The Influence of Season Variation on Hematological Parameters and Oxidative Stress for Rainbow Trout (*Oncorhynchus mykiss*)

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Bulletin UASVM Animal Science and Biotechnologies 75(1)/ 2018

Print ISSN 1843-5262; Electronic ISSN 1843-536X

DOI:10.15835/buasvmcn-asb: 003517

Abstract

The parameters studied were RBC, Ht, Hb, erythrocyte index MCV, MCH, MCHC, and oxidative stress through SOD and GPx. For each parameter, blood samples were collected at specimens weighing between 100-140g in the summer season, 250-280 g in the fall, 270-300g in the winter and 320-350g in the spring. At the specimens studied the highest values of GPx and SOD were recorded in the spring season due to the stress caused by massive precipitation. During the study, the water temperature ranged between 5.1° C in winter and 19.2° C in summer and O₂ ranged between 8.30 mg/l in the summer and 10.20 mg / l in winter. For most parameters, the highest values were recorded in the spring season and the lowest in the summer season. The data obtained in each season on *Oncorhynchus mykiss* species do not indicate pathological conditions but more an adaptive fish response to environmental conditions and can serve as a database for future research on welfare of salmonids.

Keywords: *antioxidant, alimentary behavior, hematological parameters*

INTRODUCTION

Fish like other species have an antioxidant defense system (Cristina Trenzado *et al.*, 2006) which is subject to change by alimentary behavior, phylogeny, age, water chemical composition and physiological condition (Martínez-Álvarez *et al.*, 2005). From physiological point of view the level of oxidative stress and hematological parameters gives us useful information in detection and diagnosis of metabolic dysfunctions. As well according to Aldrin and Smith (1975) we can appreciate the state of health, nutrition, stress response and adaptability to environmental conditions. Also, Ates *et al.*, (2008) sustained that fish biochemical analyses and hematological parameters can be used as sensitive biomarkers for monitoring of water quality before negative effects to appear in the aquatic environment.

MATERIALS AND METHODS

In every season (summer, autumn, winter and spring) were taken randomized from growing pool 10 specimens of rainbow trout (*Oncorhynchus mykiss*) from which we collected blood from kidney to evaluate the oxidative stress and hematological changes. The research took place in Bistrișorii Valley, Bistra, Alba County beginning with the summer of 2014 till the spring of 2015.

The hematological parameters included the count of RBC, Hb, Ht from whole blood into an anticoagulant (Li heparin) in vacutainers of 5 ml.

Erythrocytes count was made with the Gowers reagent, through a turbidimetrically reaction discernible to a wavelength of $\lambda = 546$ nm and at a temperature of 37° degrees.

Hemoglobin was dosed within 6 hours from harvesting, through a calorimetric END -POINT

Table 1. The mean values of hematological parameters on four seasons of rainbow trout (*Oncorhynchus mykiss*)

Specification	n	Summer			Autumn			Winter			Spring		
		X±sx	s	V%	X±sx	s	V%	X±sx	s	V%	X±sx	s	V%
RBC (T/L)	10	3.36±0.08	0.25	7.44	4.05±0.05	0.15	3.70	3.80±0.12	0.38	10	3.66±0.24	0.75	20.4
Ht (%)		41.2±0.84	2.65	6.43	44±1.01	3.19	7.25	41.4±0.84	2.67	6.4	44.4±1.54	4.88	10.9
Hb (g/dl)		13.16±0.08	0.27	2.05	14.01±0.08	0.28	1.99	14.04±0.13	0.41	2.92	15.12±0.17	0.56	3.70
MCV (fL)		122.65±1.49	4.72	3.84	108.97±3.36	10.65	9.77	109.62±3.17	10.05	9.16	124.2±5.36	16.96	13.65
MCH (pg)		39.28±0.76	2.43	6.18	34.64±0.35	1.13	3.26	37.25±1.22	3.88	10.41	43.72±3.40	10.8	24.70
MCHC (x10g/L)		32.03±0.51	1.62	5.05	32.0±0.73	2.34	7.31	34.0±0.76	2.42	7.11	34.52±1.55	4.89	14.16

RBC= Red blood cell; Ht= Hematocrit; Hb= Hemoglobin; MCV = Mean corpuscular volume; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; V%- coefficient of variation; s- standard deviation; sx- standard error of the average; x-mean; n- number of specimens.

reaction, read in the VIS domain at $\lambda = 546$ nm and at a temperature of 37° degrees.

The hematocrit through centrifugation was dosed from whole blood into capillary tubes at 12.000 rpm for 3 minutes and on a graduated scale there is the high of the erythrocytes column, indicated in percent (%) of volume.

Erythrocyte indices MCV (fL), MCH (pg) and MCHC (x10g/L) were determined throughout calculates formulas in conformity with Ghergariu *et al.*, (1999).

All the analyses were performed with the UV-VIS spectrophotometer, Screen Master Touch, which works in 340-620 nm wavelengths. The oxidative stress was determined based on the antioxidant enzyme GPx and SOD according to the protocol (Ghergariu *et al.*, 1999; Kádár, 2002).

Statistical calculation was made with GraphPad Prism 6 software. Bonferroni test was used for processing data, this test performed a comparison of hematological parameters depending on the season, obtaining the value of the probability index "p". The statistical validity of the data obtained by the Tukey test was verified by applying normality tests involving the calculation of the Kolmogorov-Smirnov index (Motulsky *et al.*, 1999; Motulsky, 2004).

RESULTS AND DISCUSSIONS

As regards the fishes, RBC's are the most numerous blood cells, up to 4 million/mm³ (Nicula *et al.*, 2004) which contains hemoglobin involved in oxygen delivery from gills to tissues. Fishes erythrocytes are usually mononuclear with different sizes depending on the species (Snyder *et al.*, 1999), on the active ones like the rainbow trout, the dimensions are bigger compared with the sedentary ones since they need a bigger amount of oxygen (Cocan, 2011). Within our

research the average values of the RBC are in Table 1 and differences between seasons in Table 2.

According to Sonia Mumford *et al.*, (2007), the increase of the RBC number is due to the enhancing of the hematopoietic system from the apical segment pole of the kidney, under the circumstances of temperature rise. However, in the case of our research, the highest values were recorded in the spring season when the water temperature was 10.2°C and not in the summer season when the water temperature was 19.2°C. This can be attributed due to the adaptability of rainbow trout to different environmental condition, the species developed a mechanism of hemoglobin regulation without having the need for an increase of hematopoiesis. And even if RBC values were lower in the summer, differences between seasons were not significant except for the autumn season. However as stated by Rahman *et al.*, (2013), this can be also influenced by the body weight or by the photoperiod (Solomon and Okomoda, 2012).

Ht value is used to establish the erythrocyte index, and along with these, in conformity with Pârvu *et al.*, (1984), in diagnose different anemias. According to Lientz and Smith (1974) Ht value are positively correlated with the weight of the fish and it can be influenced by its density of breeding as stated by Yarahmadi *et al.*, (2014). The values we obtained regarding the species *Oncorhynchus mykiss* can be placed between the limits quoted by Fazio *et al.*, (2016) which are 20-43%. Within our research Ht value at the rainbow trout did not present variations between seasons and the average values for each season are presented in Table 1 and 2. The obtained results fall within the limits quoted by Ates *et al.*, (2008), Cocan *et al.*, (2011) and Yldiz and Aydin (2006), with the specification that our values are for the same season and for the same fish population.

Table 2. Differences between seasons and their statistical significance on hematological parameters of rainbow trout (*Oncorhynchus mykiss*)

Specification	Summer - Autumn		Summer - Winter		Summer - Spring		Autumn - Winter		Autumn - Spring		Winter - Spring	
	d	t	d	t	d	t	d	t	d	t	d	t
RBC (T/L)	-0.68**	3.76	-0.43 ns	2.41	-0.29 ns	1.63	0.24 ns	1.35	0.38 ns	2.13	0.14 ns	0.78
Ht (%)	-2.80 ns	1.72	-0.20 ns	0.12	-3.20 ns	1.97	2.60 ns	1.60	-0.40ns	0.24	-3.00 ns	1.85
Hb (g/dl)	-0.85***	5.06	-0.88***	5.24	-1.96***	11.67	-0.03 ns	0.178	-1.11***	6.61	-1.08***	6.43
MCV (fL)	13.68 *	2.95	13.03ns	2.81	-1.55 ns	0.33	-0.64 ns	0.14	-15.23 *	3.28	-14.58*	3.14
MCH (pg)	4.64 ns	1.84	2.03ns	0.805	-4.44 ns	1.77	-2.61 ns	1.04	-9.08**	3.61	-6.47 ns	2.57
MCHC (x10g/L)	0.04 ns	0.02	-2.00ns	1.38	-2.49 ns	1.72	-2.05 ns	1.41	-2.54 ns	1.75	-0.48 ns	0.33

RBC= Red blood cell; Ht= Hematocrit; Hb= Hemoglobin; MCV = Mean corpuscular volume; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; d= the difference between seasons; t= the value of t; *=p<0.05 significant; **=p<0.01 distinctly significant; ***=p<0.001 very significant; ns= insignificant;

Table 3. The mean values of glutathione peroxidase (GPx) and superoxide dismutase (SOD) on four seasons of rainbow trout (*Oncorhynchus mykiss*)

Specification	n	Glutathione peroxidase U/gHb			Superoxide dismutase U/gHb		
		$\bar{X} \pm s_x$	s	V%	$\bar{X} \pm s_x$	s	V%
Summer	10	72.90±1.11	3.53	4.84	914.9±26.21	82.89	9.06
Autumn		79.56±0.82	2.60	3.27	865.9±23.53	74.41	8.59
Winter		91.84±2.75	8.72	9.49	841.1±18.18	57.50	6.84
Spring		95.54±3.41	10.79	5.19	919.4±8.86	28.02	5.54

V%- coefficient of variation; s- standard deviation; s_x - standard error of the average; $\bar{x}$ -mean; n- number of specimens.

The hemoglobin contains approximately 80-90% from the dry substance of the erythrocytes at the rainbow trout, meaning 7g/100 ml with variations between 4.1-10.3 g/dl, according to Pârnu *et al.*, (1984).

