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Sex Differences of Hematological and Biochemical Parameters in Healthy Rainbow Trout (*Salmo gairdneri*, R)

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A hematological study of the rainbow trout has permitted to establish a sexual difference in the parameters related to it, such as haemoglobin, haematocrit, erythrocyte and leukocyte count. The types of leukocytes were homologated to the human blood. Haemoglobin, haematocrit, erythrocyte sedimentation rate, erythrocyte and leukocyte count values were lower in female than male. The normal values of some biochemistry parameters were equally studied and in some cases they similarly showed a sexual difference. Creatinine, triglycerides, phosphatase alkaline, sodium and globulin values were higher in female than male. Establishing a sexual difference from the biochemical and hematological parameters is possible.

Key words: Sex, Hematological and biochemical parameters, Rainbow trout.

Studies of the haematology and blood biochemistry in different species of fish are of a comparative physiological interest. They contribute to a greater understanding of the variation of blood characteristics in relation to such factors as phylogenetic position, ecological habitat, food selection and mode of life. Several authors have been concerned with comparative data on haematological parameters and organic components in trout blood (1, 8). Thus far, there is still a lack of information on the sex differences in haematological parameters and organic metabolites in trout blood.

The purpose of this investigation has been to describe quantitatively some parameters in male and female trout blood. It tries to find through some haematological characteristics (haemoglobin, haematocrit) and organic constituents (total plasma protein, globulins, creatinine, triglycerides) sexual variations.

Materials and Methods

Rainbow trout (*Salmo gairdneri*, R), sexually immature, were obtained from «Los Leoneses» fish hatchery, Castrillo

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del Porma, León (Spain). Weighing from 500 g to 1,200 g and from 24 to 30 months of age. The experiment was carried out during May, when water temperature remained constant at 12-13° C. Each trout was anaesthetized in a 0.1 g/l concentration of MS-222 (tricaine methanesulfonated). Blood was drawn by cardiac puncture from the bulbous arteriosus with a 25/7 hypodermic needle and a 2 ml sterile plastic heparinized syringe. Lower than 0.2 ml samples were discarded.

The haemoglobin was determined within 6 h after withdrawal of blood. Each sample was analyzed in duplicate. The concentration of haemoglobin in g/100 ml was measured by using the cyanethemoglobin method (4, 5) and calculated from the equation: c(g/100 ml) = 36.77 × D.O. Based on the measurement of the absorbance with Varian Techtron Spectrophotometer at 546 nm.

The well mixed blood was drawn into a microhaematocrit reader (Gricel) and expressed as the volume of the erythrocytes per 100 ml. This procedure is widely used in human haematology and its value in fishery research and management was reported by SNIESZKO (12).

The erythrocyte sedimentation rate was determined by Westergreen method. Blood was drawn into the Westergreen tube and the readings were taken at 1 h and 2 h.

Total erythrocyte and leukocyte were counted by sight. Blood is diluted with a fluid that is isotonic with the erythrocytes. The diluted sample (1:200) is introduced into the Thoma hemacytometer chamber, and the erythrocytes are counted (7).

The slide smears were prepared by the May Grumwald-Giemsa method. The types of leukocytes were homologated to the human blood.

The values for erythrocyte count, haemoglobin concentration and haematocrit can be used to obtain certain erythrocyte indices that define the size and haemoglobin content of the erythrocyte. These Wintrobe indices are mean cell volume (M.C.V.), mean cell haemoglobin (M.C.H.) and mean cell haemoglobin concentration (M.C.H.C.) being calculated by using standard formulas (9).

The plasma biochemical parameters measured, with the aid of a DACOS, Coulter Electronic Inc., autoanalyzer were: urea nitrogen, glucose, creatinine, uric acid, triglycerides, cholesterol, alkaline phosphatase, total bilirubin, direct bilirubin, total protein, albumin, globulins, phosphorus, calcium, sodium, potassium and chloride.

Results

A sexual difference in trouts is observed in all the haematological parameters (table I). Male values are higher than the female ones, this relationship being altered only in the Wintrobe indices, M.C.V. and M.C.H., in which the female showed higher values that the male. The M.C.H.C. values were similar in both.

M.C.H.C. values on trout were interestingly less than one-half of those reported on mammals, while the red blood count was only about one-third of the mammal values. The low M.C.H.C. might be related to the much lower rate of oxygen consumption in trout than in mammals.

No differences were observed in most of the parameters of male and female trout. A very significant difference in the creatine, triglyceride and globulin levels with female values higher than the male, was found. Alkaline phosphatase and sodium showed a less significant difference (table II).

