

# BLOOD INDICES OF THE RAINBOW TROUT, *Oncorhynchus mykiss* (WALBAUM) IN AEROMONAS-INDUCED ULCEROUS DERMATITIS

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## Abstract

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Indices of the peripheral blood of the rainbow trout, *Oncorhynchus mykiss* (Walbaum) were studied in cases of affected skin, occurring on mass. Haematological and biochemical analyses were carried out in 8 to 10 fish from one group of fish subjectively judged as healthy and another as diseased. A significant reduction ( $P = 0.01$ ), as compared to the values obtained from clinically healthy fish, was recorded in the red blood cells count (RBC: 0.55 vs 0.95 T/L), haematocrit (PCV: 0.200 vs 0.344) and haemoglobin (Hb: 36.8 vs 65.9 g/L). As to the biochemical parameters of the blood plasma, a statistically significant ( $P = 0.01$ ) decrease occurred in total protein (TP: 10.7 vs 35.6 g/L), cholesterol (CHOL: 0.74 vs 5.75 mmol/L), triglycerides (TG: 0.37 vs 2.37 mmol/L) and calcium ( $\text{Ca}^{2+}$ : 2.09 vs 3.25 mmol/L). Increases in the urea (BUN: 1.7 vs 0.5 mmol/L) and glutamyl transferase activities (GMT: 0.48 vs 0.09  $\mu\text{kat/L}$ ) were observed in the affected fish at the same level of statistical significance. At a significance level of  $P = 0.05$  we recorded a decrease in lactate dehydrogenase activity (LDH: 40.98 vs 15.85  $\mu\text{kat/L}$ ). Electrophoresis on agarose gel showed mainly a reduction of albumins.

*Haematology, blood chemistry, aeromonad epidemic, Oncorhynchus mykiss*

Intensive rainbow trout farming requires veterinary supervision to be provided by qualified personnel on a regular basis. Clinical haematology and biochemistry also play a significant role in the complex of supporting diagnostic methods of determining the state of health of the fish. Consistent research into the biochemical processes in healthy fish and in fish affected by diseases should lead to selecting the best indices capable to give the most objective image of the current physiological state of the fish and draw attention to any departures from the optimum that would approach the boundary of pathological changes. Synthesis and analysis of the blood values for the various nosological units of diseases may provide valuable information on any specific response or on the range and nature of any pathological process. This will enable to select optimum therapeutic procedures and draw prognostic conclusions. Enhancement of knowledge in the areas of clinical and laboratory haematology and biochemistry is therefore an essential part of the educational process.

This paper is aimed at describing the changes that occurred in the blood of rainbow trout exposed on mass to *Aeromonas*-induced ulcerous dermatitis. Investigation is focused on the basic and derived indices of the red blood cell, some biochemical indices of the blood plasma, including the activities of selected enzymes, and electrophoretic analysis of plasmaproteins.

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## Materials and Methods

### Description of the Case

The outbreak of the disease in the fish kept in flow-through concrete tanks occurred early in April. The typical symptom was the formation of skin lesions mainly on the sides near the gill covers. The changes on the skin developed through the following stages: erosions devoid of pigment, prominent bulges filled with a pellucid exudation, sometimes an almost indiscernible admixture of blood, and ulcers which arose where the skin broke. Exophthalmos and inflammations around pectoral fin bases were observed in some of the fish. The changes inside the body included hyperaemia of the side of the swim bladder, small haemorrhages in the liver, splenomegaly and clear ascitic fluid. The gills showed different degrees of anaemia. The microscopic picture of the skin lesions included suppurative inflammations without distinct boundaries with ample exudation, and necrosis of the muscular fibres, indicative of myocytolysis. The average body weight of the fish was 380 g and body length 380 mm.

Primary role in the etiopathogenesis of the disease was played by *Aeromonas schubertii*. Other isolations included *Aeromonas hydrophila*, *Aeromonas media*, *Staphylococcus hyicus*, *Staphylococcus epidermidis*, *Enterobacter cloacae* and the non-saccharolytic *Acinetobacter*.

### Preparation of the Blood Samples

After Menocain-induced anaesthesia of the fish, blood samples were taken by puncture from the caudal blood vessels of 8 to 10 diseased fish and the same number of fish without symptoms of the disease. The sampling time was between 7 and 11 h A.M. Heparin film in syringe was used as anticoagulant. At the blood sampling time the water temperature was 4 °C, the content of dissolved oxygen was 88 %, water hardness 3.4°N and  $\text{COD}_{\text{Mn}}$  was 0.8 mg O<sub>2</sub>/L.

### Haematology

To determine the red blood cell count (RBC in T/L) we used Bürker's haemocytometer. Haematocrit (PCV in l/l) was determined in heparinised microhaematocrit capillaries using microhaematocrit centrifuge (13000 rpm for 3 min), and haemoglobin (Hb in g/L) by the standard photometrical method. As to the derived red blood indices, we determined the mean corpuscular volume (MCV in fl), mean corpuscular haemoglobin (MCH in pg) and mean corpuscular haemoglobin concentration (MCHC in l/l).

### Clinical Chemistry

The biochemical examination of the blood plasma was performed on the Hitachi 717 apparatus within 24 hours of maintaining the plasma at 4°C. Calcium content (Ca<sup>2+</sup> in mmol/L) was determined by flame emission photometry, and inorganic phosphate (P in mmol/L) photometrically in the UV segment of the spectrum. The following examinations were proposed: total protein (TP in g/L), glucose (GLU in mmol/L), cholesterol (CHOL in mmol/L), urea (BUN in mmol/L), uric acid (UA in µmol/L) creatinine (CREA in µmol/L), triglycerides (TG in mmol/L), total bilirubin (T-bil in µmol/L), and the activities of alanine aminotransferase (ALT in µkat/L), aspartate aminotransferase (AST in µkat/L), alkaline phosphatase (ALP in µkat/L), lactate dehydrogenase (LDH in µkat/L), glutamyl transferase (GMT in µkat/L) and hydroxybutyryl dehydrogenase (HBD in µkat/L).

The electrophoretic separation of protein was performed on agarose gel at pH 8.6, at a separation tension of 15 to 20 V/cm, temperature of 10°C and separation time of 45 minutes, using the LKB 2117 Bromma Multiphor II apparatus. The LKB Bromma 2202 Ultrosan Laser Densitometer was used for the qualitative and quantitative assessment of the protein fractions, with graphic recording using the LKB Bromma 2220 Recording Integrator. The LKB Bromma 2219 Multitemp II system served for cooling. The LKB Bromma 2197 Power Supply was used for feeding the instruments.

Statistical significance of the differences between the arithmetic means for the selected sets was verified by the *t*-test at the significance levels of  $P = 0.05$  and  $P = 0.01$ .

