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RESEARCH ARTICLE

Impact of Bioimmuno with methisoprinol on non-specific cellular and humoral defense mechanisms and resistance of African catfish (*Clarias gariepinus*) to experimental infection with iridovirus

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Abstract. The aim of the experiment was to determine the impact Bioimmuno had on the non-specific cellular and humoral defense mechanisms and resistance to experimental infection with iridovirus of African catfish, *Clarias gariepinus* (Burchell). The experiment was performed on clinically healthy African catfish weighing from 30 to 200 g. The fish were infected experimentally with iridovirus 59.90 at a dose of 10^4 $TCID_{50}$ ml^{-1}. Bioimmuno administered at a dose of 1 kg per 50 kg of feed most effectively stimulated the responses of non-specific cellular and humoral defense mechanisms, corrected delayed immunological reactions induced by iridovirus infection, and limited mortality caused by experimental viral infection of 5-45%.

Keywords: Bioimmuno, isoprinosine, immune system, fish

Introduction

Studies conducted in recent years in Poland and Europe indicate viral infections in fish are on the rise (Walker and Winton 2010). This problem is particularly prevalent among fish cultured under intense conditions at high stocking densities. Additionally, importing stocking material increases the risk of introducing dangerous pathogens into aquaculture that have not previously occurred in Poland. Iridoviruses, which cause epizootic hematopoietic necrosis (EHN), are one of these potentially dangerous infectious agents. A range of reports indicate that this disease is responsible for significant losses in worldwide aquaculture, and that it poses an increasing threat to European fish culture (Whittington et al. 2010). EHN is an infection caused by closely related iridoviruses belonging to the genus *Ranavirus* that are responsible for high mortality among various fish species, and it has already been isolated in Germany, France, and Denmark (Ahne et al. 1989, Hedrick et al. 1992, Pozet et al. 1992).

Fish are continually exposed to the impact of a variety of infectious agents in the aquatic environment. This is why it is important in fish culture to use immunostimulators to activate the non-specific cellular and humoral defense mechanisms that are essential elements of fish resistance especially when the adaptive immune system is still immature. Many publications in the available literature address the topic of applying natural and synthetic immunostimulators in animal husbandry (Morand et

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al. 1999, Siwicki et al. 2003, