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ASSESSMENT OF RANGES PLASMA INDICES IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) REARED UNDER CONDITIONS OF INTENSIVE AQUACULTURE

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Abstract

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Plasma parameters in rainbow trout (*Oncorhynchus mykiss*) from three various trout farms in the Czech Republic were assessed using automated blood plasma analyser. Non-haemolysed serum from the heart of 48 healthy, randomly selected fish (standard length, mean ± SD = 247.3 ± 24.2 mm; body mass, mean ± SD = 262.18 ± 87.28 g) was analysed for the following plasma parameters: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, acid phosphatase, lactate dehydrogenase, creatine kinase, total protein, cholinesterase, amylase, glucose, lactate, albumin, urea, cholesterol, triglycerides, lipase, Ca, Mg, P, Fe, Na, K and Cl. All data were analysed statistically such as normality assessment by means of Kolmogorov–Smirnov test and adequate statistical testing using various parametric and non-parametric tests for each variable. With regard to data distribution, 19 indices out of 23 (aspartate aminotransferase, alkaline phosphatase, acid phosphatase, lactate dehydrogenase, total protein, amylase, glucose, lactate, albumin, urea, cholesterol, triglycerides, Ca, Mg, P, Fe, Na, K and Cl) were normally distributed. The indices were affected by handling time and, accordingly to the physical and chemical properties of water. Estimates obtained were compared with previously reported ranges. The blood automated analyser proved to be a valuable and reliable instrument for the estimation of plasma parameters determining normal ranges in rainbow trout.

fish, biochemical variables, aquaculture

Development of intensive aquaculture requires that examination of the state of health of fish should also include approaches that involve clinical biochemical diagnostics. Such approaches serve to identify in an early stage the onset of any organ failure caused by the use of wrong feeds and to draw attention to changes in the abiotic factors of the environment and to any complications due to an infection or parasitic invasion. To identify as many pathological deviations as possible, it is advisable to use more than one test reflecting the basic metabolic functions (Řehulka and Minařík, 2008). Control of the physiological state of the rainbow trout, focused on biochemical examinations of the blood plasma, is an integral part of the complex methods of examining the health of the fish and at the same time, plays a role in feeding experiments – in testing diets of different composition or testing the properties of substances having a specific effect (Řehulka and Minařík, 2001). Fish blood plasma chemistry is a promising area in fish biology and clinical pathology although it requires further research particularly in assessment of normal ranges. Difficulties due to establishing normal estimates for rainbow trout *Oncorhynchus mykiss* (Walbaum) have been emphasized by many authors (Wedemeyer and Chatterton, 1970; McCarthy *et al.*, 1973; Wedemeyer and Nelson, 1975; Meade and Perrone, 1980; Hille, 1982; Roscoe Miller *et al.*, 1983). Moreover, apart from the inevitable differences in,

e.g. methodology, fish size and strains, season and physiological condition, it is often difficult to find an exhaustive set of blood chemistry estimates for rainbow trout in a single study (Manera and Britti, 2006).

The aim of the present study was to assess plasma parameters in rainbow trout (*Oncorhynchus mykiss*) from aquaculture system. Fish health status was accurately assessed by accurate necropsy to avoid including fish with subclinical pathologies that could affect the estimates.

MATERIALS AND METHODS

Experimental fish

Clinical healthy immature rainbow trout (*Oncorhynchus mykiss*) were provided from three fish farms from 19 November 2009 to 4 August 2010. A total of 5 samples were taken while every sample means 10 fish. We were collected this samples on the fish farm Pravikov in June (19. 7.) and November (19. 11.), on the fish farm Skalní mlýn in August (4. 8.) and March (11. 3.) and on the fish farm Litomyšl just in March (25. 3.). The physical and chemical properties of water are documented in Tab. I. Water saturation by oxygen, pH and temperature were measured by the portable HACH HQ40D meter (Hach Lange, Germany). Conductivity measurements were done by conductivity meter HI 98129 (Hanna Instruments, USA), and other chemical parameters were determined by standard methods (APHA, 1998). The fish were fed by commercial diets containing 40–43 % crude protein, 23–28 % crude fat, 12–20 % carbohydrates, 6–8.3 % ash, 1–3.8 % crude fibre, 1.3 % Ca and 0.9 % P.

