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INFLUENCE OF O-ANTIGEN *Aeromonas salmonicida* ON NON-SPECIFIC AND SPECIFIC IMMUNE RESPONSES IN SIBERIAN STURGEON, *Acipenser baeri* Brandt

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ABSTRACT. Investigations were carried out on Siberian sturgeon (*A. baeri* Brandt) fry rearing in recirculation systems conditions. Outer membrane antigen of *A. salmonicida* was administered in the single intra peritoneal injection . Test of NBT reduction (both cytochemical and spectrophotometric assay), lisozyme activity, phagocytic ability of PMN neutrophils, levels of γ– globulins and total protein, titres of natural and specific antibodies were determined during six weeks after injection. The results obtained showed significant elevation of the cellular and humoral response indices; production of specific antibodies began in the first week yet.

Key words: SIBERIAN STURGEON, INTENSIVE BREEDING, *Aeromonas salmonicida*, CELLULAR AND HUMORAL IMMUNE RESPONSE

INTRODUCTION

Intensive breeding of sturgeons has been developing since a few years, resulting in growing interest in the problem of effective prophylaxy against epizootic diseases, which at high fish densities may cause considerable losses. Practical experience with teleost fish has revealed that immunoprophylactic methods were the most effective in preventing fish diseases (Siwicki 1990, Grawiński 1993). Their application, however, must be preceded by immunophysiological studies. Study of immunoreactivity of sturgeons enables establishment of sensitive methods to determine changes in the fish health and their potential resistance. The first study upon effects of enter membrane antigen of *A. salmonicida* and an appropriate multi-antigen vaccine (containing the whole killed *A. salmonicida* bacteria among others) were performed in bester F_3 (Kolman et al. 1999). Siberian sturgeon is certainly the most popular species in European aquaculture. In view of this, studies were undertaken on immune response of *A. baeri* to the administration of *Aeromonas salmonicida* O-antigen.

MATERIAL AND METHODS

Studies were carried out on Siberian sturgeon fry aged 0+, initial mean body weight of 100 g. The fry was divided into 2 groups, 60 fish in each, and placed in separate rearing tanks functioning in a water recirculation system, with water treatment devices installed in (Kolman 1992). The experimental fish were given a single intraperitoneal injection of *A. salmonicida* O-antigen, at the dose of 10 mg in 0.2 ml PBS/fish. The control fish were injected PBS solution only. *A. salmonicida* O-antigen was supplied by the National Fish Health Research Laboratory in Learnesville, WV 25 430, USA. Blood was collected from live fish using a heparinized needle, poured into Eppendorf's test tubes. Blood samples were collected for the period of 6 weeks at weekly intervals, from 10 fish each time in each group. Before blood sampling, the fish were anaesthetized with „Propiscin".

Metabolic activity of the leucocytes was determined using the spectrophotometric test of nitrotetrazolium blue salt reduction (NBT) (Studnicka et al. 1985). Percentage of polymorphonuclear (PMN) NBT-positive cells was determined using the cytochemical method (Szczylik et al. 1979). The preparates were preserved with ethyl alcohol, and the cells stained using safranine solution (Van Oss et al. 1973). Phagocytar activity of the leucocytes was determined with the method described by Avtalion and Shahrabani (1975) and O'Neill (1985), and expressed as phagocytar index (PhI). A suspension of *Staphylococcus aureus* 209 P was used. The plasma was isolated centrifuging complete blood for 10 at 5000 rot/min. (centrifuge type 317 „Mechanika Precyzyjna", produced in 1982, Warsaw) and stored in -20^0C until analyses. Lysozyme activity (LZM) in blood plasma was determined with turbidimetric method described by Studnicka et al. (1986), using *Micrococcus lysodeikticus* (Sigma) suspension in phosphate buffer . Extinction was determined using spectrophotometer Eskalal, Smith Kline Instruments, USA. Egg white lysozyme (Sigma) was used as the standard. Titre of natural antibodies (hemagglutinins) in the plasma was determined with the micromethod (Siwicki and Buczek 1987). Titre of specific antibodies in the serum was assayed with method of microagglutination (Cossarini-Dunier et al. 1987). Levels of total protein and of γ-globulin fraction in the plasma were determined according to Siwicki and Anderson (1993).

