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THE HUMORAL EFFECTS OF EPIN IN SIBERIAN STURGEON (*ACIPENSER BAERI* BRANDT)

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ABSTRACT. Immersing Siberian sturgeon fry of an average body mass of 40 ± 4 g in epin solution (0.1 mg l^{-1}) resulted in an increase of ceruloplasmin level over the entire experimental period ($P < 0.001$). The differences between the treated and control groups gradually increased reaching the maximum in the fourth and fifth weeks of the study when average levels of ceruloplasmin in the epin-treated group were 2.5 and 5.7-fold higher, respectively. In the control group, ceruloplasmin levels were considerably higher in the first four weeks in comparison to the last two weeks ($P < 0.01$). The bacteriolytic activity of lysozyme increased up to 50% compared to the control group ($P < 0.05$) in the first and fourth weeks. The sturgeon in the epin-treated group also showed from 10 to 65% higher concentrations of γ-globulin levels than the control fish, and in the second week the difference was statistically significant ($P < 0.01$). Simultaneously, total protein concentrations also increased ($P < 0.05$).

Key words: *ACIPENSER BAERI*, IMMUNOMODULATION, EPIBRASSINOLIDE, LYSOZYME, CERULOPLASMIN, γ-GLOBULIN, TOTAL PROTEIN

INTRODUCTION

The technology of intensive production of fish stocking material necessitates the development of efficient prevention methods against infectious and invasive diseases. Fish reared at high stocking densities are subjected to continuous stress that impairs their immune mechanisms (Ellis 1981, Tripp et al. 1987, Wendelaar 1997). The application of immunomodulatory drugs is a promising method of enhancing the immunological status of fish. A non-specific immune response in sturgeon, *Acipenser baeri* Brandt fry was successfully stimulated using substances such as ISK, levamisole, and glucan (Kolman H. et al. 1998a). It was also observed that they may positively affect fish growth and survival (Kolman R. et al. 1998, Kolman R. et al. 2000). Natural immunomodulators are of special interest (Kolman H. et al. 2000) since they are harmless to consumers. This group includes the plant growth regulator epibrassinopid, which is known by the commercial name of epin. Its positive effect has been observed in sturgeon early embryonic stages (Egorov 1997, Egorov and Vitvitskaya 1997). The aim of the present study was to assess the effect of epin on some immunophysiological indices and the growth rate of Siberian sturgeon fry.

MATERIAL AND METHODS

The study was carried out on three-month-old Siberian sturgeon fry with an average body weigth of 40 ± 4 g. They were reared in tanks in a water recirculation system. The fish were divided into four groups of 100 individuals each. Two of them were control groups and the other two were immersed in a 0.1 mg l^{-1} epin solution for 30 min. The fish were kept in a water recirculation system comprised of four 500 l tanks with a 1.0 m^2 bottom area and a full treatment system and automatic thermoregulation (Kolman 1992). During the experiment, the sturgeon fry were fed continuously with Aller Kristall-3700 trout starter using automatic band feeders. The daily feeding rates were changed weekly according to feeding diagrams (Kolman et al. 1996). Blood was sampled from the heart of 10 live fish from each tank using an insulin syringe. The fish were anaesthetized with a Propiscin solution applied to the gills.

The blood serum was analyzed for lysozyme bacteriolytic activity, ceruloplasmin level, and total protein concentration and its γ-globulin fraction. The lysozyme bacteriolytic activity was measured with the turbidimetric method (Studnicka et al. 1986) using a *Micrococcus lysodeicticus* suspension from the Sigma company. The ceruloplasmin level was evaluated according to Rice (1986), and the total protein concentration and its γ-globulin fraction were determined with micromethods described by Siwicki and Anderson (1993). The extinction values were determined using an Elisa Epoll spectrophotometer. The results are presented in graphs as arithmetic averages and standard deviations. The significance of the differences was evaluated with Student's t-test, at $P < 0.05$.