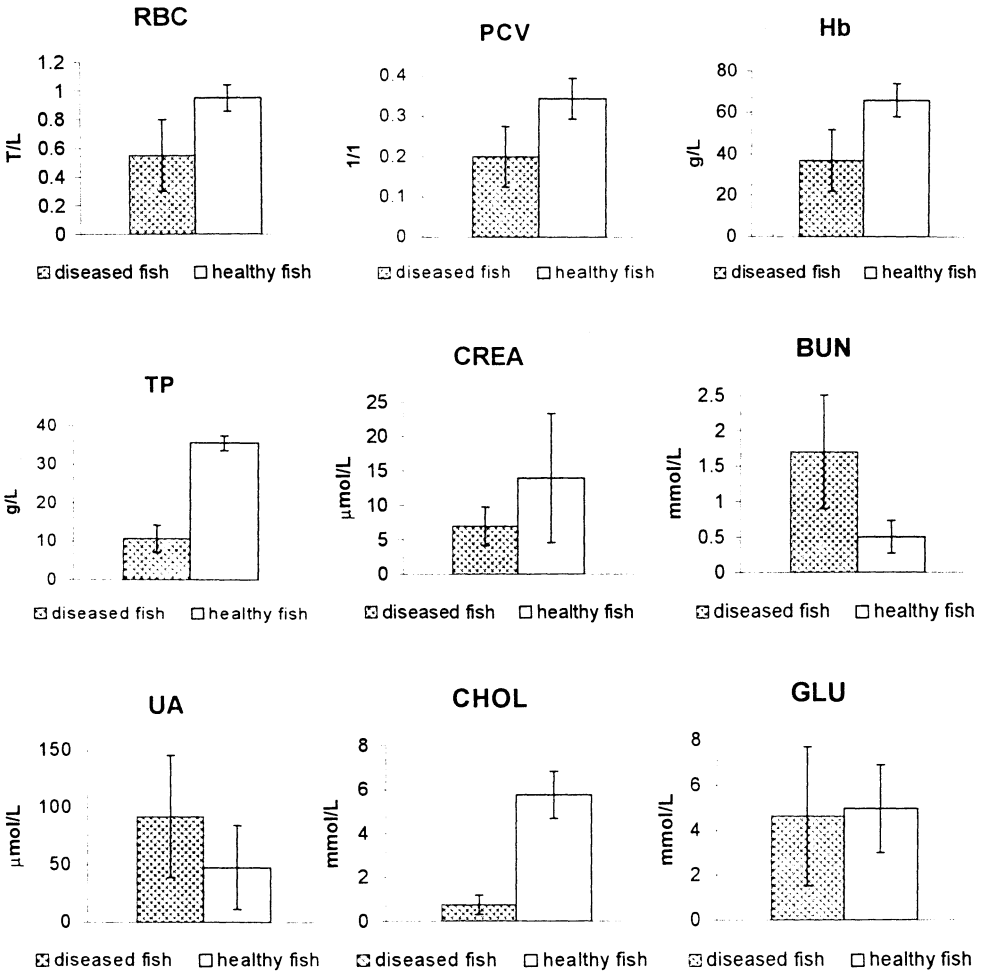
## Results

### Haematology

When interpreting the indices under study, we preferred graphic representation. The average values of the various indices for the diseased and healthy fish are indicated in Fig. 1. The different degrees of anaemia in the diseased fish were accompanied by a reduced ( $P = 0.01$ ) RBC (0.55 vs 0.95), PCV (0.200 vs 0.344) and Hb (36.8 vs 65.9). The lowest individual values of RBC (0.17), PCV (0.090) and Hb (14) were indicative of severe anaemia. The blood indices (MCV, MCH, MCHC) were not significantly different between the groups.

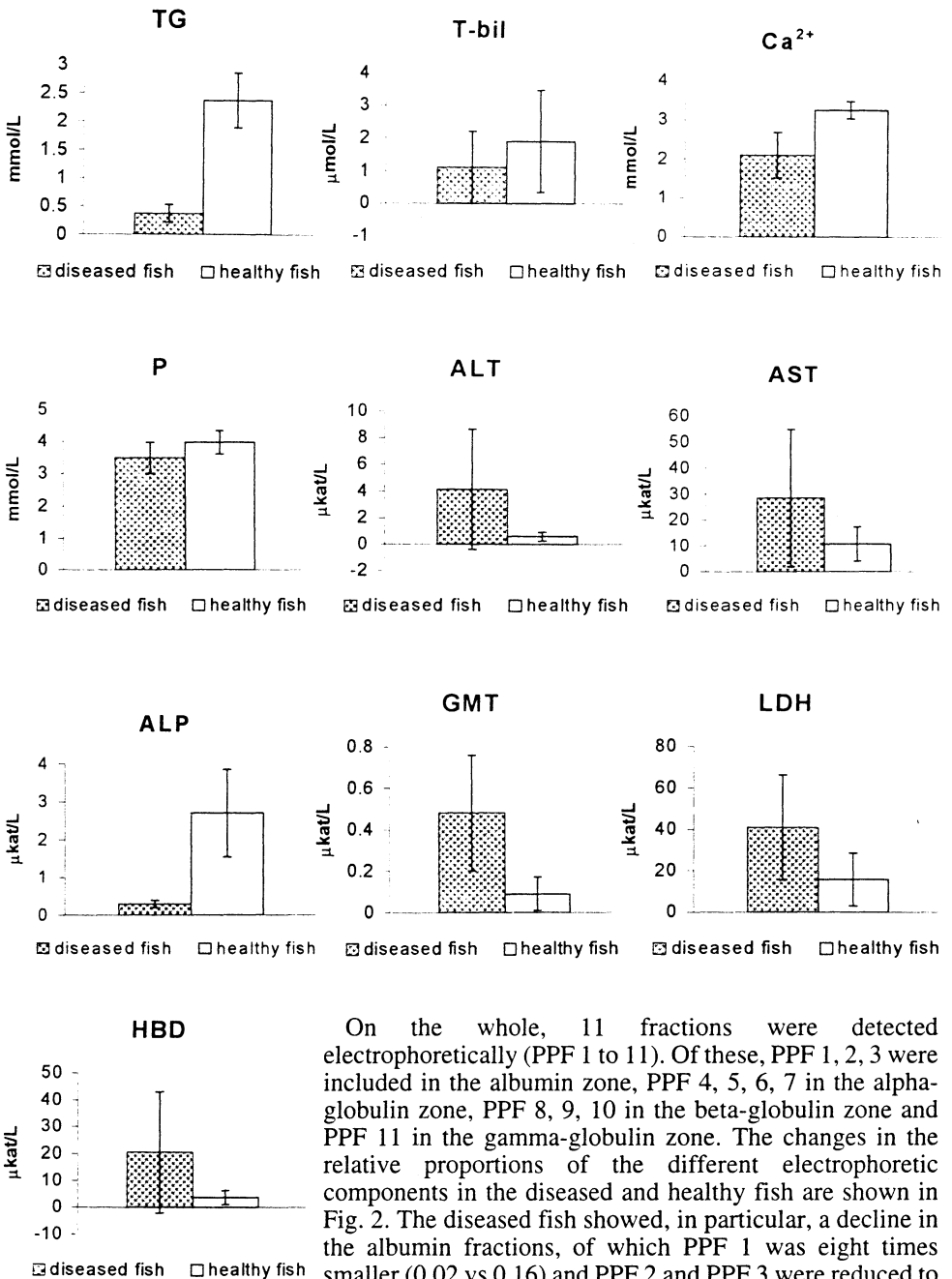
Fig 1.

