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SOME BLOOD PARAMETERS OF ONE-YEAR-OLD RAINBOW TROUT, *SALMO GAIRDNERI* RICHARDSON, REARED UNDER DIFFERENT CONDITIONS (*)

BY

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ABSTRACT

Some haematological parameters of one-year-old rainbow trout reared under different methods in Portugal (earth ponds, floating net cages, concrete raceways and metalic silos) were investigated. In the metalic silos specimens an outbreak of *Aeromonas hydrophila* has been detected about one month before blood collection. Microhematocrit values and number of erythrocytes/mm^3 were significantly different ($P < 0.01$) in all the populations. Haemoglobin, blood specific gravity and serum specific gravity values were not significantly different in net cages and earth pond specimens but were different regarding the other populations ($P < 0.01$). The values of the same parameters were also different ($P < 0.01$) when comparing metalic silos specimens with concrete raceways ones. The lowest values were those of metalic silos fish. In this population were found the most elevated values for thrombocytes, erythroblasts and erythrocytes/leukocyte ratio.

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INTRODUCTION

The blood parameters of *S. gairdneri* have been studied from several points of view: determination of normal values (PAPOUTSOGLOU and PAPOUTSOGLOU, 1979; WEDEMEYER and NELSON, 1975; WEDEMEYER and CHATTERTON, 1970; HAIDER, 1972), effect of pathological and environmental conditions (AMEND and SMITH, 1974; NOMURA and KAWATSU, 1977; BARHAM *et al.*, 1980), effect of age and season (HAIDER, 1970, 1971).

All these studies demonstrated that the haematological features are extremely sensitive to several factors. Even prophylactic treatments carried out on fish farms, like the use of malachite green can cause rapid changes of normal blood values (GRIZZLE, 1977; HLAVEK and BUKLEY, 1980). For this reason the examination of fish blood can serve as a method of assessing fish health, even if the examined parameter is only the microhematocrit value (SNIESZKO, 1960).

In this paper some of the blood parameters of one-year-old rainbow trout specimens are studied. Samples were collected from populations reared in Portugal under different conditions: metalic silos, concrete raceways, floating net cages and earth ponds. Fish reared in metalic silos experienced an outbreak of *Aeromonas hydrophila* one month before blood collection.

MATERIALS AND METHODS

Fifteen one-year-old specimens of rainbow trout were collected from the Caniçada Experimental Unit of Aquaculture belonging to the Ecological Centre from the INIC (floating net cages), from Posto Aquícola do Marão (concrete raceways and earth ponds) and from Inha trout farm (metalic silos).

All the samples were collected within a one month period, between January and February 1983 in order to avoid time dependant variation in the studied parameters (HAIDER, 1971).

Blood was collected on the spot from unanaesthetised fish by caudal puncture (LYSAK, 1958). The anticoagulant used was EDTA. Blood smears were made immediatly after sampling.

Other determinations were made at the laboratory within a 2-3 hour period.

Smears were stained by May-Grunwald Giemsa stain. Microhematocrit values were determined by centrifugation at 4,000 rpm for twenty minutes. Haemoglobin concentration was determined according to the cyanmet-haemoglobin method. Erythrocytes were counted using a Neubauer counting chamber. Determination of microhematocrit values and number of eryhtrocytes was carried out twice and average values calculated. Percentage values of lymphocytes, basophils and neutrophils were estimated by counting about 200 cells under immersion oil objective. Percentage values of erythroblasts in relation to erythrocytes was calculated by counting between 300 and 400 cells. Number of thrombocytes on about 60 microscopical fields (× 1,000 times magnification) was counted. The erythrocytes/leucocyte ratio was estimated in 4 microscopical fields. Blood specific gravity and serum specific gravity values were determined according to the method of PHILLIPS *et al.*, 1950*a*, 1950*b*.

All the specimens were measured (± 1 cm) and weighed (± 0.1 g). Between 5 and 8 specimens of each place were dissected in order to assess the presence of macroscopic parasites. The gut contents were examined and the general condition of the fish assessed.

In all the trout farms the ammonia and oxygen levels of the water were measured. In earth ponds oxygen concentration was not measured. Water temperature was measured daily and the average value for January is given.