RESULTS

The experimental rearing of Siberian sturgeon fry lasted 63 days. Over this period, the average fish body weight increased to 234.8 ± 11.3 g in the control groups and to 245.7 ± 10.2 g in the epin group, which was 4.6% higher (Fig. 1). Differences in final fish body weight were already apparent in the first weeks of rearing; in the first week after treatment relative daily growth rates in the control and epin groups were 6.6 and 7.2%, respectively. However, the difference gradually decreased and at the end of the experiment the relative daily growth rate was equal to 1.5% in both groups of fish (Fig. 2).

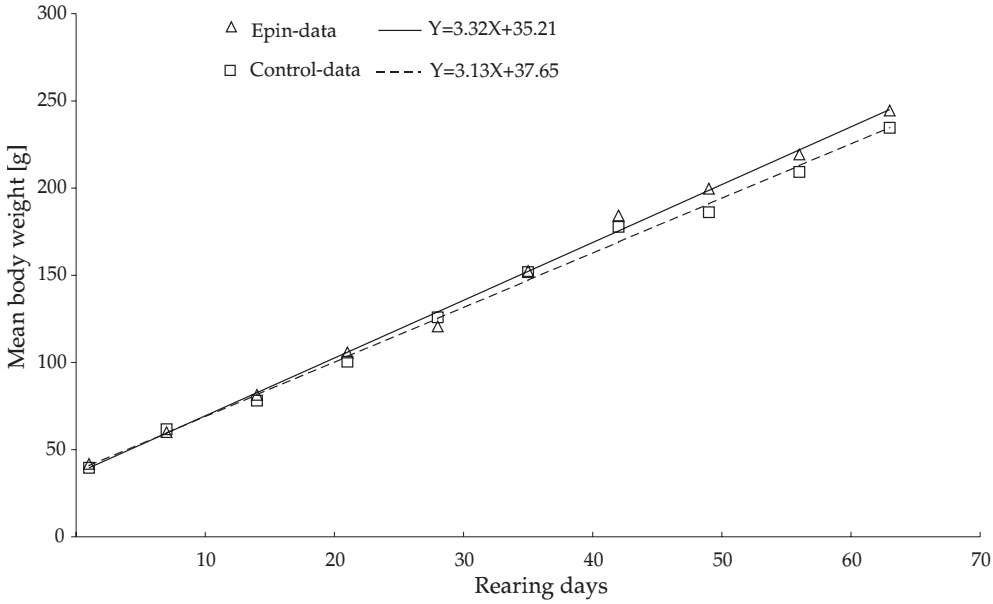

Fig. 1. Changes of body weight in control and experimental groups of Siberian sturgeon.