RESULTS

1 week after *A. salmonicida* O-antigen administration to Siberian sturgeon there was a statistically significant increase ($p \leq 0.05$) of the % of PMN NBT-positive cells

compared with the control group: from a mean level of 38 % to about 50 % (fig. 1). The trend was maintained until the end of the experiment, and in weeks 3, 4 and 6 the differences between the experimental group and the control were statistically significant. NBT-reduction ability in the blood (optical density) also increased in the experimental group compared to the control, and statistically significant differences were observed in weeks 1 (increase from 0.8 to 1.2 mg/ml), 2 (from 0.82 to 1.22 mg/ml), 4 (from 1.16 to 1.35 mg/ml) (fig. 2). Increase of lysozyme activity ($p \leq 0.01$) was observed in weeks 1 and 6 after the injection, respectively from 2.4 to 4.3 μg/ml and from 7.3 to 10 μg/ml (fig. 3). Moreover, after 1 week there was a highly significant ($p \leq 0.001$) increase of total protein level, from 19.5 to 24 g/l (fig. 4), and in week 2 - of γ-globulin in blood plasma (fig. 5), from 12.6 to 17.3 g/l. Level of this protein fraction remained very high compared with the control, especially in weeks 2, 4 and 5. Maximal levels of total protein and γ-globulins were observed in week 3: about 34 and 18.3 g/l respectively. In week 6 after O-antigen injection, phagocytar index increased in the experimental group compared to the control, from 15 to 26 (fig. 6). O-antigen administration resulted also in a statistically highly significant ($p \leq 0.001$) increase of natural antibody titre (haemagglutinins) in the experimental group, from the mean of 2.8 to 5.26 (fig. 7), as well as of specific antibody titre from a zero level to 3.6 in week 1 (fig. 8). Antibody titre increased until week 3, when maximal levels were observed (respectively 6.2 for natural and 5.4 for specific), and then decreased gradually.

DISCUSSION

Knowledge on specific and non-specific immune responses of cellular and of humoral type is far less comprehensive in fish than higher animals (Ellis 1978, 1982, Siwicki 1990, Vichman 1996). Chondrostean sturgeons have some unique characteristics of immune system (Fange 1986, Sigel et al. 1986, Lundqvist et al. 1993, 1998), but it seems that they posses all types of defence response like other vertebrate (Lukyanenko 1971, 1989, Vikhman 1996, Kolman et al. 1999). As shown by other organisms, specific responses may be directly related with phagocytosis, intracellular processing of pathogens in phagocytes and antigen (fragment of pathogen) presentation. An interaction between antigen-presenting cell and T-lymphocytes (CD4) initiate synthesis of interleukins the last cell, which liberate interleuking capable of simulating (among other cells) B-lymphocytes (CD. 19) to differentiation and producing antibodies (Falkiewicz and Liberek 1996a, b, Jakóbisiak 1998). Neutrophilic granulo-

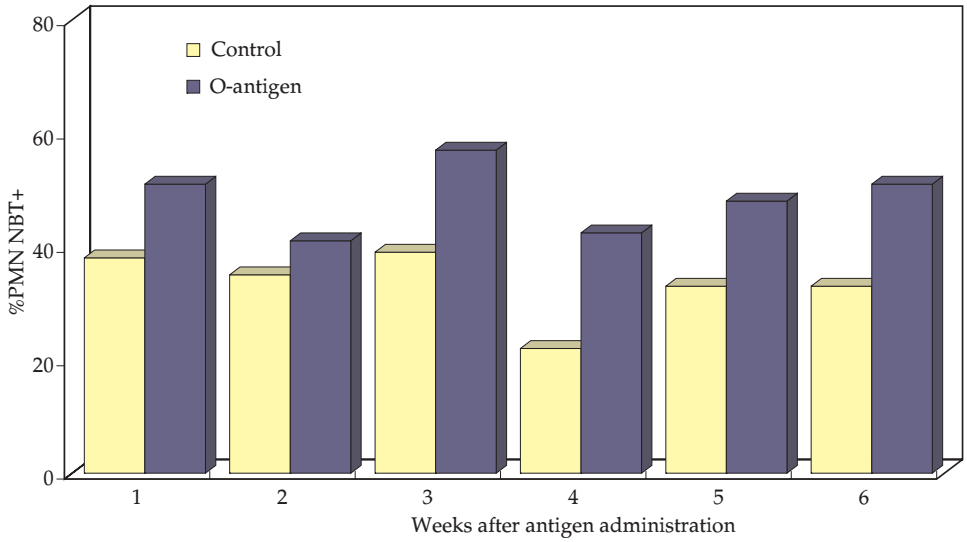

Fig. 1. Percentage of NBT-positive cells in blood of Siberian sturgeon (*A. baeri* Brandt) after administration of *A. salmonicida* O-antigen.

