9.91$	$94.41^{a} \pm 11.10$	0.000
Superoxide dismutase [U · g^{-1} Hb]	SOD	$908.50^{a} \pm 50.32$	$873.32^{a} \pm 44.81$	$888.80^{a} \pm 38.99$	$974.23^{b} \pm 38.52$	0.000

Presented values are means ± standard deviation (n = 10); SD = standard deviation; values with different letters marking significant differences.

seasonal differences in certain parameters can stem from older ages and the increased BW of huchens.

Hematological profile. The RBC indices help diagnose and treat anemia for each species. The values of these indices are affected by age (Lone et al. 2012), population density (Yarahmadi et al. 2014), and species (Xu et al. 2012).

In fish, the number of RBC differs from species to species (Ahmed et al. 2020) because of locomotor activity, which is higher in active species ($3.0–4.2 \times 10^{12} \cdot L^{-1}$) compared with less active species ($0.5–1.5 \times 10^{12} \cdot L^{-1}$) (Soldatov 2005, Witeska 2013).

Analysis of seasonal variation in hematological parameters revealed that the number of RBC was highest in the summer and lowest in the spring. There was no significant variation in RBC values among seasons, and there was only a slight increase in summer when the water temperature was slightly higher (18.6°C). Despite the lowest number of RBC being in the spring, anemia was not detected because the body compensated for the lower number of RBC (including the ability to carry oxygen) by the increased values of MCV and MCHC. We also noticed that PCV did not significantly differ and Hb was relatively constant between the two seasons that showed the maximum and minimum differences in RBC (summer and spring).

According to Morgan et al. (2008), the increase in RBC in the summer months appeared to stem from the reduced oxygen availability; consequently, more RBC are needed to absorb available oxygen. Seasonal variation in RBC observed in *H. hucho* was similar to that observed by Morgan et al. (2008) in the *Oncorhynchus mykiss* (Walbaum, 1792).

Changes in metabolism, including reductions in erythropoiesis, occur because of lower water temperatures as well as decreases in photoperiod during the winter and autumn seasons (Pedro et al. 2005, Jamalzadeh and Ghomi 2009). In colder seasons, the activity of huchens is reduced, which could be responsible for the observed decrease in PCV. The concentration of PCV was significantly lower during winter and autumn compared with the summer and spring. Our findings are similar to those of Pedro et al. (2005) showing a decrease in PCV in the autumn and winter in tench, *Tinca tinca* (Linnaeus, 1758). Cerbu et al. (2017) obtained values slightly higher than ours in *H. hucho*. There was no significant seasonal variation in Hb concentration.

Seasonal variation affected RBC indices, with the exception of MCH. MCV provides a useful indicator for the classification of anemia. Seasonal variation was observed in MCV: specifically, a significant increase in the autumn and a decrease in spring. In contrast to the seasonal variation observed in MCV, the highest value of MCHC was observed in the winter, and the lowest value was observed in summer.

Regarding the fluctuations of the seasonal hematological parameters, we found that the concentrations of PCV tended to decrease in the autumn and winter because of decreases in the volume of RBCs. This fluctuation did not affect the oxygen transport capacity, as an increase in the MCHC was observed during the same seasons.

Protein profile. TP is involved in the specific defenses of organisms by contributing to the maintenance of acid-basic balance and helping protect RBC integrity. TP can also provide an energy source for organisms in emergencies (Kádár 2002). According to Kaneko et al. (1997) and Domingo-Roura et al. (2001), TP concentration increases with the development and growth of individuals and is a general trend in animals. In our research, TP concentration showed a linear increase with the age of huchen. TP concentration varied among seasons and increased in spring after a long period of cessation of food intake. ALBs can transport and bind substances and metabolites in the bloodstream, such as free bilirubin and other substances. Their synthesis occurs in the liver (Kádár 2002). The ALB value increased with the BW of the fish and showed the same degree of variation as TP. Similar to our findings, Siddiqui (1976) obtained higher TP and ALB values as the BW of fish increased.

GG plays an essential role in body immunity. Hyperglobulinemia is a potent immune response in fish under infectious and immunological diseases. The highest levels of GG were observed during the spring and autumn. This increase can be attributed to the immune response of fish (Bowden et al. 2007) to changes in medial parameters from the rainy season affecting the microbiome of mountain waters.

UREA is the product of protein metabolism and a form of nitrogen protein excretion (Kádár 2002). The highest plasma concentration of UREA was in the summer (when more feed is consumed) and the lowest was found in the spring, which was related to the lack of feed consumption in the previous season. UREA and TP were negatively correlated across seasons. TP is used in the process of animal growth, and UREA is a product of protein catabolism that increases following food intake. Thus, when the TP value was the lowest in the summer, the value of UREA was the highest; the opposite pattern was observed in the spring.

CRN is correlated with an increase in muscle development (Munro and Allison 1964, Pirlich et al. 1996). As expected, the CRN level increased with the age of the huchens. Because of the cessation of voluntary feed intake by fish, the values of CRN increased. Therefore, a reduction in metabolic processes occurs in response to lower temperatures along with a decreased rate of renal excretion. When physical activity resumes in the spring, values remained similar to those observed during the winter. These values are similar to those of Sandnes et al. (1988) (41 ± 2 μmol $\cdot L^{-1}$) in *Salmo salar* Linnaeus, 1758 and higher compared with those of *O. mykiss* (17.68 μmol $\cdot L^{-1}$) (Lone et al. 2012).

Lipid profile. CHOL and TG concentrations may vary among season (Kopp et al. 2011). Analysis of seasonal variation in the lipid profile revealed a constant growth of CHOL from summer to autumn because of the increase in feed intake. Once temperatures had increased in the spring, there was a rapid decrease in the lipid profile because of the intensification of the physical activity of the fish. Kopp et al. (2011) observed a similar pattern of seasonal variation of CHOL plasma concentration in rainbow trout (*O. mykiss*).

The TG concentration decreased in the spring and increased in the winter because of a reduction in the voluntary feed intake. Consequently, the adipose cells broke down and mobilized their TG into fatty acids and glycerol.

Carbohydrate profile. Blood GLC concentration is a physiological parameter used to monitor fish stress (Wu et al. 2019) and may vary with species, age, season, and food. Analysis of seasonal variation in the blood GLC level revealed a pattern similar to that observed for the lipid profile. The highest feeding rate was in the summer; as a result, there was an increase in the level of GLC in the blood recorded in the autumn. Blood GLC levels were higher when the voluntary feed intake of the fish decreased in the winter along with the concomitant agglomeration of lipids in the fat tissue and constant growth of TG. In the spring, GLC levels were the highest, and there was a sudden decrease in the lipid profile once physical activity had resumed. Xu et al. (2012) obtained similar values (4.61 ± 1.12 mmol $\cdot$ L^{-1}) in *Hucho taimen* (Pallas, 1773).

Mineral profile. Seasonal changes had a small effect on Ca^{2+} values. Specifically, Ca^{2+} values were lower in the spring and higher in the winter. This variation stems from the increase in water suspensions because of the massive snow melting in the mountains. Several studies in the literature have obtained values similar to ours, such as *O. mykiss* (2.13 ± 0.39 mmol $\cdot$ L^{-1} and 3.14 ± 0.36 mmol $\cdot$ L^{-1}) (Kopp et al. 2011), *Hucho taimen* (3.18 ± 0.32 mmol $\cdot$ L^{-1}) and *Brachymystax lenok* (Pallas, 1773) (3.40 ± 0.28 mmol $\cdot$ L^{-1}) (Xu et al. 2012).

Ca^{2+} plays an essential role in bone formation, maintaining osmotic pressure, nerve impulse transmission, blood coagulation, and protection against toxic substances. Ca^{2+} is an activator of different enzymes (cholinesterase, lipase, and alkaline phosphatase) (Kádár et al. 2002). Ca^{2+} assimilation in fish primarily depends on its concentration in water and less from forage; it is completed through either the gills or intestines.

In the body, PO_4^{3-} is present in two forms, organic and non-organic phosphorus, which are linked to magnesium and calcium. PO_4^{3-} contributes to mechanisms regulating Ca:P balance, heart rhythm, bone structure, and carbohydrate metabolism. Plasma concentrations of PO_4^{3-} were highest in the summer when dietary intake was higher. In addition to the decrease in the exogenous input of PO_4^{3-} in the autumn, we observed a decrease in plasma concentrations of PO_4^{3-}. When the renal excretion rate was lower in the winter, we found that plasma concentrations of PO_4^{3-} were slightly increased compared with those observed in the autumn. Once exogenous inputs increased in the spring, we observed a small increase in the plasma concentration of PO_4^{3-}.

The low values of plasmatic Fe^{3+} in the summer stemmed from the increase in RBC number. Fe^{3+} is found in the body in the form of hemoglobin, myoglobin, ferritin, and transferrin and plays an essential role in oxygen transport. Fe^{3+} concentrations can vary greatly between species (Nicula et al. 2010). Our values were within limits (10.09 ± 2.27 µmol $\cdot$ L^{-1} and 25.15 ± 4.88 µmol $\cdot$ L^{-1}) observed in the literature (Kopp et al. 2011) in *O. mykiss*.