The parameters studied are shown as average values with the standard deviation. Obtained values were compared by Student's test.

RESULTS

Water ammonia and oxygen concentrations, water temperature, total length, total weight and condition factor of specimens are presented in table 1.

TABLE 1 — Water ammonia (ppm) and oxygen concentration (mg/1) values, January average water temperature (°C), total length (cm), total weight (g) and condition factor average values.

	Ammonia	Oxygen	Temperature	TL	TW	CF
Metalic silos	0.2	8.52	8.0	20.0 ± 1.2	94.9 ± 20.3	1.15 ±0.08
Concrete raceways	0.05	12.1	7.6	18.0 ± 1.7	64.8 ± 21.7	1.06 ±0.07
Floating net cages	0	12.4	9.3	20.7 ± 1.8	112.7 ± 31.1	1.23 ±0.06
Earth ponds	0.05	—	7.6	19.4 ± 1.4	101.3 ± 19.3	1.37 ±0.18

The general condition of specimens from floating cages was good with most fish showing full gastrointestinal tracts when examined. All fish groups were fed pellets.

Specimens from metalic silos appeared normal externally. However almost all the animals examined had damaged gills, which appeared pale and showed petechial haemorrhages. The livers appeared abnormally pale or sometimes very dark in colour. When handling the specimens they lost easily their scales. About one month before examination of specimens an outbreak of *Aeromonas hydrophila* has been detected in this population (PEIXOTO CORREIA, personnal comunication).

Specimens from concrete raceways appeared in better condition. However one had pale and damaged gills and others showed petechial haemorraghea in the gut and excess mucus production in the intestine.

Finally, specimens from the earth pond (they were put in the earth pond one month before blood collection) showed a more yellowish external body colour than the other fish examined. General body condition was good. The gut was extremely full, mostly with trout eggs and larvae (the pond had held spawners previously), insect larvae and small leeches. Their gall bladers were almost empty and the gut contents weighed up to 26 g, which could explain the elevated condition factor values of these specimens. No macroscopic parasites were observed in any of the fish examined.

The values for haemoglobin, haematocrit, number of erythrocytes, blood specific gravity and serum specific gravity are shown in table 2. Results of blood cells counts are shown in table 3.

TABLE 2 — Blood average values for analysed specimens. Hb- haemoglobin (g/100 ml). Ht- microhematocrit (%). RBC- number of erythrocytes (10^6/mm^3). BSG- blood specific gravity. SSG- serum specific gravity.

	Hb	Ht	RBC	BSG	SSG
Metalic silos	1.66 ±0.84	20.3 ±10.8	0.565 ±0.321	1.021 ±0.006	1.0118 ±0.0015
Concrete raceways	3.05 ±0.75	34.4 ± 4.65	0.913 ±0.161	1.032 ±0.0019	1.012 ±0.0018
Floating net cages	3.24 ±0.82	43.5 ± 3.27	0.956 ±0.130	1.0303 ±0.0013	1.0135 ±0.0010
Earth ponds	3.29 ±0.71	40.0 ± 5.1	1.056 ±0.154	1.0306 ±0.002	1.013 ±0.003

TABLE 3 — Diferential counting of leucocytes (percentage values of lymphocytes, basophils and neutrophils). Number of thrombocytes in about 60 microscopical fields. Percentage values of erytroblasts in relation with erythrocytes. Erythrocytes/leukocyte ratio values.

	Lymphocytes	Basophils	Neutrophils	Thrombocytes	Erythroblasts	Erythrocytes/ /leukocytes ratio
Metalic silos	83.5	3.0	9.8	123	25.6	31.7
Concrete raceways	93.7	0.42	4.9	8	10.8	50.9
Floating net cages	86.7	2.8	7.1	40	6.7	36.9
Earth ponds	92.4	0.32	6.2	35	7.4	36.6

Statistical analysis of data showed significant different values ($P < 0.01$) for haemoglobin, haematocrit, number of erythrocytes, blood specific gravity and serum specific gravity values. Values of haemoglobin, serum specific gravity and blood specific gravity from floating net cages and earth ponds specimens were not statistically different ($P > 0.01$).