For each fish (n = 48) randomly selected, the following biometrical data were measured or calculated (mean ± SD): total length (TL), standard length (SL), body mass (W), liver mass (WL), hepatosomatic index (HSI) and Fulton's condition factor (F) (Fulton, 1904). The biometrical data of fish

I: *Water characteristics in the fish farms (the average values of monitored parameters from different data sampling)*

Date of measurement	19. 11.	11. 3.	25. 3.	19. 7.	4. 8.
Temperature (°C)	2.6	3.3	8.0	20.4	11.3
pH	7.26	6.55	7.87	7.34	7.79
Oxygen saturation (%)	97	101	89	79	87
Conductivity ($mS.m^{-1}$)	18.6	18.6	60.6	16.3	42.6
Total nitrogen ($mg.l^{-1}$)	4.3	10.0	11.6	2.0	5.6
Total phosphorus ($mg.l^{-1}$)	0.25	0.51	0.09	0.11	0.14
Total organic carbon ($mg.l^{-1}$)	16.9	12.4	13.6	12.4	13.5
Chemical oxygen demand (Cr) ($mg.l^{-1}$)	15.6	13.1	6.4	27.4	10.6
Chemical oxygen demand (Mn) ($mg.l^{-1}$)	10.3	5.8	3.2	11.2	4.7
Biochemical oxygen demand ($mg.l^{-1}$)	0.49	2.36	1.47	5.39	2.28
Acid neutralization capacity ($mmol.l^{-1}$)	0.46	0.21	3.97	0.67	2.91
Ammonium ions ($N–NH_4^+$) ($mg.l^{-1}$)	0.11	0.35	0.08	0.16	0.09
Nitrite nitrogen ($N–NO_2^-$) ($mg.l^{-1}$)	0.01	0.04	0.01	0.36	0.00
Nitrate nitrogen ($N–NO_3^-$) ($mg.l^{-1}$)	4.0	8.8	5.9	1.0	5.4
Orthophosphates ($P–PO_4^{3-}$) ($mg.l^{-1}$)	0.19	0.38	0.05	0.00	0.10
Chlorides (Cl^-) ($mg.l^{-1}$)	15.4	8.2	17.6	18.4	20.9
Sulphates (SO_4^{2-}) ($mg.l^{-1}$)	11	17	49	17	43
Calcium (Ca^{2+}) ($mg.l^{-1}$)	6.7	14.8	121.0	12.5	73.1
Magnesium (Mg^{2+}) ($mg.l^{-1}$)	4.1	5.2	6.9	6.6	7.3
Total iron ($mg.l^{-1}$)	0.20	0.06	0.03	0.24	0.06
Potassium (K^+) ($mg.l^{-1}$)	3.3	1.7	3.5	2.8	3.0
Natrium (Na^+) ($mg.l^{-1}$)	4	12	21	15	18

II: *Biometrical data of fish (N = 50, Mean ± SD), explanation of abbreviations used in text*

Parameters	TL (mm)	SL (mm)	W (g)	WL (g)	F	HSI
19. 11.	249.7 ± 13.1	227.1 ± 13.4	206.7 ± 32.0	2.64 ± 0.32	1.76 ± 0.15	1.52 ± 0.33
11. 3.	278.3 ± 12.2	249.2 ± 10.4	225.6 ± 34.7	2.61 ± 1.02	1.45 ± 0.12	1.31 ± 0.09
25. 3.	274.5 ± 12.9	244.8 ± 11.5	236.1 ± 24.3	2.75 ± 0.45	1.61 ± 0.15	1.35 ± 0.10
19. 7.	261.8 ± 17.7	230.3 ± 15.5	241.4 ± 57.1	2.95 ± 0.77	1.95 ± 0.19	1.44 ± 0.13
4. 8.	316.7 ± 20.4	285.5 ± 19.7	416.3 ± 80.4	4.27 ± 0.93	1.77 ± 0.12	1.45 ± 0.10

are documented in Tab. II. A complete necropsy was performed on each sampled fish in order to exclude pathology, which could have biased the determination of normal ranges of plasma parameters. Only fish without gross lesions (i.e. lesions that can be seen with the naked eye) and visible parasites were assessed.