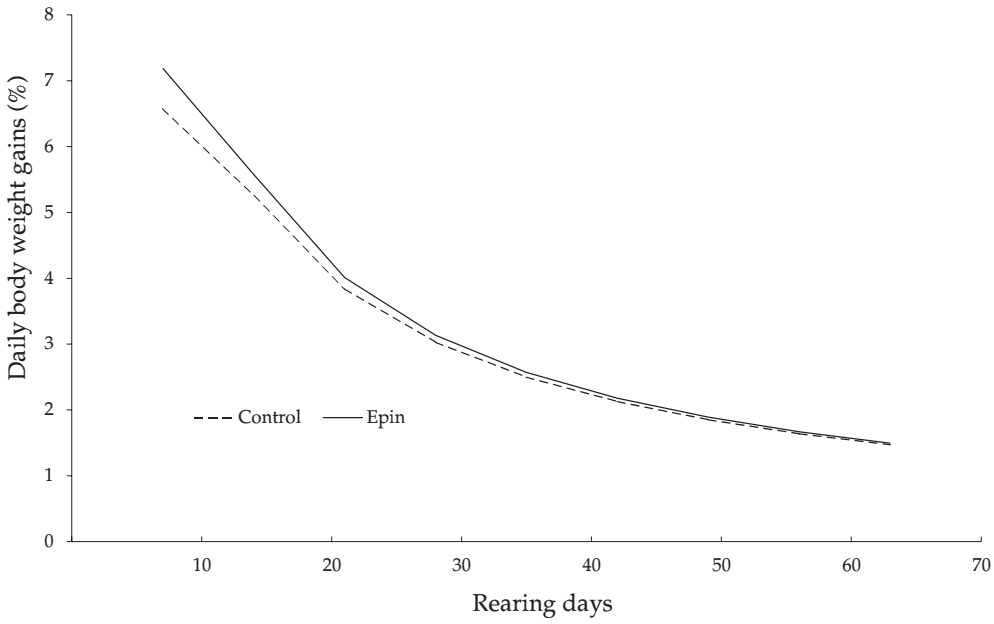

Fig. 2. Changes of growth rate in control and experimental groups of Siberian sturgeon.

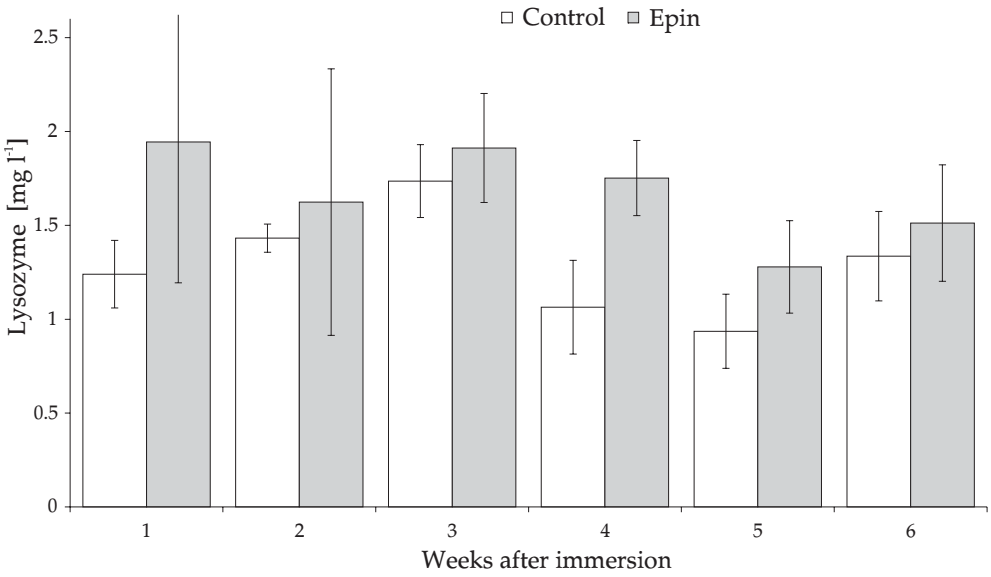

Fig. 3. Effect of epin on lysozyme bacteriolytic activity in Siberian sturgeon blood serum.

