

# Innate immunity in yellow forms inheritance of rainbow trout (*Oncorhynchus mykiss*)

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## Abstract

The aim of the present study was to investigate possible pleiotropic effects of the genotypes controlling the albino and palomino coloration on innate immunity in rainbow trout. The innate immune defence in fish consists of a large number of humoral and cellular factors. In our study we examined the metabolic activity of blood phagocytes by their respiratory burst activity (RBA), lysozyme and ceruloplasmine activity in serum and total Immunoglobulin (Ig) levels in serum. The results showed that the wild coloured rainbow trout has a higher metabolic activity of blood phagocytes, compared to the albino and palomino coloured trout. The lysozyme and ceruloplasmine activity in serum and total Ig levels in serum indicated a similar pattern. The results showed that lysozyme and ceruloplasmine activity and total Ig levels in serum were statistically significantly higher in wild coloured trout, compared to albino and palomino trout. The current study strongly demonstrated that genotypes controlling coloration determined the innate immunity in fish

**Key words:** rainbow trout, yellow forms, innate immunity.

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## Introduction

Colour mutations are a common in vertebrates, especially in fish. Additionally, albino, blue, brown, red and skin transparency mutations are reported in many fish species. In rainbow trout the following colour variants are known: albino, golden, yellow, palomino, blue and green, with golden and yellow probably representing the some colour phenotype. Kohlmann and Fredrich [1] reported highly significant lower survival in albino fry. Also the growth of albino fish was lower than in wild coloured fish from fry till sexual maturity. Clark [2] found yellow coloration to be controlled by a recessive allele and observed that the yellow fish were less active, slower growing and more sensitive to the light than the wild coloured ones. Yellow and wild-coloured rainbow trout were used in crossbreeding experiments to determine the pattern of yellow colour inheritance. The observed colour phenotypes and their relative frequency in different progeny groups can be explained by a system of two gene loci with two alleles each. Yellow colour (allele a) is caused by absence of the dominant allele A con-

trolling colour. Among the yellow fish (aa) the second gene locus allele B controls palomino and black eye colour. Albino and red eye colour (allele b) is caused by the absence of the dominant allele B controlling colour development. Many authors have shown that albinism in rainbow trout is due to an autosomal recessive allele [3]. The inheritance of golden and normally pigmented phenotypes is a single-locus trait with two alleles that act with additive effects – the homozygous genotypes GG and G'G' being normally pigmented and golden, respectively, while GG' shows the intermediate palomino phenotype.

The innate immune defence in fish consists of a large number of humoral and cellular factors, which play an important role as the first line of defence against a wide range of infective agents such as bacteria, viruses and parasites [4-7].

The aim of the present study was to investigate possible pleiotropic effects of the genotypes controlling the palomino and albino coloration on the innate immunity in rainbow trout (*Oncorhynchus mykiss*).

## Materials and methods

### Animals and experimental design

For this study healthy rainbow trout from three progeny families of known genotypes, originating from crossing designed to investigate the yellow colour inheritance: Wild (genotype Aabb), Palomino (genotype aaBb) and Albino (genotype aabb) were used. The fish, weighing 180-200 g, were held in plastic tanks in 14°C spring water and fed twice daily with commercial pellets. The blood was separated from 10 healthy fish of each group: Wild, Palomino and Albino. A portion of blood was used for cell separation by centrifugation in Gradisol G (Polfa) and remaining portion was used for serum separation.

### Assay procedure

The metabolic activity of blood phagocytes by their respiratory burst activity (RBA) stimulated by Phorbol myristate acetate (PMA, Sigma) was determined with a microcolorimetric method presented by Siwicki and Anderson [8].

The lysozyme activity in the serum was measured in a turbidimetric assay described by Siwicki and Anderson [8]. The standard used was hen egg white lysozyme (Sigma, USA) and a *Micrococcus lysodeicticus* (Sigma, USA) suspension in phosphate buffer.