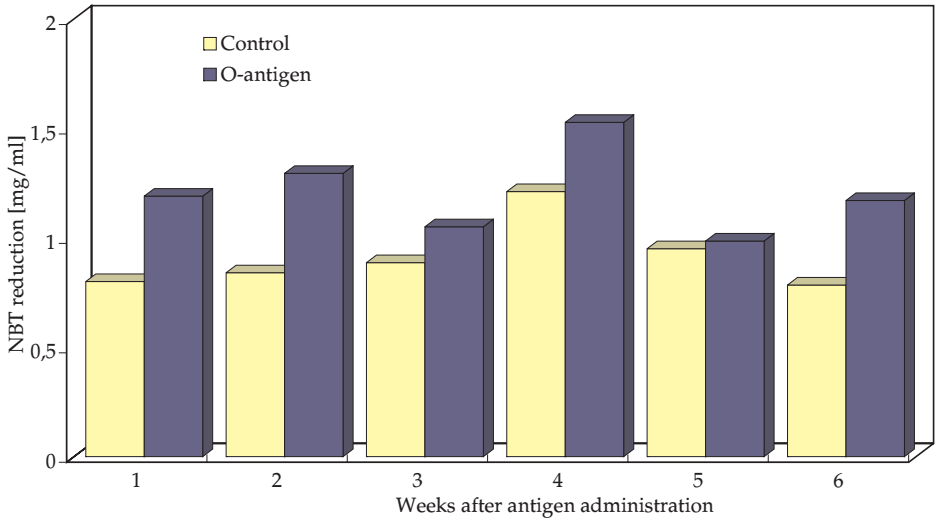

Fig. 2. Increase of NBT-reduction ability in blood of Siberian sturgeon after intraperitoneal injection of *A. salmonicida* O-antigen

cytes are engaged in presenting the antigen to T-lymphocytes (Falkiewicz and Liberak 1996a, b). We showed that bacterial antigen administration to Siberian sturgeon stimulated metabolic activity (Figs 1-3), and so did phagocytic capacity of neutrophils. Yhe levels of products of hyphoidal effector cells were also elevated (Fig. 7, 8).

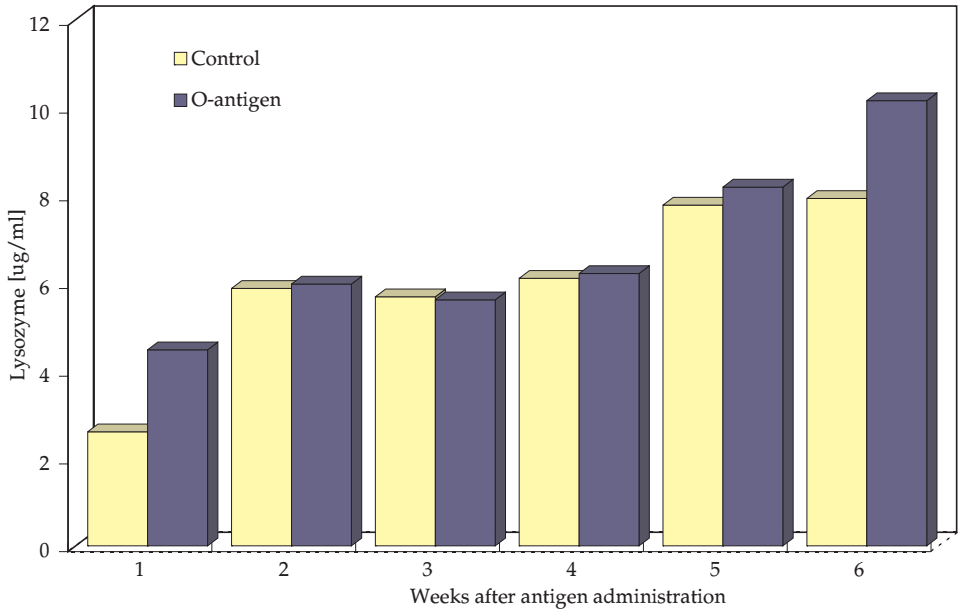

Fig. 3. Increase of lysozyme activity in blood serum of Siberian sturgeon after intraperitoneal injection of *A. salmonicida* O-antigen