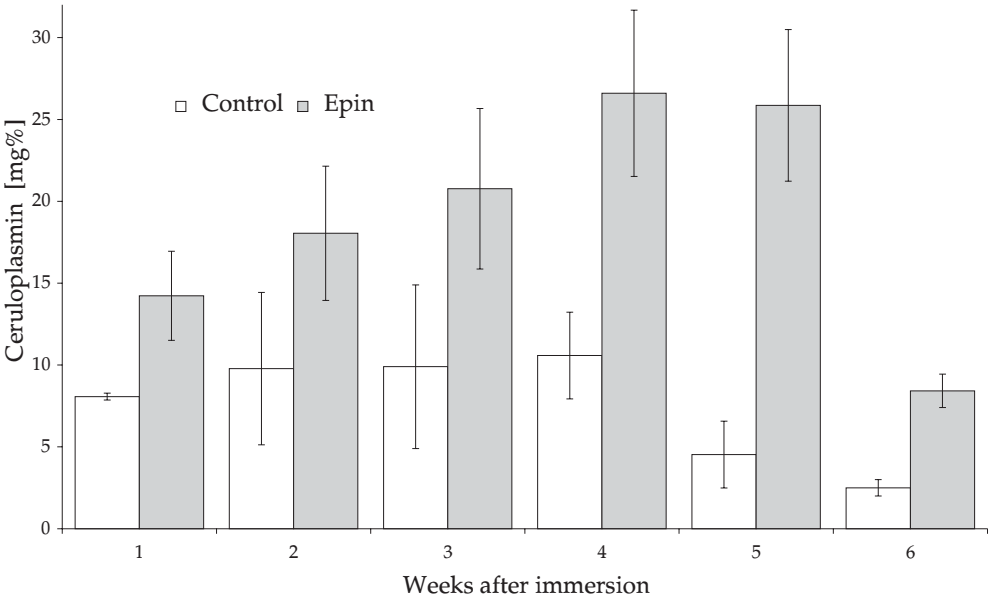

Fig. 4. Effect of epin on the ceruloplasmin level in Siberian sturgeon blood serum.

A statistically significant increase of lysozyme activity ($P < 0.05$) and ceruloplasmin level ($P < 0.001$) was already apparent in the first week of the study; differences in the levels of these indicators between groups were 57 and 76%, respectively (Figs. 3 and 4). Lysozyme activities significantly differed between the groups only in the first, fourth and fifth weeks (Fig. 3). The ceruloplasmin level in the treated fish increased until the fifth week, when it reached a level over 500% higher in comparison to that in the control group. Epin caused a 10-65% increase of γ-globulin level in the blood serum of the treated fish (a significantly higher value was observed in the second week, $P < 0.05$) in comparison to that of the control group (Fig. 5). The total protein level was higher in the epin-treated fish over the first three weeks of the experiment (Fig. 6).

DISCUSSION

Lysozyme is produced mainly by phagocytes (Ellis 1977, MacArthur and Fletcher 1985, Kolman H. et al. 1998b, c), and data obtained by various authors indicate that it plays a multiple role in fish immunity (Kolman H. et al. 2000). The role of ceruloplasmin is similar to that of interferon and transferrin; it inhibits bacterial development by depriving it of essential nutrients, i.e. copper ions (Aleksander 1985). It is also involved in oxidative protection (Kushner and Mackiewicz 1993). Analyses of liver and digestive tract homogenates of Siberian sturgeon revealed ceruloplasmin activity in the liver supernatant (Kolman H., unpublished data). The ceruloplasmin level was higher in actively feeding fish at higher temperatures in comparison to starving fish during wintering (Kolman et al. 1999). It was also observed that the blood ceruloplasmin concentration in Siberian sturgeon increased with fish growth; this might have been related to the increase of liver mass (Kolman H. et al. 1988b, c). An increased ceruloplasmin level in the control group over the first four weeks of the present study was related to manipulation stress (Fig. 4). The ceruloplasmin concentration was also elevated in Siberian sturgeon fry infested with *Diplostomum* sp. larvae. These findings suggest that ceruloplasmin belongs to the acute phase proteins in Acipenseridae (Kolman H et al. 1998d, 1999). The particularly high ceruloplasmin level in the epin-treated fish might have resulted from the stimulatory effect of activated leukocytes upon hepatocytes (Kolman H. et al. 2000), and could also be related to its metabolism in sturgeon.

