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NONSPECIFIC CELLULAR AND HUMORAL DEFENCE MECHANISMS IN PIKEPERCH (*SANDER LUCIOPERCA*) GROWN IN AN INTENSIVE SYSTEM OF CULTURE

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ABSTRACT. Monitoring the condition of fish and protecting them from diseases are two of the most important aspects of prevention in intensive fish culture. Nonspecific cellular and humoral defense mechanisms were studied for the first time in pikeperch (*Sander lucioperca*) grown in an intensive system of culture using two sizes of fish weighing approximately 50 g and 500 g. The respiratory burst activity and potential killing activity of blood phagocytes and pronephric macrophages were measured in each group. The proliferative response of lymphocytes stimulated by mitogen ConA and LPS was also examined in the two groups of fish. The lysozyme and ceruloplasmine activities in the plasma and the total protein with Ig levels in the serum were also determined. These results will be used in controlling the influence of diets and xenobiotics on nonspecific cellular and humoral defense mechanisms and for infectious disease prevention in pikeperch raised in different systems of culture.

Key words: PIKEPERCH (*SANDER LUCIOPERCA*), AQUACULTURE, NONSPECIFIC CELLULAR AND HUMORAL IMMUNITY

INTRODUCTION

The immune system is a highly evolved system that functions to provide organisms with the ability to resist pathogenic agents, destroy neoplastic cells and reject nonself components. Generally, defense mechanisms are characterized by two pathways. The first is nonspecific immune responses mediated by mononuclear (MN) phagocytes (blood monocytes and tissue macrophages) and granulocytes which can recognize foreign material nonspecifically. The second is specific immune responses mediated by several effector lymphocytes which are directed and specific for an eliciting antigen. The nonspecific defense mechanisms are comprised of two types of responses: cellular with phagocytosis and humoral presented by lysozyme, C-reactive protein (CRP), complement levels and inflammatory responses. Lysozyme and CRP are important in the initial destruction of invasive agents and in some cases can serve as an early biomarker indicating the deterioration of some protective mech-

anisms. Several aspects of defense mechanisms amenable for use as specific biomarkers are conserved phylogenetically. Nonspecific cell-mediated and humoral responses are a very important part of immunological mechanisms and perform a key role in the regulation of immune response. Assays of these responses can provide useful measures of immunosuppression induced by environmental contamination, stress and system of culture (Wedemeyer 1996).

Defense mechanism parameters have been used in various laboratory and field experiments to analyze the influence of different systems of culture, diets and xenobiotics on nonspecific cellular and humoral defense mechanisms, specific immune responses and disease resistance in fish.

The present study examined the nonspecific cellular and humoral defense mechanisms of pikeperch (*Sander lucioperca*) grown in an intensive system of culture; this species is a potential candidate for aquaculture (Kestemont and Mélard 2000).

MATERIAL AND METHODS

The pikeperch were reared in six circular (71 cm in diameter, 72 cm deep) fiberglass tanks with a water volume of about 200 liters each. The tanks were part of a recirculation system equipped with biological and mechanical filters. The water temperature was maintained at a constant level of about 22°C. Flow rates ranged from 3 to 6 l min^{-1}. The oxygen concentration did not fall below 7.5 mg O_2 l^{-1} (input) and 4.5 mg O_2 l^{-1} (output). The concentration of total ammonia nitrogen (TAN = NH_4^+-N + NH_3-N) did not exceed 0.4 mg TAN l^{-1} in the outflow. Twenty-four hour illumination was applied, and the light intensity above the surface of the experimental tanks was 30-80 lux.

The fish were fed for 18 h per day (0900 – 0300 h) with a commercial trout feed (TROUVIT, Nutreco Aquaculture, Holland) using automatic band feeders manufactured by Fish Technic, GmbH, Germany. Initially, NUTRA 0 (54% protein, 18% fat, 8% carbohydrates, 0.8-1.4 mm) was used, and finally the feed size was increased (CLASSIC 5; 46% protein, 14% fat, 21.5% carbohydrates, 5.0-5.5 mm). The initial daily feed ration was 3.5% of stock biomass, but during rearing it was reduced to 1.2%. Rations and food sizes were adjusted weekly to compensate for changes in fish biomass and size. The average fish biomass was about 9 kg m^{-3} at the beginning of the experiment and 25 kg m^{-3} at its conclusion.

In order to determine the nonspecific cellular and humoral defense parameters in intensive culture conditions, 20 healthy pikeperch of approximately 50 g and 20

healthy pikeperch of approximately 500 g were examined. The fish were anaesthetized with PROPISCIN, IFI Olsztyn, Poland (1.5 ml l^{-1}) (Kazuń and Siwicki 2001), weighed (W ± 0.1 g), blood was drawn from the caudal vein (Vacutainer systems) and the pronephros was removed for cell separation.