Sampling and measuring of biochemical indices

Immediately after catching the fish from a fish farm, blood samples were collected. Inadequate and haemolytic plasma samples were eliminated. Fish blood was taken by cardiac puncture using heparinised syringes. Heparin at a concentration of 50 I.U. per 1 ml was used for blood stabilization. The blood was centrifuged at 400 g for 15 min at 4 °C, and plasma supernatant was stored at –80 °C until the day of analyses. Biochemical analyses were performed by the ADVIA 1650 automatic analyser (Siemens, USA) using commercially available reagents. Serum enzymatic activities were determined at 37 °C. Alanine aminotransferase (ALT) activity determination was based on the kinetic assessment of NADPH consumption coupled with the generation of pyruvate (Expert panel, 1976). Aspartate aminotransferase (AST) activity was determined by kinetic measurement of NADH consumption coupled with the formation of oxaloacetate (Expert panel, 1976). Lactate dehydrogenase (LDH) was determined as the formation of NADH during conversion of L-lactate to pyruvate (Hajzler and Jagelkova, 1988). Alkaline phosphatase (ALP) was determined by a modification of the enzymatic method using AMP (adenosine monophosphate) buffer (Tietz *et al.*, 1980). Acid phosphatase (ACP) activity was determined by an enzymatic method with 1-naphtylphosphate (Hillmann, 1971). Cholinesterase (CHE) was determined by a modification of the kinetic test with butyrylthiocholine (Gary, 1971). The creatine kinase (CK) method is based on the procedure described by Szasz *et al.* (1976), modified by IFCC.

Total serum protein (TP) was determined by the biuret reaction (Doumas *et al.*, 1981). Glucose (GLU) concentration was determined by the glucose hexokinase method at 37 °C with an endpoint reading at 340 nm (Barham and Trinder 1972). Calcium (Ca) and magnesium (Mg) concentration were determined by the modified colorimetric methods with arzenazo III (Ichaylova and Ilkova, 1971; Skavrada, 1999). Phosphorus (P) was determined by an endpoint method with sample blanking using ammonium molybdenate reagent (Kratochvila and Garcic, 1977). Total bilirubin (BIL) was determined by the oxidation reaction with potassium ferricyanide (O'Leary *et al.*, 1993). Iron (Fe) was determined by the photometric method with ferene (ferroin-type reagent) without deproteination (Higgins, 1981). The concentration of lactate (LACT) in plasma was measured by the enzymatic method according to Shimojo *et al.* (1989). Albumin (ALB) was determined by the photometric method with bromocresol green (Doumas *et al.*, 1971). Urea (UREA) concentrations were determined by the kinetic enzymatic method with urease (Roch-Ramel, 1967). The cholesterol (CHOL) was determined by the CHOD-PAP method after enzymatic hydrolysis and oxidation (Roeschlau *et al.*, 1974). Electrolyte levels (Na, K, Cl) were analysed by ion selective electrodes (Eisenman, 1967). Amylase (AMS) was determined by the method using ethylidene blocked *p*-nitrophenyl-maltoheptaoside as a substrate (Jensen and Wydeveld, 1958). The triglycerides (TRIG) were assessed by the Fossati three-step enzymatic reaction with a Trinder endpoint (Fossati and Prencipe, 1982). The lipase (LIP) activity was determined by the enzymatic colorimetric assay according to Neumann *et al.* (1987).

Statistical analyses

During the mathematical and statistical processing of the results, the selected biochemical indices were characterized by arithmetic mean values and standard deviation. Also plasma parameter values were assessed for normality by the Kolmogorov-Smirnov test ($\alpha = 0.05$) in order to evaluate the normal ranges correctly. The correlations between biochemical parameters and basic water physico-chemical characteristics such as temperature, pH, etc. were analysed by Spearman's rank directional correlations. P values ($\alpha = 0.001$) were considered to be statistically significant. All the calculations were made using the statistical package Statistica 8.0 for Windows (StatSoft).

RESULTS

The blood plasma indices are summarized in Tab. III. Electrolytes, with the noticeable exception of potassium and iron, showed the lowest coefficients of variation whereas enzymes (namely creatine kinase, lipase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase and amylase) the highest. The Kolmogorov–Smirnov test showed that 19 indices out of 23 were normally distributed. The values of alanine aminotransferase, creatine kinase, cholinesterase and lipase were not normally distributed.