The rising protein level in blood serum was probably related to the increase in acute phase proteins (Koj 1985), and was correlated with the increase of the level of

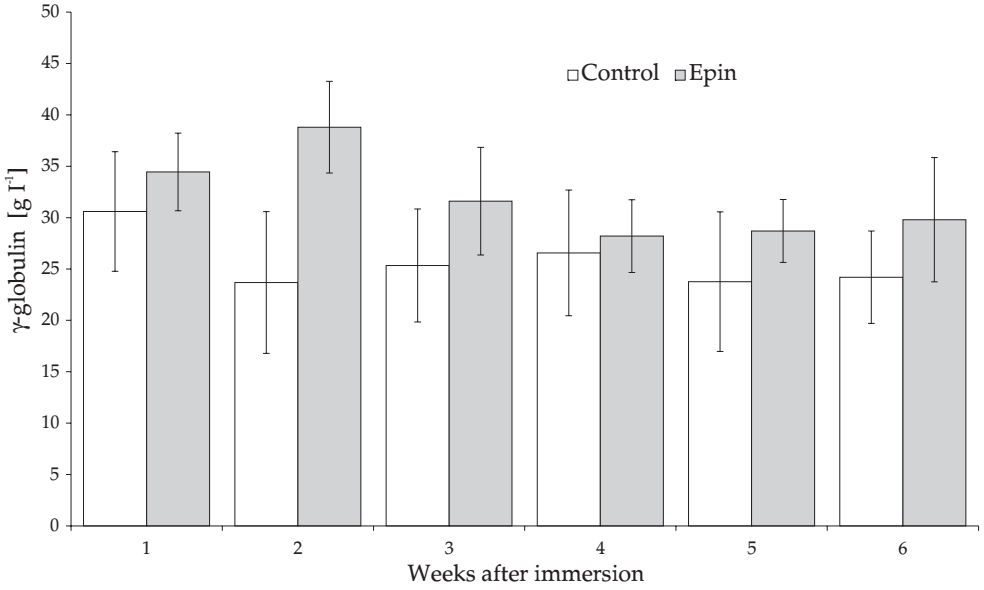

Fig. 5. Effect of epin on the γ-globulin level in Siberian sturgeon blood serum.

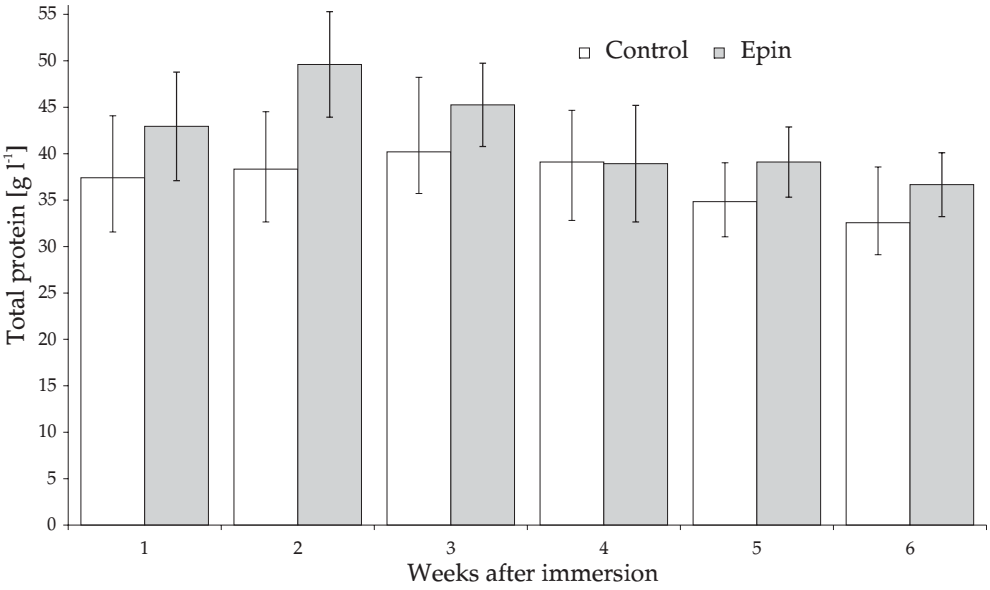

Fig. 6. Effect of epin on the total protein level in Siberian sturgeon blood serum.