Discussion

The range of haemoglobin obtained in the rainbow trout was similar to the value

Table I. *Comparison of hematological values from male and female healthy rainbow trout.* Values are means ± S.D. Number of specimens in parenthesis. Student's «t» test was used for comparison of the means.

PARAMETER	MALE	FEMALE
Haemoglobin, g/100 ml	9.4 ± 1.3 (15)	7.1 ± 0.7 (14)**
Haematocrit, %	56 ± 2 (91)	48 ± 1.3 (66)***
E.S.R. Westergreen, mm/h	1 – 3 (15)	0.5 – 1 (14)N.S.
Erythrocytes/mm^3 range	2,300,000-1,050,000 (15)	1,230,000-560,000 (14)
M.C.V., μm^3	300	575
M.C.H., pg	50	85
M.C.H.C., %	17	15
Leukocytes/mm^3 range	503,000 - 300,000 (15)	370,000 - 200,000 (14)
Lymphocytes, % range	97 - 100	91 - 99
Neutrophils, % range	0 - 1	1 - 6
Monocytes, % range	0 - 2	0 - 2
Eosiniphils, % range	0 - 1	0 - 1
Basophyls, % range	—	0 - 1

N.S. non significant; * $p < 0.05$, ** $p < 0.02$, *** $p < 0.001$.
M.C.V. (pg) = Hct. (%) × 10/erythrocyte count (millions/mm^3)
M.C.H. (μm^3) = Hb. (g/100 ml) × 10/erythrocyte count (millions/mm^3)
M.C.H.C. (%) = Hb.(g/100 ml) × 100/Hct. (%).

found by other authors (2, 3, 6). This range was slightly higher than the value reported by MILLER *et al.* (8) (5 ± 1.6 g/100 ml) and SCHIFFMAN *et al.* (10) (6.5 g/100 ml). The haemoglobin value varies not only within the different kinds of trout, but within those of fish (3).

The finding of a sexual difference in the hematocrit was confirmed by SANO (9), who reported a variation in rainbow trout (*Salmo gairdneri*, R) with gonad development, the male varying between 22-46 % and the female between 19-38 %. The present hematocrit values agree fairly well with those of SNIESZKO (12) (35-57 %) who studied the seasonal changes in haematology, and with those of GIORGETTI *et al.* (3) who gave a mean value of 45 ± 11 %. The values proposed by SCHIFFMAN *et al.* (10), 31,8 % and MILLER *et al.* (8) 32 ± 5,5 %, were lower than the results in the present paper. The hematocrit did not vary among the different kinds of trout: 39-44 % for brown trout (*Salmo trutta*, L) and 45-50 % for brook trout (*Salmo fontinalis*, M) (12); 20-43 % for brown trout (1); and 43 ± 5 % for brown trout, and 38 ± 3 % for marbled trout (*Salmo trutta marmoratus*) (3).

The erythrocyte sedimentation rate can be used as an indication of the disease conditions in fish. SCHUMACHER *et al.* (11), using a Westergreen type method gave values of 10-28 mm/h in brook trout (*Salvelinus fontinalis*, L) suffering furunculosis as compared to 4-8 mm/h with healthy fish. BLAXHALL *et al.* (1), using the same method obtained for brown trout (*Salmo trutta*, L) values between 1-5 mm/h. Low values were obtained here with healthy rainbow trout (*Salmo gairdneri*, R).

The indices of Wintrobe M.C.V., M.C.H. and M.C.H.C. that relate the haemoglobin, hematocrit and red blood cell counts found by DENTON *et al.* (2) and MILLER *et al.* (8), for the rainbow trout fell in with the values presently reported.

Erythrocyte and leukocyte counts suffer from a fair degree of inherent error when carried out visually. The present

Table II. *Comparison of plasma biochemical values from male and female healthy rainbow trout.* Values are means ± S.D.. Number of specimens in parenthesis. Student's «t» test was used for comparison of the means.