Values of TP, ALB, GLU, CHOL and Ca significantly increased with the increasing weight of fish, on the other hand value of Cl and cholinesterase decreased. There was no confirmed influence of physicochemical parameters of the water to values of aspartate aminotransferase, creatine kinase, lipase, lactate, triglycerides and Na in fish plasma. Minor effects were observed in the values glucose, urea, phosphorus and alkaline phosphatase. Eight indices out of 23, were affected by handling time and, accordingly to temperature with highest values in July and August for total protein, albumin, alanine aminotransferase, lactate dehydrogenase, amylase, iron and calcium and with the highest values in March and November for cholinesterase. Likewise

III: *Values of rainbow trout plasma indices from aquaculture (Mean ± SD), explanation of abbreviations used in text*

Indices	19. 11.	11. 3.	25. 3.	19. 7.	4. 8.
ALP (μkat.l^{-1})	3.35 ± 2.12	0.90 ± 0.44	1.85 ± 0.73	0.60 ± 0.14	1.79 ± 0.62
ACP (μkat.l^{-1})	0.09 ± 0.01	0.07 ± 0.01	0.10 ± 0.01		
ALT (μkat.l^{-1}) *	0.26 ± 0.05	0.14 ± 0.03	0.32 ± 0.19	0.77 ± 0.49	0.29 ± 0.10
AST (μkat.l^{-1})	7.43 ± 1.77	7.12 ± 2.41	11.53 ± 7.83	14.16 ± 6.71	10.64 ± 1.69
LD (μkat.l^{-1})	4.88 ± 1.89	3.94 ± 1.26	8.95 ± 6.57	16.74 ± 4.01	9.10 ± 3.06
CK (μkat.l^{-1}) *	14.07 ± 4.91	6.05 ± 1.73	22.63 ± 13.71	23.79 ± 51.89	27.39 ± 12.12
CHS (μkat.l^{-1}) *	21.68 ± 2.23	22.19 ± 1.24	21.05 ± 4.81	7.76 ± 0.82	8.01 ± 0.39
AMS (μkat.l^{-1})	4.67 ± 1.71	6.15 ± 2.27	6.07 ± 4.75	13.24 ± 3.00	7.61 ± 4.26
LIP(μkat.l^{-1}) *	0.45 ± 0.46	0.25 ± 0.20	0.16 ± 0.02	0.14 ± 0.01	0.14 ± 0.01
GLU (mmol.l^{-1})	5.05 ± 0.75	4.84 ± 0.99	5.58 ± 1.26	5.34 ± 0.47	6.63 ± 0.58
TP (g.l^{-1})	27.80 ± 4.56	26.21 ± 2.89	29.30 ± 2.94	38.95 ± 4.05	39.41 ± 3.55
ALB (g.l^{-1})	7.91 ± 1.47	5.10 ± 1.12	6.71 ± 1.31	10.33 ± 1.59	11.43 ± 1.61
LACT (mmol.l^{-1})	3.78 ± 1.64	2.44 ± 0.52	4.46 ± 1.47	4.37 ± 1.52	3.81 ± 1.83
CHOL (mmol.l^{-1})	5.71 ± 1.45	3.72 ± 0.64	5.52 ± 0.89	6.54 ± 0.90	7.83 ± 1.40
TRIG (mmol.l^{-1})	3.28 ± 1.29	3.24 ± 1.23	3.77 ± 0.98	2.90 ± 1.21	5.31 ± 0.99
UREA (mmol.l^{-1})	0.72 ± 0.13	0.54 ± 0.09	0.72 ± 0.17	0.75 ± 0.18	0.76 ± 0.13
P (mmol.l^{-1})	4.07 ± 0.61	2.77 ± 0.34	3.33 ± 0.42	3.17 ± 0.39	3.27 ± 0.37
Ca (mmol.l^{-1})	2.13 ± 0.39	2.50 ± 0.19	2.61 ± 0.15	3.06 ± 0.18	3.14 ± 0.36
Mg (mmol.l^{-1})	1.41 ± 0.17	0.93 ± 0.05	0.75 ± 0.18	1.22 ± 0.09	0.97 ± 0.07
Fe (μmol.l^{-1})	13.28 ± 5.59	10.09 ± 2.27	20.76 ± 7.52	25.15 ± 4.88	21.85 ± 4.03
Na (mmol.l^{-1})	170.94 ± 4.28	172.97 ± 3.27	169.91 ± 3.87	172.24 ± 2.85	169.31 ± 3.28
K (mmol.l^{-1})	2.07 ± 0.90	0.84 ± 0.19	1.74 ± 0.44	1.00 ± 0.20	1.90 ± 0.69
Cl (mmol.l^{-1})	139.97 ± 4.88	140.94 ± 6.42	135.33 ± 3.03	134.88 ± 1.42	130.04 ± 3.30