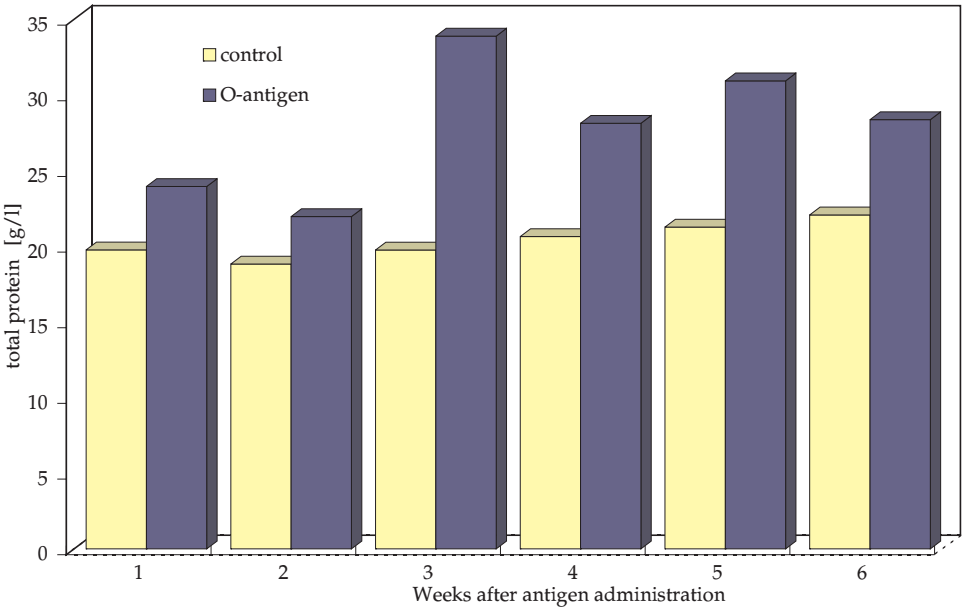

Fig. 4. Increase of total protein in blood serum of Siberian sturgeon after intraperitoneal injection of *A. salmonicida* O-antigen

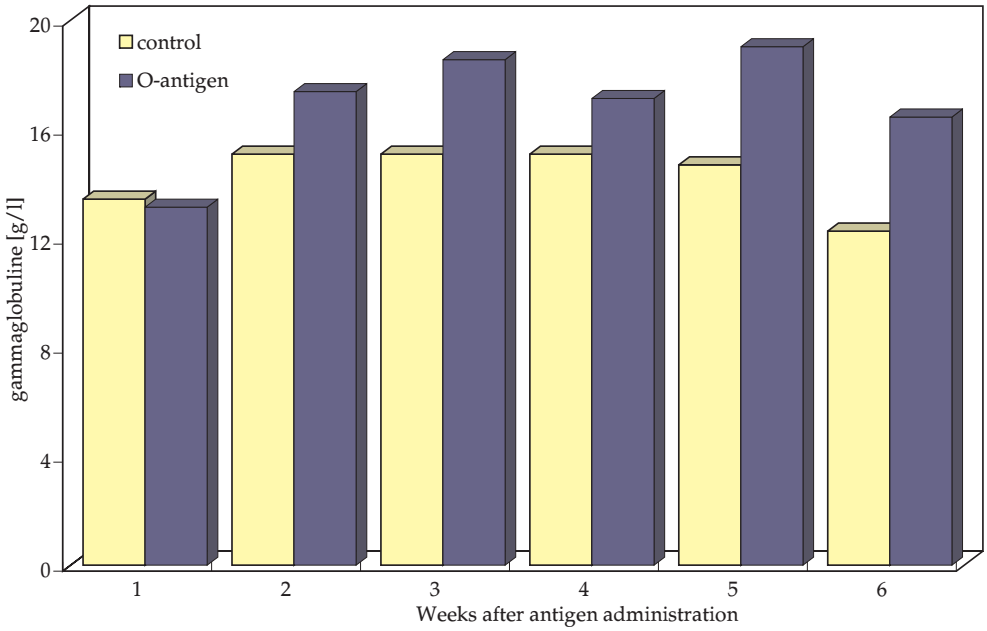

Fig. 5. Increase of γ-globulin in blood serum of Siberian sturgeon after intraperitoneal injection of *A. salmonicida* O-antigen

