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SOME HUMORAL EFFECTS IN SIBERIAN STURGEON (*ACIPENSER BAERI* BRANDT) AFTER LYSOZYME DIMER APPLICATION IN BATH

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ABSTRACT. The present study was undertaken to evaluate humoral indices response in sturgeons reared under controlled conditions and treated with exogenous lysozyme dimer in immersion. Fish of average body weight 40±4 g were bathed for 30 minutes in 0.1 mg l^{-1} of dimer (*Lydium* KLP-602). After the treatment, blood was sampled from the fish for 7 weeks, at weekly intervals. Lysozyme activity, the level of γ-globulins, ceruloplasmin, and total protein were measured in blood serum. Average body weight of fish increased 5 fold (to 210±12 g) during the experiment. In treated fish the bacteridytie activity of endogenous lisozyme, γ-globulins, and ceruloplasmin levels increased already in the first week after immersion by 27%, 40%, and 60% respectively. Increase of these parameters was observed until the end of the experiment. The results indicate that lysozyme dimer stimulated humoral responses in fish.

Keywords: CHONDROSTEI, HUMORAL IMMUNITY, γ-GLOBULINS, LYSOZYME, CERULOPLASMIN, *LYDIUM* KLP-602

INTRODUCTION

The role of lysozyme in non-specific defences (Aleksander 1985) in eucaryotic organisms is more important than predicted from its functioning as N-acetylmuramid-glycan-hydrolase (E.C. 3.2.1.17) (Benathen and McKenzie 1998). It is a cationic protein reacting with such biomolecules as nucleic acids, lipopolysaccharide bacterial toxins, and acid mediators of inflammatory process (Muraguchi et al. 1988, Takada et al. 1994). Lysozyme particles may polymerise, depending on their concentration, temperature, and pH of the environment (Sophianopoulos, Holde 1964), which results in a modification of it's effects. Pleiotropic effects of lysozyme dimer were observed in vaccine-induced immune reactions, and in pathogenesis of diseases of various animal species (Kiczka 1994, Borzemska et al. 1996, Mukezamfura et al. 1996, Dembiński and Mieczyńska 1997, Obmińska-Domoradzka et al. 1998, Sobolewski et al. 1997, Siwicki et al. 1997 a, b, 1998 a, b, Samorek-Salamonowicz et al. 1999, Klein et al. 1999, and others). It was proved that stimulating effects of lysozyme on phagocytic abilities were enhanced when it was applied as a dimer (Kiczka et al. 1994). In teleost fishes exogenous dimer compensated for reduced level of endogenous lysozyme and enhanced

other defensive functions (Klein et al. 1999, Siwicki et al. 1998 a, b, 1999 a). Exogenous lysozyme of natural origin and multifunctional modulator properties meets the requirements of non-specific prophylaxy in sturgeons under aquaculture conditions. The results of our previous studies showed that bacteriolytic activity of lysozyme depended on various factors, such as type of the environment, water temperature, body weight, and health of sturgeons (Kolman et al. 1998 a, b, c, 1999 a, b). These results, together with the data of other authors, inspired preliminary studies on the effects of lysozyme dimer upon selected humoral reactions in healthy sturgeon juveniles reared under optimum conditions.

MATERIAL AND METHODS

The study was performed on Siberian sturgeon fry reared in 500 l tanks, in water recirculation and purification system (Kolman 1992). Water temperature was 20±1^{o}C, water pH 7.5-8.0, and DO level at water outflow was over 6 mg l^{-1}. Concentration of total ammonia did not exceed 0.3 mg l^{-1}, and the level of nitrite was below 0.2 mg l^{-1}. The fish were reared in 4 tanks – 2 experimental groups, and 2 controls, 100 fish per tank. After 2 weeks of adaptation, when the fish reached body weight 40±4 g, the experimental groups were immersed for 30 minutes in 0.1 mg l^{-1} of lysozyme dimer (active agent of *Lydium* KLP-602, NIKA Health Products, Princeton, USA). Control fish were immersed in water without *Lydium*.

During the experimental rearing the fish were fed trout pellets Aller Kristall-3700, supplied continuously using conveyor belt feeders. Daily feeding rates were changed weekly, according to fish weight and feeding schedule (Kolman R. et al. 1996). Blood was collected weekly, for 7 weeks, from 15 randomly selected live fish of each group, anaesthetised using *Propiscin*. Blood serum was frozen and stored in –18^{o}C until analyses. Lysozyme activity, ceruloplasmin, γ-globulin, and total protein levels were evaluated according to the standard methods (Kolman et al. 1999 a, b). The results were shown as arithmetic means for the experimental groups and standard deviations. Significance of the differences between the groups was evaluated using t-Student's test, assuming p=0.05.