the γ-globulin fraction. An elevated level of this protein fraction indirectly indicates the epin-induced increase of immunoglobulin concentration in sturgeon blood. Epin probably affects effector cells or directly stimulates B-lymphocyte proliferation or general haematopoiesis similarly to the way other immunomodulators have been shown to work (Kolman H. et al. 1998a). Therefore, epin may exert an immunomodulatory effect when B-lymphocyte activity is suppressed, as it is often observed in fish under stress. Various stressors affect leukocyte functions which result in disturbances in the equilibrium between specific and non-specific immune mechanisms. Weytz et al. (1998a) reported that cortisol, the main stress hormone in fish, causes an increase in lymphocyte apoptosis accompanied by inhibited apoptosis of neutrophils entering into the blood stream (Weytz et al. 1998b). This results in the topographic translocation of leukocytes in the fish immune system and immunological imbalance (Yin et al. 1995, Kolman H. et al. 1998d). Epin affects fish in various ways; this is evident in its stabilizing influence on the biological membranes of various types of cells (Egorov 1997, Egorov and Vitvitskaya 1997, Zagrijchuk and Tikhomirov 1999, Khabumugisha and Tikhomirov 1999).

The long-term effect of epin on sturgeon with regard to the analyzed indices suggests that it stimulates regulatory mechanisms at various levels. The results of the present study indicate that epin has an immunostimulatory effect and it influences immunoglobulin and/or immunological complex levels in the blood serum as well as non-immunoglobulin humoral immune factors. The positive effects of epin on the sturgeon growth rate indicate that it may be used to increase the economic efficiency of sturgeon stocking material production.

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STRESZCZENIE

EFEKTY HUMORALNE U JESIOTRA SYBERYJSKIEGO (*ACIPENSER BAERI* BRANDT) PO PODANIU EPINU W KĄPIELI

Celem badań było określenie wpływu epinu na wybrane wskaźniki immunofizjologiczne u narybku jesiotra syberyjskiego. Narybek podzielono na cztery grupy po 100 szt. w każdej, z których dwie stanowiły kontrolę, a pozostałe dwie poddano działaniu epinu w immersji. Eksperymentalny podchów narybku jesiotra syberyjskiego trwał 63 dni. W ciągu tego czasu średnia masa ryb w grupie kontrolnej wzrosła do 234,8 ± 11,3, a w grupie „epin" do 245,7 ± 10,2 g, tzn. była wyższa o 4,6%. Różnica w końcowej masie ryb powstała w pierwszych tygodniach chowu: w pierwszym tygodniu po kąpieli względny dobowy przyrost średniej masy ryb w grupach „kontrola" i „epin" wynosił odpowiednio 6,6 i 7,2% d^{-1}. Różnica ta stopniowo malała i w końcowej fazie eksperymentu względny dobowy przyrost masy w obu grupach ryb był równy i wynosił 1,5% d^{-1}. Statystycznie istotny wzrost aktywności lizozymu ($P < 0,05$) i ceruloplazminy ($P < 0,001$) w surowicy ryb z grupy „epin" stwierdzono już w pierwszym tygodniu badań. Różnice pomiędzy grupami w wartościach tych wskaźników wynosiły odpowiednio 57 i 76%. Statystycznie istotne różnice w aktywności lizozymu występowały w pierwszym, czwartym i piątym tygodniu. Poziom ceruloplazminy u ryb doświadczalnych stopniowo wzrastał do piątego tygodnia, w którym był on wyższy od kontroli o ponad 500%. Epin powodował wzrost ilości γ–globulin w surowicy od 10 do 65% (w drugim tygodniu statystycznie istotny przy $P < 0,05$) w porównaniu z kontrolą (rys. 5) oraz wzrost białka ogólnego ($P < 0,05$) obserwowany w pierwszych 3 tygodniach eksperymentu. Wyniki badań wykazały, że epin po jednorazowym zastosowaniu w kąpieli stymulująco wpłynął na poziom badanych wskaźników u jesiotrów.

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