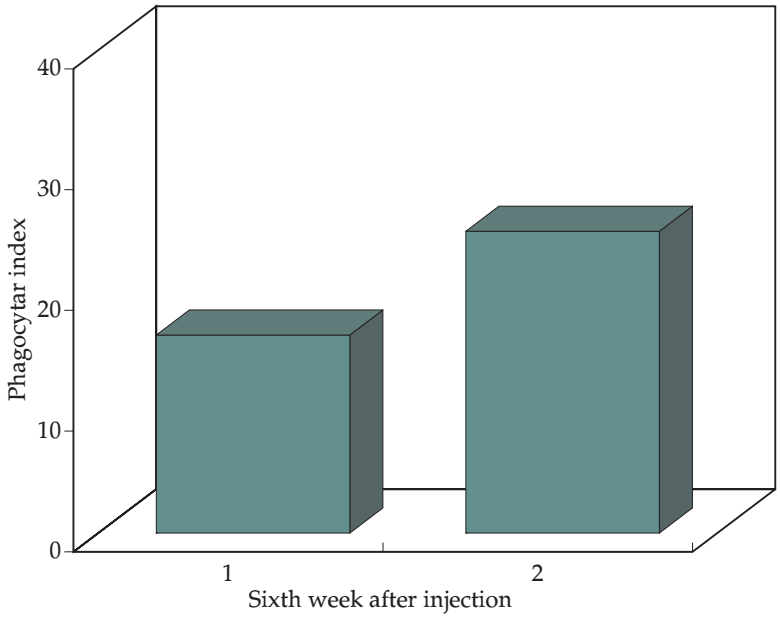

Fig. 6. Increase of phagocytar index in blood serum of Siberian sturgeon after intraperitoneal injection of *A. salmonicida* O-antigen

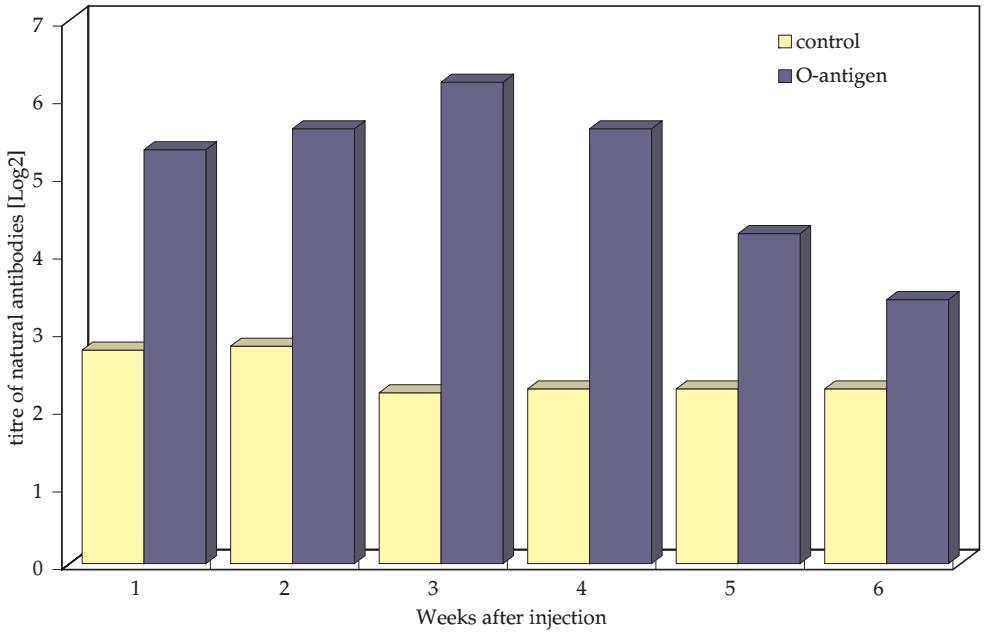

Fig. 7. Increase of the titre of natural antibodies in blood serum of Siberian sturgeon after intraperitoneal injection of *A. salmonicida* O-antigen