RESULTS

Fish body weight increased over 5-fold during the experiment (from 40±4 g to 210±12 g), and no differences in growth rate were observed between the groups

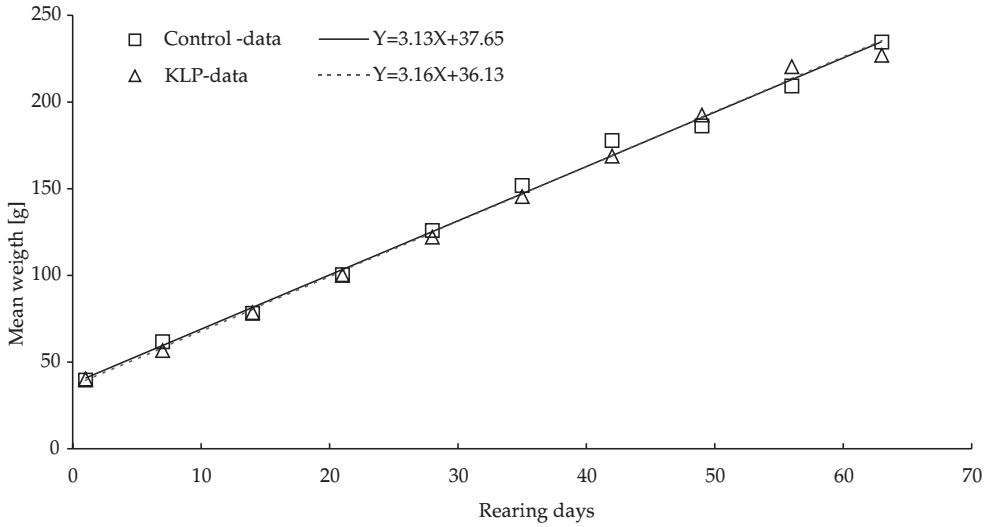

Fig. 1. Changes of average body weigth in control and experimental groups of Siberian sturgeon

(Fig. 1), although the values of proteins indices differed significantly. *Lydium* treatment resulted in significant increase (about 20%) of total protein level only in the first week of the experiment (p=0.05) (Fig. 2). In the subsequent weeks the differences of protein level between the treated fish and the control were insignificant. Also the level of γ-globulin increased in the first week (p=0.01) in the experimental group comparing to the control, from 16.2 to 20.6 g l^{-1} (27%), and the difference lasted in weeks III, VI, and VII (Fig. 3).

Treatment with the dimer resulted in 40% increase of bacteriolytic activity of lysozyme in the first week of the study. Higher lysozyme activity in the serum of treated fish (p=0.01) lasted until week V post immersion (Fig. 4).

Changes of ceruloplasmin level in sturgeons treated with lysozyme dimer were more dynamic. In the control fish, Cp concentrations were higher in the first 4 weeks (p=0.05) comparing to the last 3 weeks. In the experimental fish, Cp level increased by 60% (from 0.61 to 0.95 μmol l^{-1}) already in the first week. The highest differences between the groups were observed in weeks III, IV, and V of the experiment, when Cp levels in the treated fish exceeded control values by 200%, over 200%, and 400% respectively (Fig. 5). Maximum values of the indices in the experimental groups were observed at different time: total protein level in week I – about 45 g l^{-1}, LZM in week II – 2.6 mg l^{-1}, γ-globulin level in week III – 23 g l^{-1}, and Cp in week IV – 2.35 μmol l^{-1}.