SD – standard deviation, * – Null hypothesis (Kolmogorov–Smirnov test) was rejected

higher pH increased values of acid phosphatase, cholesterol, albumin and iron in fish plasma, just Cl decreased significantly. Conversely, higher oxygen saturation of water increased the value of Cl and reduced value of wide range of biochemical indices (ACP, ALB, ALT, AMS, CHS, LD, TP, Ca and Fe).

Chemical parameters of water had the biggest impact on the content of magnesium in plasma of trout. Higher content of organic matter and iron caused significant increase in values of magnesium, contra to higher acid neutralization capacity, conductivity and higher value of total nitrogen, sulphates, Na, Mg and Ca which caused the decline. Higher content of magnesium, chlorides, sulphates, sodium and temperature of water decreased content of calcium in fish plasma. Higher content of phosphorus and oxygen in the water increase value of chloride in plasma of trout again the higher values of pH, sulphates, natrium, chlorides and magnesium caused decline. By enzymatic indices of plasma was most influenced cholinesterase. Higher content of phosphorus and nitrogen increased value of CHS in fish plasma, decrease of values occurred in the case of higher content of organic substances, oxygen, pH, temperature, Mg and Cl.

DISCUSSION

Taking into account the use of older methods of determination, the inconsistency of the units used, and in many cases the determination of indices in the plasma, our results allow for only tentative comparisons, particularly with the results presented by Wedemeyer and Chatterton (1970), McCarthy *et al.* (1973), Wedemeyer and Nelson (1975), Meade and Perrone (1980), Hille (1982) and Roscoe Miller *et al.* (1983).

Our results are comparable to works using an automatic blood analyser for determination of indices in plasma. Comprehensive survey about normal distribution of blood plasma chemistry in rainbow trout reported Manera and Britti (2006). The authors concluded that seven of the 19 evaluated plasma indices are not normally distributed (urea, creatinine, alanine aminotransferase, alkaline phosphatase, creatine kinase, lactate dehydrogenase and Cl). Although they did not estimate all the indices of the present survey, many discrepancies appeared. In contrast to the present data, they reported urea, alkaline phosphatase, lactate dehydrogenase and chlorides estimates as not normally distributed.

In the present investigation, ranges of lactate, triglycerides, Ca and Mg appeared the same as reported in the literature (Manera and Britti, 2006; Meka and McCormick, 2005; Řehulka and Minařík, 2008; Řehulka and Minařík, 2001; Řehulka and Párová, 2000a, 2000b; Velisek *et al.,* 2008; Velisek *et al.,* 2009). Our observed ranges of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, creatine kinase, total protein, cholinesterase, glucose, albumin, urea, cholesterol, P, Fe, Na, K and Cl appeared to shift towards the lower or higher limits of intervals than those reported in the literature (Manera and Britti, 2006; Řehulka and Párová, 2000a, 2000b; Velisek *et al.,* 2008; Velisek *et al.,* 2009). Considering these recorded differences, it should be noted that plasma parameters may be affected by a wide range of factors both methodological and individual. However, pathological conditions can be in presented study excluded. There is no information in the literature on normal ranges of amylase, lipase, acid phosphatase and iron in rainbow trout. Our results bring new information concerning variation these parameters in healthy fish reared under the conditions of intensive aquaculture.

Evaluating the results of biochemical tests from literature, it can be said that the level of protein and lipid metabolism of rainbow trout corresponded to the contents of protein and lipid components in the diet (Řehulka and Párová, 2000a, 2000b). These conclusions are supported by our results, when there was no demonstrated effect of physico-chemical parameters on values of triglycerides and lipase in fish plasma and their fluctuations depends mainly on the used diet. On the contrary, in our case the values of protein were significantly affected by physical and chemical parameters. Increased content of albumin and total protein in fish plasma was caused by increasing water temperature and chloride and reduced by increased values of nitrogen, phosphor and dissolved oxygen. These factors according to the level of feed intake and fish metabolism intensity and do not exclude impact of used diet on proteins in fish plasma.

The changes of biochemical parameters in the blood plasma of the rainbow trout are also depending on ambient factors such as stress, chemical factors (Meka and McCormick, 2005; Řehulka and Minařík, 2001) and sex of fish (Řehulka and Minařík, 2008). The values of total protein, albumin, urea, glucose, cholesterol, triglycerides, Ca and P in blood plasma were reaching the highest levels in summer, when is higher food intake by fish that was confirmed by our results. There is no enough data to compare the impact of other physical and chemical parameters on changes of biochemical indices of trout plasma. Generally prevailing view that the food intake is primary source of electrolytes to maintain acid-base balance of rainbow trout.

Trout which lives in hypoosmotic environment compensates the electrolytes absence by active intake through the gill epithelium associated with high water discharge by kidney. The importance of dietary ions intake is increasing at low pH, when decreased intake of Na and Cl thought gill epithelium (D'Cruz and Wood, 1998). Based on our results it is clear that physical and chemical composition of water can have an influence on the changes of some parameters of blood plasma and that impact can be more remarkable than previously thought.

According to existing knowledge concerning biochemical indices and their fluctuation in the plasma is influenced by a number of exogenous and endogenous factors, which must be respected in the clinical interpretation of laboratory data. To distinguish between physiological and pathological deviations, more information will be needed to elucidate interindividual and intraindividual variability. Our study suggests that the determination of blood plasma indices belongs to the rational indication of laboratory examination, if we are to evaluate and interpret in an exhaustive manner the physiological response of the organism. The finding that the plasma levels of biochemical indices decreased or increased in the case injury of fish suggests that tests for blood plasma chemistry should be included in the methods of examination and evaluation of the state of health of rainbow trout in aquaculture.

SUMMARY

The aim of the present study was to assess plasma parameters in rainbow trout (*Oncorhynchus mykiss*) from aquaculture system. Clinical healthy immature rainbow trout were provided from three fish farms from 19 November 2009 to 4 August 2010. Plasma parameters were assessed using the automated blood plasma analyser. Non-haemolysed serum from the heart of 48 healthy, randomly selected fish (standard length, mean ± SD = 247.3 ± 24.2 mm; body mass, mean ± SD = 262.18 ± 87.28 g) was analysed for the following plasma parameters: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, acid phosphatase, lactate dehydrogenase, creatine kinase, total protein, cholinesterase, amylase, glucose, lactate, albumin, urea, cholesterol, triglycerides, lipase, Ca, Mg, P, Fe, Na, K and Cl. All data were analysed statistically such as normality assessment by means of Kolmogorov–Smirnov test and adequate statistical testing using various parametric and non-parametric tests for each variable. Electrolytes, with the noticeable exception of potassium and iron, showed the lowest coefficients

of variation, whereas enzymes (namely creatine kinase, lipase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase and amylase) the highest. The Kolmogorov–Smirnov test showed that 19 indices out of 23 (aspartate aminotransferase, alkaline phosphatase, acid phosphatase, lactate dehydrogenase, total protein, amylase, glucose, lactate, albumin, urea, cholesterol, triglycerides, Ca, Mg, P, Fe, Na, K and Cl) were normally distributed ($\alpha = 0.05$). The values of alanine aminotransferase, creatine kinase, cholinesterase and lipase were not normally distributed.

Based on our results it is clear that physical and chemical composition of water can have an influence on the changes of some parameters of blood plasma and that impact can be more remarkable than previously thought. Estimates obtained were compared with previously reported ranges. The blood automated analyser proved to be a valuable and reliable instrument for the estimation of plasma parameters determining normal ranges in rainbow trout.

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