Na^+ concentration varied among seasons; the highest values were in the spring, and the lowest values were in the summer. When Na^+ was at its lowest, K^+ levels were at their highest. Na^+ in the extracellular space was the most important cation, and it plays a role in maintaining blood osmolarity and homeostasis (Kádár et al. 2002, Kulkarni 2015).

The K^+ concentration was influenced by season and showed a constant decrease from summer to spring. K^+ is an essential cation of the intracellular space because it is involved in plasma osmolarity and the regulation of blood pressure (Kádár et al. 2002).

Enzymatic profile. The activity of ALT and AST plasma enzymes is used to diagnose the dysfunction of organs, such as the liver (ALT), kidney, pancreas, muscle, heart (AST), and spleen (Lusková 1997). The values of these enzymes may vary depending on the species, medial parameters, and the influence of the seasons (Kopp et al. 2011, Xu et al. 2012). AST values decreased in the autumn and winter because of the reduced muscular activity of fish. According to Ciereszko et al. (1998) and Kopp et al. (2011), the water temperature is directly proportional to the AST value.

The highest value of ALT was recorded in the winter because of the glucose-alanine cycle (Hemre et al. 2002), which was confirmed by the increased value of GLC. The ALP value was highest in the spring because of gluconeogenesis at the hepatic level, along with decreases in plasma concentrations of CHOL and TG. ALP varied by sex and age and was primarily found in the liver, bones, and intestines (Kádár et al. 2002).

The high level of α-Amy in the spring stemmed from the production of large quantities of glucose. In the summer, the increase in α-Amy increased because of the high exogenous input of polysaccharides in food. Patterns of variation in both α-Amy and LIP matched temperature oscillations in the water. α-Amy and LIP are mainly produced by the pancreas but can also be produced by other organs, such as kidneys, duodenum, and spleen. It is eliminated from the kidneys in the blood plasma. These enzymes provide useful information for diagnosing pancreatic dysfunction (Kádár et al. 2002).

LDH catalyzes the reversible transformation of pyruvate into lactate (Kádár et al. 2002). LDH is found in several tissues, such as muscles, liver, heart, kidneys, and blood vessels. The increased activity of this enzyme has been noted in diseases of the muscles and liver as well as during increased physical exertion. The most significant change was observed in the spring because of the increase in physical activity and also because the BW almost doubled during the period of study. Cocan et al. (2015) reported that there was a simultaneous increase in BW and lipids deposited between the basement membrane and the plasmalemma of the skeletal muscle cells in *O. mykiss*. Because of growth and the lipids deposited pericellularly, compression atrophy occurs in a manner similar to myodystrophy. In addition, the increased plasma concentrations of LDH can be attributed to the intensification of muscular activity (spring) after a long period of reduced inactivity (winter). This pattern is also related to changes in several processes of dystrophia similar to those described for Rainbow trout by Cocan et al. (2015).

CK is found in the cytoplasm of skeletal and cardiac muscle cells and the central nervous system (Kádár et al. 2002). Unexpectedly, values of CK value were at their lowest in the spring and highest in the autumn. A similar pattern of variation but with higher values was observed by Kopp et al. (2011) (355.88 $U \cdot L^{-1}$ in spring and 827.64 $U \cdot L^{-1}$ in autumn) in *O. mykiss*.

TB is a nitrogenous compound that arises from the oxidative degradation of hemoglobin as well as other hemoproteins. It varies depending on age and species (Kádár et al. 2002). TB is closely bound to ALB; therefore, seasonal variation in TB was similar to that of ALB.

Oxidative stress. Like aerobic organisms, fish have an antioxidant defense system that is influenced by dietary, behavior, phylogeny, age, the chemical composition of water, and physiological states (Alonso-Alvarez et al. 2004, Martínez-Álvarez et al. 2005, Trenzado et al. 2006). Glutathione peroxidase (GPx) protects proteins and nucleic acids from the action of oxidizing molecules. Superoxide dismutase (SOD) is the first enzyme that acts against free oxygen radicals (Otto and Moon 1996).

The activity of (GPx) was significantly lower in the autumn than in the other seasons. SOD activity was significantly higher in the spring than in the other seasons most likely because of changes in medial parameters (massive melting of snow, rain precipitation, and fluctuations in water temperature).

CONCLUSIONS AND FUTURE PERSPECTIVES

This paper represents the first study providing comprehensive data on the blood and hematologic biochemical parameters, which cover all seasons, comparatively. As such, the values represent reference data for the *H. hucho* species. Furthermore, the study is a starting point for future investigation that would favor the bioconservation and increase the spread of the *H. hucho* species.

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