Its value may present differences depending of species, season, forage given, age etc. In our research, the Hb value ranged from 13.16±0.08 g/dl in summer and 15.12±0.17 g/dl in spring. Thus, resulting very significant differences among the seasons summer-autumn d=-0.85, summer-winter d=-0.88, summer-spring d=-1.96, autumn-spring d=-1.11 and winter-spring d=-1.08. The values we obtained are bigger than Cocan *et al.*, (2011) which were 7.42-11.32 g/dl for the same species.

Mean corpuscular volume (MCV) represents the volume filled with only one erythrocyte, whose analysis helps in identify precocious anemias as well as in their classification. MCV value is influenced by the age of the fish that is why in the case of samplings the hematopoiesis tissues change and the organs such as the spleen and the kidney keep developing (Lone *et al.*, 2012; Rijkers and Muiswinkel, 1977). In what our research is concerned, the MCV presented the highest values in spring 124.2±5.36 fl and in summer 122.65±1.49 fl and the lowest in autumn

124.2±5.36 fl and 109.62±3.17 fl during winter, leading to significant differences between seasons: summer-autumn d=13.68, autumn-spring d=-15.23 and winter-spring d=-14.58. MCV values were positively correlated with the temperature and they were higher than 88 fl cited by Lone *et al.*, (2012) for the same species.

In terms of the MCH values the lowest were during autumn 34.64±0.35 pg and winter 37.25±1.22 pg and the highest in spring 43.72±3.40 pg and summer 39.28±0.76 pg. In this way a significant difference resulted between autumn and spring of d=-9.08. In accordance with Lone *et al.*, (2012) the MCH value is influenced by the age of the fish that is why in the case of rainbow Trout saplings it is between 18-25 pg, and at the adult fish is 58.49 pg. As stated by Wedekind and Schreckenbach (2003) the MCH values influenced by the water temperature and Yarahmadi *et al.*, (2014) claims that it depends on the density of breeding. The values we obtained can be placed between the limits quoted by Fazio *et al.*, (2016), meaning 23.16-68.06 pg. The Concentration of red blood cell hemoglobin (MCHC) includes values within 32.03±0.51 (x10g/L) in summer and 34.52±1.55 (x10g/L) in spring, situated between the limits cited by Yarahmadi *et al.*, (2014).

Table 4. Differences between seasons and their statistical significance on oxidative stress by glutathione peroxidase (GPx) and superoxide dismutase (SOD) of rainbow trout (*Oncorhynchus mykiss*)

Season	Glutathione peroxidase U/gHb		Superoxide dismutase U/gHb	
	d	t	d	t
Summer – Autumn	-6.66 ns	2.04	49.00 ns	1.70
Summer – Winter	-18.94 ***	5.82	73.72 ns	2.56
Summer – Spring	-22.64 ***	6.96	-4.58 ns	0.15
Autumn – Winter	-12.28 **	3.77	24.72 ns	0.86
Autumn – Spring	-15.98 ***	4.91	-53.58 ns	1.86
Winter – Spring	-3.70 ns	1.13	-78.30 ns	2.72

d= the difference between seasons; t= the value of t; **=p<0.01 distinctly significant; ***=p<0.001 very significant; ns= insignificant;

Such as aerophiles organisms, the fish have an antioxidant defense system (Cristina Trenzado *et al.*, 2006) being influenced by the alimentary behavior, phylogeny, age, chemical composition of the water and physiological condition (Martínez-Álvarez *et al.*, 2005). In the Table 3, medium values and the variability of the GPx (Glutathione peroxidase) and SOD (superoxide dismutase) are presented for each season, and in Table 4 there are presented the statistic differences of those between seasons.

As regards the glutathione peroxidase (GPx), these are protecting the proteins and the nucleic acids from the action of the oxidative molecules, and its quantity might be found in bigger proportion than in SOD and the values of these metalloenzymes might vary depending on the zone they were collected (heart, liver, kidney, muscles, etc.). In our research the GPx values between seasons were: influenced by the water temperature and it is situated in the limits cited by Cocan (2011) for the same species of 43.77 U/gHb during winter and 116.53 U/gHb during summer.

Superoxide dismutase (SOD) of rainbow trout is the first enzyme which acts against oxygen radicals (Mccord and Fridovich, 1969) which gives the best answer at oxidative stress (Winston and Giulio, 1991). As expected, the lower values were recorded in the winter cold season and the highest during summer and spring when there was plentiful precipitation which slightly changed the water chemical composition. The values we obtained are higher than what Trenzado *et al.*, (2006) had for this species *Oncorhynchus*, of 834.69 U/gHb and lower than 1309.9 U/gHb quoted by Cocan (2011) in summer on rainbow trout.

CONCLUSIONS

The values of hematological parameters and oxidative stress presented variations among the 4 seasons, interpreted as being the adaptability reaction of the fish at the environmental conditions.

Future research is needed for other species of salmonids with the purpose to offer a database that can be used in monitoring the physiological status of the fish as well as aquatic environment to have a more efficient fishery production.

Acknowledgments. This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.

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