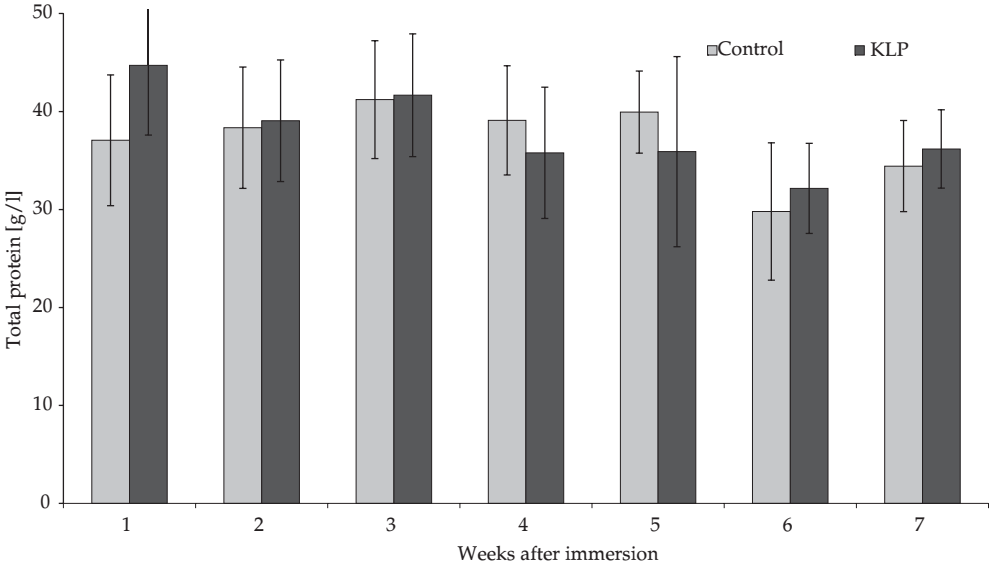

Fig. 2. Effects of lysozyme dimer upon total protein content in serum of Siberian sturgeon

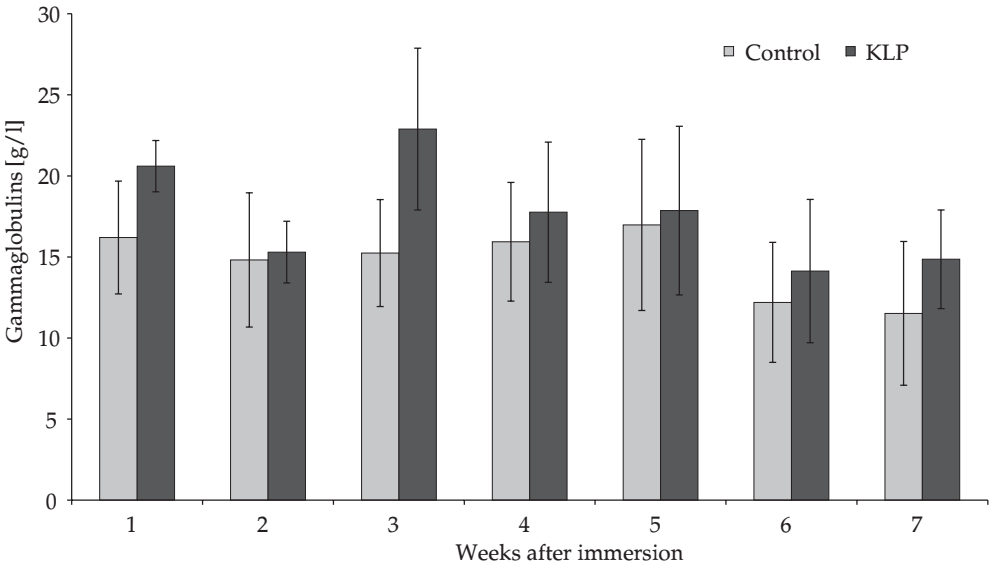

Fig. 3. Effects of lysozyme dimer upon γ - globulins level in serum of Siberian sturgeon