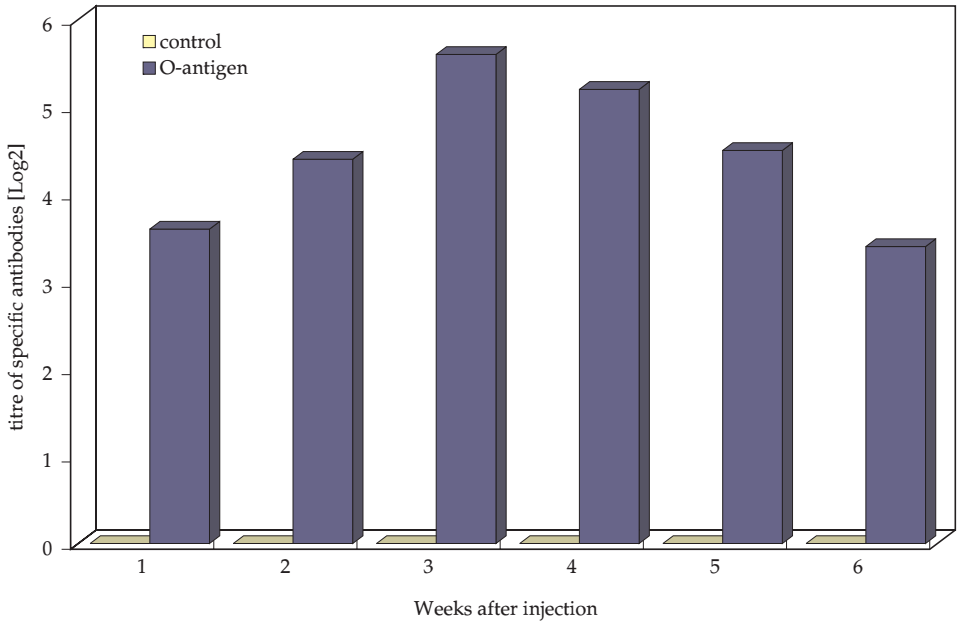

Fig. 8. Increase of the titre of specific antibodies in blood serum of Siberian sturgeon after intraperitoneal injection of *A. salmonicida* O-antigen

Since week 2, the tendencies of changes in protein and γ-globulin levels in the blood serum, and of the titre of natural and specific antibodies (Fig. 4, 5, 7, 8) took place in a synchronic way, suggesting that these antibodies may be the components of γ-globulin serum protein fraction, similarly as in vertebrate fish (Siwicki and Buczek 1987, Siwicki 1990). It is postulated that presence of natural antibodies in an organism may reflect their immunological readiness (Siwicki and Buczek 1987, Diwicki et al. 1994, Siwicki and Studnicka 1986). The results obtained with Siberian sturgeon (Fig. 8) confirm the thesis that animals possessing natural antibodies capable of more quick producing specific antibodies after immunization (Lukyanenko 1971). Class M pentameric antibodies are sythesized in sturgeons (Adkison et al. 1986, Partula and Charlemagne 1993, Landqvist et al. 1996, 1998). Immunoglobulins of this class are produced in verterbrate as the first during ontogenesis as well as an immune response. As far as regards the sturgeons, the fact that specific antibodies were produced already one week after antigen administration, and that their production increased gradually until week 3 suggests that there must be some mechanisms modulating epitopes affinity of the antibodies towards a strange antigen or stimulating elevation of their synthesis (Kolman et al. 1999).

Based on the experiments it can be concluded that administration of *A. salmonicida* O-antigen to Siberiam sturgeon, similar as in bester (Kolman et al. 1999), stimulated immunophysiological mechanisms which determined the increase of non-specific cell and humoral immunity response and induced specific response.

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STRESZCZENIE

WPŁYW O-ANTYGENU *Aeromonas salmonicida* NA SWOISTĄ I NIESWOISTĄ ODPOWIEDŹ ODPORNOŚCIOWĄ U NARYBKU JESIOTRA SYBERYJSKIEGO (*Acipenser baeri* Brandt)

Badania prowadzono na narybku jesiotra syberyjskiego (*A. baeri* Brandt) podchowywanym w warunkach zamkniętego obiegu wody. Otoczkowy antygen *Aeromonas salmonicida* wprowadzono w jednorazowej iniekcji dootrzewnowej. W ciągu sześciu tygodni po podaniu antygenu oznaczano odsetek komórek PMN NBT-dodatnich, zdolność redukcji NBT, IF, aktywność lizozymu, poziom białka całkowitego i frakcji γ – globulinowej, miano przeciwciał naturalnych i swoistych. Stwierdono wzrost badanych wskaźników nieswoistej odporności komórkowej i humoralnej. Wyprodukowanie przeciwciał swoistych stwierdzono już w I tygodniu po podaniu antygenu.

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