Haematological and biochemical values (arithmetic mean  $\pm$  standard deviation) of peripheral blood in the rainbow trout, *Oncorhynchus mykiss*, in cases of infectious ulcerous dermatitis: RBC (red blood cell count), PCV (haematocrit), Hb (haemoglobin), TP (total protein), CREA (creatinine), BUN (urea), UA (uric acid), CHOL (cholesterol), GLU (glucose), TG (triglycerides), T-bil (total bilirubin),  $\text{Ca}^{2+}$  (calcium), P (inorganic phosphate), ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase), GMT (glutamyl transferase), LDH (lactate dehydrogenase), HBD (hydroxybutyryl dehydrogenase).



### Clinical Chemistry

A highly significant ( $P = 0.01$ ) decrease was recorded in TP (10.7 vs 35.6), CHOL (0.74 vs 5.75), TG (0.37 vs 2.37) and  $\text{Ca}^{2+}$  (2.09 vs 3.25). Increases in the BUN (1.7 vs 0.5) and GMT (0.48 vs 0.09) activities were observed in the affected fish at the same level of statistical significance. At a significance level of  $P = 0.05$  we recorded a decrease in LDH activity (40.98 vs 15.85). More or less balanced values were recorded for GLU (4.63 vs 4.96), T-bil (1.1 vs 1.9) and P (3.50 vs 3.98).



**Fig. 1 continued**

only observed in PPF 7 (0.107 vs 0.061) and PPF 11 (0.069 to 0.128 vs 0.041 to 0.078). It is interesting to note that PPF 8 was only recorded in the sick fish.

On the whole, 11 fractions were detected electrophoretically (PPF 1 to 11). Of these, PPF 1, 2, 3 were included in the albumin zone, PPF 4, 5, 6, 7 in the alpha-globulin zone, PPF 8, 9, 10 in the beta-globulin zone and PPF 11 in the gamma-globulin zone. The changes in the relative proportions of the different electrophoretic components in the diseased and healthy fish are shown in Fig. 2. The diseased fish showed, in particular, a decline in the albumin fractions, of which PPF 1 was eight times smaller (0.02 vs 0.16) and PPF 2 and PPF 3 were reduced to almost a half (0.22 vs 0.37). A significant decline was also recorded in PPF 9 (0.046 vs 0.085). A clear increase was

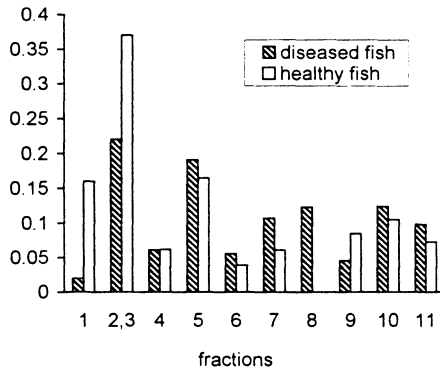


Fig. 2.

Results of blood plasma electrophoresis in the rainbow trout, *Oncorhynchus mykiss*, affected by infectious ulcerous dermatitis.

## Discussion

The increase in the level of BUN was probably associated with the endogenous catabolism of proteins which increases with fasting, infection and losses of blood. Hypoproteinaemia grew with the extent of the skin lesions and had been induced mainly by the blood losses and by the reduction of the albumin fractions (hypoalbuminaemia). The reduction of ALP activity is, in our opinion, a consequence of severe anaemia and the increase in ALT activity is due to damage to the liver cells. The intensified activities of AST, LDH and HBD may be associated with the dermatomyositis. The decline in albumin levels led to an alleviation of the oncotic pressure of the blood plasma and to an increase in the permeability of the capillary endothelium for fluid and protein. This resulted in the production of exudation in the skin lesions and inside the body cavity.