DISCUSSION

The fish reared in metalic silos clearly showed different blood parameter values from the other groups. The blood values for this group were lower than those of the other groups of fish and also lower than normal values cited in the literature. The microhematocrit showed an average value of 20.3 % (range: 4.5 %-42 %), with a corresponding value of number of erythrocytes of 565,000/mm³. As a consequence haemoglobin concentration was only of 1.66 mg/ml, and blood specific gravity was 1.021. However serum specific gravity was 1.0118.

All these values are extremely low indicating an advanced stage of anaemia. These findings could be explained in a number of ways. This population had an outbreak of *A. hydrophila* which was detected about one month before blood collection. The effect of diseases upon the blood picture of fish is well known. Several authors have shown that a disease can alter the normal blood parameters of fish (BARHAM *et al.*, 1980; HARBELL *et al.*, 1979; IKEDA and MINAMI, 1982; RICHARDS and PICKERING, 1979; LISAK *et al.*, 1977).

Comparing with the other populations, differential white blood cells count from silos specimens showed an increase in the number of basophils and neutrophils and a decrease in lymphocytes. Thrombocytes were very abundant indicating that the fish were under stress (WEDEMEYER *et al.*, 1976). The percentage of erythroblasts in relation to erythrocytes was very high (25.6 %), indicating the intense erythropoiesis. The low erythrocyte/leukocyte ratio value (31.7) shows the severe anaemia present in this fish population.

On the other hand the ammonia level in silos was quite high — 0.2 ppm. PAPOUTSOGLOU and PAPOUTSOGLOU (1979) have shown that rainbow trout fed on different diets show different blood characteristics. The specimens from metalic silos were feed on pellets made at the fish farm, while the other populations were feed on commercial pellets. This fact could also play a role on the observed blood parameters of this population. So it would seem that the low values can be attributed to several factors concurring simultaneously.

According to the values found the blood picture of fish from earth ponds and floating cages ones can be considered opposite to those of concrete raceways specimens.

The haemoglobin content, microhematocrit, serum specific gravity values and number of erythrocytes/mm^3 were higher in the first two groups of fish. The higher value of blood specific gravity for the concrete raceways specimens is difficult to explain. Differential counting of leucocytes from these

populations specimens showed values not very much different each other. Nevertheless floating cages specimens had less lymphocytes and more neutrophils and basophils. The number of thrombocytes was higher and in concrete raceways speciments the erythroblasts percentage and the erythrocytes/leucocyte ratio values were higher.

Overall it seems that the specimens reared in earth ponds and floating cages had «better» haematological values. The method of feeding of these specimens may explain this fact. Earth pond specimens fed largely on trout eggs, larvae and invertebrates. It is believed that a small proportion of natural food was available to the floating cages specimens, penetrating through the net mesh. This food supplement is thougth to have affected the blood picture of the fish.

The haematocrit and blood cells count agree with the value usually considered as «normal» by several authors (WEDEMEYER and YASUTAKE, 1977; BLAXHALL and DAISY, 1973), but no not agree with those pointed out by other authors also as «normal» ones (SNIESZKO, 1960). Haemoglobin values are lower than those referred by other authors for the same species.

The concept of «normal» value is difficult to define and is complicated by different workers using different methods of analysis (especially concerning haemoglobin determination). STEUKE and SCHOETTGER (1967) showed that the method of sampling trout blood measurement of hematocrit influences the haematocrit value. NOMURA and KAWATSU (1977) found variation of haematocrit values for rainbow trout samples maintained under different conditions.

It has been demonstrated that the fish blood is very sensitive to a wide range of factors. It could happen that a population apparently in good health status is on the begining of a bacterial outbreak, for instance. If blood picture is analysed before symptoms appear, the values found will not be the «normal» ones.

Also there exists a great variability among individual fish within the same population. Microhematocrit values in this study showed a range of 33.5 %-56 % (earth pond), 27 %-40 % (concrete raceways) and 36 %-43.5 % (net cages). Should all these values be considered as «normal»? It is also possible that fish from different regions have different «normal» values due to genetic factors.

Therefore in our opinion a «normal» value should be defined as a value obtained from healthy fish by means of standardized methods which urge to be defined. These values should be complemented by an attempt to characterize the factors possibly involved in its determination.

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