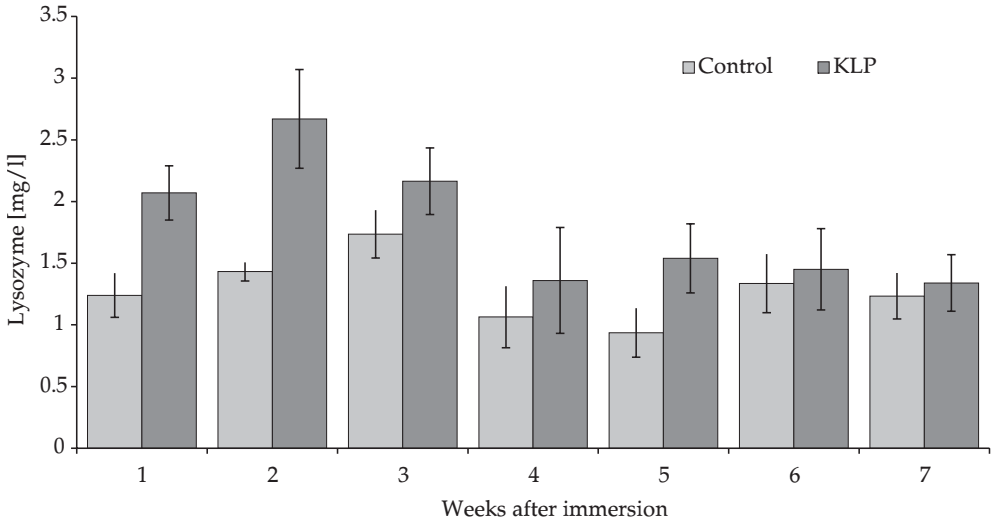

Fig. 4. Changes of bacteriolytic activity of endogenous lysozyme in serum of Siberian sturgeon

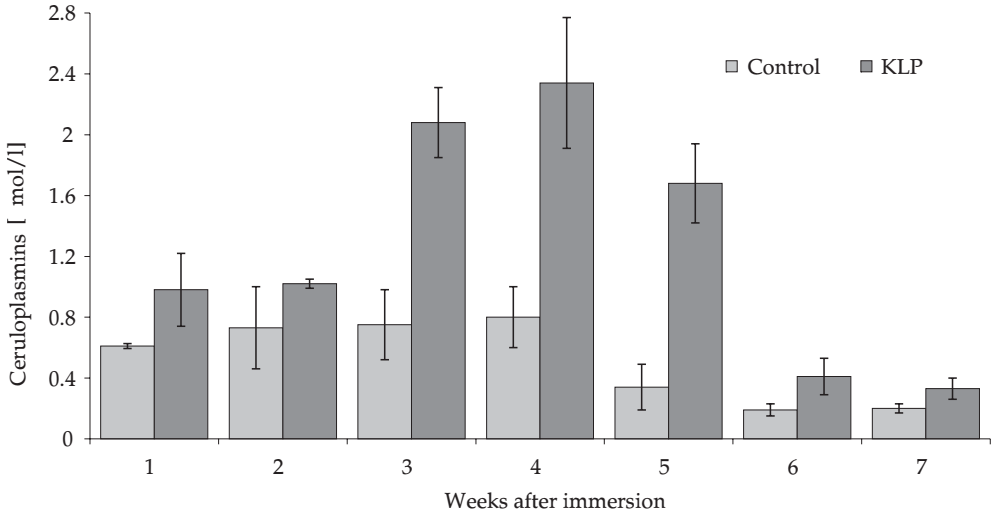

Fig. 5. Effects of lysozyme dimer upon ceruloplasmin level in serum of Siberian sturgeon

DISCUSSION

In sturgeons, concentrations of blood components correlated with body weight, this being related to the serum water content (Kolman et al. 1998 b, c, d). Thus, changes of the level of the measured parameters must be considered together with the

increase of body weight. Unlike other immunomodulators (Kolman R. et al. 1998, 1999), *Lydium* KLP-602 did not affect sturgeon growth (Fig. 1). This suggests that the observed differences did not result from basic growth-related metabolic processes, but from changes in immunological system of the fish. Changes in production of non-specific defensive factors and/or their elution from appropriate cells, were observed also after sturgeon treatment with bio-immuno preparation or 3-hydroxy-3-methylbutyrate (Kolman et al., in print), and other immunomodulators (Kolman et al. 1998 d). Their effects were accompanied by changes in white blood cell system, such as the increase of neutrophil, and large lymphocyte levels in circulating blood, and increased potential phagocytic ability of circulating polymorphonuclear cells (Kolman 1999, Kolman et al. 1999 c).

Treatment with exogenous lysozyme dimer resulted in an increase of bacteriolytic activity of sturgeon endogenous lysozyme (Fig. 4). Similar effect was observed also in terrestrial animals, and might have been related to the activation of phagocytes (Kiczka et al. 1994, Siwicki et al. 1997, 1998, 1999, Sobolewski et al. 1997). Studies carried out on rainbow trout showed also that *Lydium* stimulated activity of PMN and MN cells, this being observed as an increase of oxygen radicals production by blood phagocytes, increase of myeloperoxidase activity in neutrophilic granulocytes, and increased phagocytic ability (Siwicki et al. 1998 a). Moreover, activation of phagocytes by *Lydium* in piglets *in vivo* was accompanied by an increase of the level of IL-1, IL-2, TNFa, and IFNγ cytokines, and in rainbow trout *in vitro* – IL-1, IL-2, TNFα, and IL-6 (Klein et al. 1999, Siwicki et al. 1999). Activation of T lymphocytes by *Lydium* resulted in enhanced synthesis of IFNγ, and IL-2 (Klein et al. 1999).