A technique developed by Secombes (1990) and modified by Siwicki et al. (1996) was used to study the metabolic activity of blood and pronephric phagocytes by their respiratory burst activity stimulated by Phorbol myristate acetate (PMA, Sigma). The plates were read with a microreader at OD 620 nm (MRX Dynex Technologies, GB).

A technique developed by Rook et al. (1985) and modified by Siwicki and Anderson (1993) was used to measure the potential killing activity of blood and pronephric phagocytes. The protocol used to obtain adherent cells was similar to that used in the respiratory burst activity. The phagocytes were stimulated with 100 µl of 0.2% NBT solution in PBS containing living *Aeromonas hydrophila* (1×10^8 ml^{-1}) which was added and incubated 30 min at 22°C.

The proliferative ability of the lymphocytes was determined by the MTT colorimetric assay method according to Mosmann (1983) and modified by Siwicki et al. (1996) for this fish species. The mitogens concanavaline A (ConA, Sigma) at a concentration of 64 µg ml^{-1} or lipopolysaccharide (LPS, Sigma) at a concentration of 160 µg ml^{-1} were used for the stimulation of lymphocytes.

The lysozyme activity in the plasma was measured in a turbidimetric assay presented by Siwicki and Anderson (1993). The standard used was hen egg white lysozyme (Sigma) and a *Micrococcus lysodeikticus* (Sigma) suspension in phosphate buffer.

The ceruloplasmine activity in the plasma was determined according to Siwicki and Studnicka (1986) which was modified for micro-methods. The plasma was incubated in microplates for 15 minutes in acetate buffer containing 0.2% p-phenylenediamine (PPD, Sigma). Some sodium azide (0.02 %) was used to stop the reaction. The ceruloplasmine activity was measured at 540 nm on the microreader.

Total protein level in serum was measured by the colorimetric Lowry micro method (Sigma, Diagnostic Kits).

Total immunoglobulin levels in the serum were also measured using the Lowry micro method modified by Siwicki and Anderson (1993). This method requires first precipitating the immunoglobulin out of the serum with polyethylene glycol (10 000 kDa).

In the statistical analysis, the means and standard deviations for all test values were derived using the Student *t*-test. The significance level used was P = 0.05.

RESULTS AND DISCUSSION

In the current study the level of some nonspecific cellular and humoral defense mechanisms is analyzed for the first time in healthy pikeperch from intensive culture. This basic examination provides very important information about physiological levels of nonspecific cellular and humoral protection against pathogens in pikeperch grown in intensive recirculation systems of culture. Two different sizes of fish were examined, and no statistically significant different levels were observed between the two groups of pikeperch of approximately 50 g and 500 g concerning nonspecific cellular and humoral defense mechanisms (data not presented). The metabolic activity of blood phagocytes and pronephric macrophages measured by respiratory burst activity are presented in Table 1. The respiratory burst activity observed in pronephric macrophages was higher in comparison to that of blood phagocytes. Similarly, the potential killing activity of pronephric macrophages was higher in comparison to that of the blood phagocytes of pikeperch (Table 1).

TABLE 1

Nonspecific cellular and humoral defense mechanism levels in healthy pikeperch (*Sander lucioperca*) grown in an intensive system of culture (N = 40)

Immunological parameters	Mean ± SD
Nonspecific cellular defense mechanisms:	
Respiratory burst activity of blood phagocytes (OD 620 nm)	0.36 ± 0.05
Respiratory burst activity of pronephric macrophages (OD 620 nm)	0.48 ± 0.04
Potential killing activity of blood phagocytes (OD 620 nm)	0.32 ± 0.05
Potential killing activity of pronephric macrophages (OD 620 nm)	0.46 ± 0.05
Lymphocyte proliferation stimulated by ConA (OD 620 nm)	0.48 ± 0.06
Lymphocyte proliferation stimulated by LPS (OD 620 nm)	0.32 ± 0.05
Nonspecific humoral defense mechanisms:	
Lysozyme activity in plasma (mg l^{-1})	45.5 ± 5.9
Ceruloplasmine activity in plasma(IU)	22.8 ± 3.5
Total protein level in serum (g l^{-1})	64.5 ± 4.0
Total Ig level in serum (g l^{-1})	16.0 ± 2.5

The activity of pronephric lymphocytes analyzed by the proliferative response on mitogens ConA and LPS are presented in Table 1. The analyses of the results showed that the proliferative response on the ConA mitogen was higher in comparison to that of LPS. These results suggest that the activity of T-lymphocytes observed in

pikeperch was higher in comparison to B-lymphocytes after stimulation by selected mitogens.

The nonspecific humoral defense mechanisms presented by lysozyme and ceruloplasmine activity in plasma and total protein with total immunoglobulin (Ig) levels in serum are shown in Table 1. The results indicate that the lysozyme and ceruloplasmine activity in the plasma of pikeperch is similar to that observed in rainbow trout (*Oncorhynchus mykiss*) (Siwicki et al. 1994). The results also indicate that the activity of lysozyme in the plasma of pikeperch is five to seven times higher compared to the that observed in European catfish (*Silurus glanis*) and eight to ten times higher in comparison with that in carp (*Cyprinus carpio*) (Studnicka and Siwicki 1986, Morand et al. 1998). The results of total protein and Ig are very difficult to interpret and to compare with other species, because commercial pellets with very restricted levels of protein and fat were used in the current study. However, the relation between total protein and Ig level is about 25% and is similar to that observed in healthy rainbow trout (Siwicki et al. 1994, 1996).