The ceruloplasmine activity in the serum was determined spectrophotometrically according to the method presented by Siwicki and Studnicka [9]. The plates were then read in a micro-reader (MRX DYNEX Technologies, Worthing, UK) at a wavelength 540 nm.

The total immunoglobulin (Ig) levels in serum were measured by colorimetric Lowry micro methods (Sigma Diagnostic Kits) with modification presented by Siwicki and Anderson [8]. This technique consists at first in precipitating the total Ig from the serum with polyethylene glycol (10 000 kDa) and after centrifugation, removing the supernatant and determining the amount of remaining proteins by the micro reader at a wavelength 540 nm (MRX DYNEX Technologies, Worthing, UK).

The results were statistically analyzed with STAT-ISTICA PL (StatSoft, Cracow, Poland). Single-factor analysis of variance (ANOVA) and the Tukey test (when statistically significant differences were determined among groups  $p \leq 0.05$ ) were applied.

## Results and discussion

The colour genes controlling the palomino and albino phenotype in the spring spawning rainbow trout strain reared at the Department of Salmonid Research IFI in Rutki were found to have strong detrimental pleiotropic effects on growth and vitality [10]. The differences in mean body weights between albino, palomino, and wild coloured rainbow trout reached after 5 and 9 months of rearing were sta-

tistically highly significant with albino showing the poorest, palomino an intermediate and wild coloured rainbow trout the highest growth. An identical ranking of colour phenotypes was observed for survival. The immunological study showed that wild coloured rainbow trout has a higher blood phagocyte and lymphocyte activity compared to the albino and palomino coloured rainbow trout [14].

In our study we observed statistically significant ( $p < 0.05$ ) higher innate immunity parameters in wild coloured rainbow trout, compared to albino and palomino trout. The metabolic activity of blood phagocytes, lysozyme and ceruloplasmine activities in serum and total immunoglobulin (Ig) level in serum are presented in Table 1. The results of our study showed that wild coloured rainbow trout has significantly ( $p < 0.05$ ) higher phagocytic activity of blood leucocytes. But statistically different results between albino and palomino coloured fish were not observed. The lysozyme and ceruloplasmine activities and total Ig levels in serum indicated a similar pattern. The results showed that lysozyme and ceruloplasmine activity and total Ig levels were statistically significantly ( $p < 0.05$ ) higher in wild colored trout, compared to albino and palomino rainbow trout. Siwicki *et al.* [11] described that albino and palomino colored rainbow trout has lower cell-mediated immunity compared to the wild coloured trout and suggested that genotypes controlling coloration determined nonspecific cellular defense mechanisms in rainbow trout. In this study, we noted pleiotropic effects of the genotypes controlling the palomino and albino coloration on the innate immunity presented by cellular and humoral factors which play an important role as the first line of defense against a wide range of infective agents. The results showed that the wild colored fish has a higher innate immunity. The current study strongly demonstrated that genotypes controlling coloration determined the innate immunity in lower vertebrates.

**Table 1.** The metabolic activity of blood phagocytes (RBA), lysozyme and ceruloplasmine activities in serum and total immunoglobulin (Ig) levels in serum in wild, palomino and albino coloured rainbow trout ( $n + 10$ ; mean  $\pm$  SD; \* statistically significant to wild color fish  $p < 0.05$ ).

Immunological parameters:	Male colour (known genotype):		
	Wild (Aabb)	Palomino (aaBb)	Albino (aabb)
RBA (OD 620 nm)	0.47 $\pm$ 0.04	0.39 $\pm$ 0.05*	0.36 $\pm$ 0.05*
Lysozyme activity (mg/l)	12.8 $\pm$ 1.0	9.40 $\pm$ 1.2*	8.70 $\pm$ 1.5*
Ceruloplasmine activity (IU)	86.5 $\pm$ 2.5	80.5 $\pm$ 2.0*	78.0 $\pm$ 3.5*
Total Ig level in serum (g/l)	9.80 $\pm$ 0.4	8.50 $\pm$ 0.5*	7.90 $\pm$ 0.6*

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