Stimulation of antibody production by *Lydium* was observed in mice (Obmińska-Domoradzka et al. 1998), and in geese (Samorek-Salamonowicz et al. 1999). Mechanism of this response is induced by IL-6 (released from activated phagocytes) which stimulates, among others, differentiation of B lymphocytes into antibody-producing cells (Muraguchi et al. 1988, Kiczka 1994, Kiczka et al. 1994, Obmińska-Domoradzka et al. 1998, Klein et al. 1999). Similar effect was also observed in rainbow trout – the level of γ-globulin after intraperitoneal *Lydium* injection increased considerably in a dose-dependent way (Siwicki et al. 1998 a). These results suggest that the increase of γ-globulin level in the experimental sturgeons (Fig. 2) resulted from enhanced synthesis of natural antibodies (Kolman et al. 1999 a, b).

Similarly as in other animals, ceruloplasmin normally occurs in sturgeon blood, and its level depends on fish age, body weight, physiological state (Kolman et al. 1998 a, b, c, d). The increase of Cp level after treatment with *Lydium* (Fig. 5) might have been caused by activation of hepatocytes, and was probably related to IL-6 release from active phagocytes (Heinrich et al. 1990, Obmińska-Domoradzka et al. 1998). Statistically significant increase of Cp level in the control sturgeons in the first 4 weeks post immersion might be due to manipulation stress (Heinrich et al. 1990, Solter et al. 1991). The increase of the differences between experimental and control fish in weeks III and V after treatment suggests additive stimulation of hepatocytes by endogenous stimuli, generated by *Lydium* in neuro-hormonal and immunological systems.

Stimulatory effect observed in sturgeon encourages to undertake further studies on fish of various age and immune status, taking into consideration also other immunological indices.

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STRESZCZENIE

NIEKTRÓRE EFEKTY HUMORALNE U JESIOTRA SYBERYJSKIEGO (*ACIPENSER BAERI* BRANDT) PO PODANIU DIMERU LIZOZYMU W KĄPIELI

Celem badań było określenie reaktywności układu odpornościowego u jesiotrów po podaniu dimeru lizozymu egzogennego pochodzenia (*Lydium* KLP-602). Biopreparat podano jesiotrom o średniej masie ciała 40±4 g w jednorazowej kąpieli w ciągu 30 min. w roztworze o stężeniu 0,1 mg na 1 litr wody z basenów podchowowych. Ryby kontrolne kąpano w wodzie bez preparatu. Następnie przez 7 tygodni w odstępach tygodniowych od ryb przyżyciowo pobierano krew. W surowicy oznaczano aktywność lizozymu, poziom γ-globulin, ceruloplazminy i białka całkowitego. W czasie trwania eksperymentu jesiotry z grup doświadczalnych i kontrolnych charakteryzowały się podobnym tempem wzrostu, a ich średnia masa wzrosła ponad 5-krotnie. Maksymalne wartości badanych wskaźników odpornościowych u ryb doświadczalnych występowały w różnym czasie: w I tygodniu - białko całkowite, w II tyg. - LZM, w III tyg. - γ-globuliny, w IV tyg. - Cp. Wzrost poziomu białka całkowitego wynosił ok. 20 %, natomiast bakteriolityczna aktywność lizozymu w I tyg. - o ok. 40 % z utrzymywaniem się tendencji wzrostowej do V tyg. Poziom frakcji γ-globulinowej wzrósł statystycznie istotnie w I tyg. z 16,2 do 20,6 g/l (tj. o 27%), a następnie przewaga ta utrzymywała się w III, IV i VIII tyg. W skutek stresu manipulacyjnego poziom Cp u kontrolnych ryb był statystycznie istotnie wyższy w pierwszych 4 tyg. po kąpieli niż w pozostałych 3 tygodniach. Istotny wzrost poziomu Cp nastąpił już w I tyg. (o ok. 60 %), a największe różnice (o kilkaset %) pomiędzy rybami doświadczalnymi a kontrolnymi stwierdzano w III, IV i V tyg. Uzyskane wyniki wskazują na efekty stymulujące funkcje odpowiednich komórek efektorowych, odpowiedzialnych za syntezę i uwolnienie badanych czynników. Potwierdzają to wyniki badań prowadzonych na innych zwierzętach po podaniu *Lydium*.

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