PARAMETER	MALE	FEMALE
Urea-Nitrogen, mg/100 ml	9.25 ± 0.5 (15)	9.2 ± 1 (11)$^{N.S.}$
Glucose, mg/100 ml	87 ± 12 (10)	87 ± 7 (14)$^{N.S.}$
Creatinine, mg/100 ml	0.5 ± 0.08 (15)	0.7 ± 0.1 (14)***
Uric acid, mg/100 ml	1.02 ± 0.36 (15)	1.3 ± 0.6 (14)$^{N.S.}$
Triglycerides, mg/100 ml	952 ± 227 (17)	1512 ± 318 (14)**
Cholesterol, mg/100 ml	369 ± 52 (15)	334 ± 50 (14)$^{N.S.}$
Phosphatase alkaline, U.I./l	92 ± 12 (15)	119 ± 14 (14)*
Bilirubin total, mg/100 ml	0.36 ± 0.13 (15)	0.46 ± 0.17 (14)$^{N.S.}$
Albumin, g/100 ml	1.13 ± 0.17 (15)	1.25 ± 0.16 (14)$^{N.S.}$
Phosphorus, mg/100 ml	22 ± 5 (15)	19 ± 6 (14)$^{N.S.}$
Calcium, mg/100 ml	12 ± 0.7 (15)	12.4 ± 1.1 (14)$^{N.S.}$
Sodium, mEq./l	133 ± 11 (15)	159 ± 15 (14)*
Chloride, mEq./l	105 ± 10 (14)	117 ± 4 (13)$^{N.S.}$
Globulins, g/100 ml	0.9 ± 0.4 (10)	1.85 ± 0.2 (11)**
Total protein, g/100 ml	3.9 ± 0.5 (15)	3.86 ± 0.3 (14)$^{N.S.}$

N.S. non significant; * $p < 0.05$; ** $p < 0.02$; *** $p < 0.001$.

values of erythrocyte counts obtained for rainbow trout accord fairly well with the values reported by other authors (2, 8, 12). The leukocyte count was higher than values reported by BLAXHALL *et al.* (1) for brown trout (63,000/mm^3).

Leukocyte differential counts found by us for rainbow trout were similar to those reported by BLAXHALL *et al.* (1), for brown trout. Although, a higher number of lymphocytes and the existence of neutrophils and basophils were observed, they were not in the brown trout.

The three biochemistry parameters that have shown a significant sexual difference: creatinine, triglycerides and globulins can be counted on to determine the sex, as well as the haematological parameters. It is in the previous three parameters, as shown in results, that the female trouts had values significant higher than the male trouts.

A remarkable difference has been found between the values of urea (measured as nitrogen), creatinine, triglycerides, cholesterol, total bilirubin and globulins and those reported by GIORGETTI *et al.* (3), which were much lower. Similarly the value for phosphorus was much higher than that provided by MILLER *et al.* (8), 22.5 mg/100 ml versus 8.7 ± 1.5 mg/100 ml.

The glucose value fell within those reported by MILLER *et al.* (8) (144 ± 41 mg/100 ml), and GIORGETTI *et al* (3) (54.5 ± 29.5 mg/100 ml). The uric acid, sodium and albumin values, to the contrary, were lower than the ones reported by these authors.

The calcium and chloride values were equal to those reported by GIORGETTI *et al.* (3), although the given intervals were not coincident. In calcium the mean value was higher than the one reported by previous authors, 9.93 ± 2.93 mg/100 ml and in chlorides the mean values were lower, but the validity ranges had common points. The plasma total protein value higher than that given by MILLER *et al.* (8) for the same parameter but it was almost the same as the one reported by GIORGETTI *et al.*

The values for the alkaline phosphatase were higher than the ones reported by MILLER *et al.* (8) 50 ± 23 U.I./l, and interval was almost coincident with the

one proposed by GIORGETTI *et al.* (3), 125.6 ± 26.4 U.I./l. These authors reported a work about biochemistry parameters similar to the present work and also studied the variations of the alkaline phosphatase with the weight of the animal. They found that as the animal weight increases the alkaline phosphatase value decreases, so that for animals weighing 560 g the alkaline phosphatase value was 143.5 ± 10 U.I./l and for animals weighing 810 g the alkaline phosphatase value fell to 90 ± 7.2 U.I./l (3). Keeping this in mind and assuming that the animals weighed between 500 and 1,000 g, those values were similar to the ones proposed by the present authors. Nevertheless, the values reported by MILLER *et al.* do not agree with this theory, since their animals weighed 250 g and the alkaline phosphatase value proposed by them was much lower than expected.

It may be concluded that there exists a sexual difference in the haematic parameters and some biochemical values.

Resumen

Se estudian en la trucha arco iris algunos parámetros hematológicos como hemoglobina, hematocrito, recuento eritrocitario y leucocitario y su relación con el sexo. Los tipos de leucocitos fueron homologados a los de la sangre humana. Los valores obtenidos para la hemoglobina, hematocrito, velocidad de sedimentación, recuentos eritrocitario y leucocitario son menores en hembras que en machos. Igualmente, los valores de creatinina, triglicéridos, fosfatasa alcalina, sodio y globulinas son mayores en las hembras que en los machos. Se concluye que es posible establecer una diferencia sexual en algunos parámetros hematológicos y bioquímicos.

Palabras clave: Trucha, Parámetros hematológicos.

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