Harbell et al. (1979) indicated similar decreases in RBC, PCV, Hb, TP and ALP in the coho salmon, *Oncorhynchus kisutch*, in an experimental infection with a highly virulent strain of *Vibrio anguillarum*, and the same author described an increase in LDH and AST levels - another finding similar to ours. Also recorded by Waagbø et al. (1988) in Atlantic salmon suffering from coldwater vibriosis ("Hitra disease") were lower levels of TP, CREA, TG, CHOL and ALP activity and increased activities of ALT and AST in the serum. Racicot et al. (1975) drew attention to the increased activities of LDH, AST and ALT in *Aeromonas* infections in the rainbow trout. A decrease in total protein and changes in the blood serum protein fractions separated by polyacrylamide gel electrophoresis were described in *Salmo trutta* (L.) and the Atlantic salmon (*Salmo salar* L.), diseased with ulcerative dermal necrosis (UDN), by Mulcahy (1969, 1971). A decline in total protein is described by the same author also for a salmon with simple fungal infection (*Saprolegnia ferax*), for salmon fingerlings with fin rot and for salmon fingerlings with furunculosis.

The results presented here, supported by the findings of the quoted authors, have shown that certain infectious skin lesions are accompanied by a number of changes in peripheral blood, including mainly a reduction of the level of total protein and changes in blood protein fractions. As suggested by the significant differences between the diseased and healthy fish as to the red blood cell corpuscle parameters and as to a whole range of various biochemical characteristics, research into the area of clinical haematology and biochemistry may contribute to an enhancement of knowledge in humoral pathology, which is needed in order to improve diagnostic work and to select the best preventative and therapeutic procedures.

## Změny v krvi pstruha duhového, *Oncorhynchus mykiss* (Walbaum) při aeromonádové ulcerózní dermatitidě

Předmětem studia byly hematologické a biochemické ukazatelé periferní krve pstruhů duhových při hromadném infekčním kožním onemocnění. U nemocných ryb došlo oproti rybám bez klinických příznaků onemocnění k signifikantnímu ( $P = 0.01$ ) snížení počtu erytrocytů (0,55 vs. 0,95 T/L), hematokritu (0,200 vs. 0,344 l/l) a hemoglobinu (36,8 vs. 65,9 g/L). Z biochemických ukazatelů krevní plazmy bylo zaznamenáno statisticky významné ( $P = 0.01$ ) snížení celkové bílkoviny (10,7 vs. 35,6 g/L), cholesterolu (0,74 vs. 5,75 mmol/L), triacylglycerolů (0,37 vs. 2,37 mmol/L) a kalcia (2,09 vs. 3,25 mmol/L). Na stejné hladině významnosti byla zvýšena hladina močoviny (1,7 vs. 0,5 mmol/L) a aktivita glutamyltransferázy (0,48 vs. 0,09  $\mu$ kat/L). Signifikantní ( $P = 0.05$ ) snížení aktivity bylo zaznamenáno u laktátdehydrogenázy (40,98 vs. 15,85  $\mu$ kat/L). Elektroforézou na agarosovém gelu byl zjištěn především pokles albuminů.

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### References

- HARBELL, S.C., HODGINS, H.O., SCHIEWE, M.H. 1979: Studies on the pathogenesis of vibriosis in coho salmon *Oncorhynchus kisutch* (Walbaum). *Journal of Fish Diseases* 2: 391-404
- MULCAHY, M.F. 1969: Serum protein changes in UDN-infected Atlantic salmon a possible method of diagnosis. *J. Fish Biol.* 1:333-338
- MULCAHY, M.F. 1971: Serum protein changes associated with ulcerative dermal necrosis (UDN) in the trout *Salmo trutta* L. *J.Fish Biol.* 3:199-201
- RACICOT, J.G., GAUDET, M., LERAY, C. 1975: Blood and liver enzymes in rainbow trout (*Salmo gairdneri*) with emphasis on their diagnostic use: Study of  $\text{CCl}_4$  toxicity and a case of *Aeromonas* infection. *J. Fish Biol.* 7:825-835.
- WAAGBØ, R., SANDNES, K., ESPELID, S., LIE, Ø. 1988: Haematological and biochemical analyses of Atlantic salmon, *Salmo salar* L., suffering from coldwater vibriosis ("Hitra disease"). *Journal of Fish Diseases* 11: 417-423.