Basic information regarding cellular and humoral defense mechanisms in healthy pikeperch reared in intensive culture is very important in monitoring fish condition and in the early diagnosis of infection diseases. Actually, it is very difficult to compare these results, because little information exists regarding the metabolic ability or potential killing activity of blood and pronephric phagocytes. Additionally, the lymphocyte proliferation stimulated by selected mitogens provides researchers with preliminary information about the activity of T and B cells in this fish species and suggests that this activity is similar to that in salmonids. This information will simplify monitoring fish health and make possible the early diagnosis of infectious diseases. This also means that controlling the influence of diet, system of culture and environmental contamination on nonspecific defense mechanisms and disease protection will be possible.

REFERENCES

Kazuń K., Siwicki A.K. 2001 – Propiscin – a new safe anaesthetic for fish – Arch. Pol. Fish. 9: 183-190.

Kestemont P., Mélard C. 2000 – Aquaculture – In: Percid fishes, systematics, ecology and exploitation (ed. J.F. Craig). Blackwell Science, Osney Mead, Oxford OX2 0EL, UK, pp. 191-224.

Morand M., Siwicki A.K., Pozet F., Vinaize J.C., Keck N. 1998 – Effects of dimerized lysozyme (KLP-602) on the cellular and humoral defence mechanisms in sheatfish (*Silurus glanis*): *in vitro* and *in vivo* study – Vet. Research 30: 411-418.

Mosmann T. 1983 – Rapid calorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays – J. Immunol. Meth. 65: 55 – 63.
Rook G.A.M., Steele J., Dockrell H.M. 1985 – A simple method for the solubilization of reduced NBT, and its use as a calorimetric assay for activation of human macrophages by gamma – interferon – J. Immunol. Meth. 82: 161 – 167.
Secombes C.J. 1990 – Isolation of salmonid macrophages and analysis of their killing activity – Techn. Fish. Immunol. Meth. 1: 137 – 154.
Siwicki A.K., Anderson D.P. 1993 – Nonspecific defence mechanisms assay in fish. II. Potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and total immunoglobulin (T- Ig) levels in serum. Fish Diseases Diagnosis and Prevention's Methods. FAO-Project GCP/INT/526/JPN, IFI Olsztyn: 105 – 112.
Siwicki A.K., Anderson D.P., Rumsey G.L. 1994 – Dietary intake of immunostimulants by rainbow trout effects nonspecific immunity and protection against furunculosis – Vet. Immunol. Immunopathol. 41: 125-139.
Siwicki A.K., Miyazaki T., Komatsu I., Matsusato T. 1996 – *In vitro* influence of heat extract from firefly squid *Watasenia Scintillans* on the phagocyte and lymphocyte activities in rainbow trout *Oncorhynchus mykiss* – Fish Pathology 31: 1-7.
Siwicki A.K., Studnicka M. 1986 – Ceruloplasmin activity in carp (*Cyprinus carpio*) – Bamidgeh 38: 126-129.
Studnicka M., Siwicki A.K. 1986 – Lysozyme level in carp (*Cyprinus carpio*) – Bamidgeh 2: 22-26.
Wedemeyer G.A. 1996 – Physiology of fish in intensive culture systems – Chapman & Hall, New York, NY 10003, USA, 232 pp.

STRESZCZENIE

NIESWOISTE KOMÓRKOWE I HUMORALNE MECHANIZMY OBRONNE U SANDACZA (*SANDER LUCIOPERCA* (L.)) POCHODZĄCEGO Z INTENSYWNEGO PODCHOWU

Stałe monitorowanie stanu kondycyjnego i odporności na choroby jest istotnym elementem ochrony zdrowia ryb. Celem niniejszych badań było określenie nieswoistych komórkowych i humoralnych mechanizmów obronnych u sandacza pochodzącego z intensywnego podchowu. Badania tego typu nie były dotychczas prowadzone na tym gatunku. Materiał badawczy stanowiły dwie grupy wielkości ryb, o masie ciała 50 i 500 g. Analizowano aktywność fagocytów izolowanych z krwi. Równocześnie oznaczono odporność proliferacyjną limfocytów stymulowanych mitogenami ConA i LPS. W surowicy krwi oznaczono aktywność lizozymu i ceruloplazminy oraz poziom białka całkowitego i frakcji gamma-globuliny (Ig). Uzyskane wyniki będą stanowiły podstawę do określenia wpływu różnych systemów chowu ryb oraz zróżnicowanej diety na nieswoiste mechanizmy obronne i odporność przeciwzakaźną.

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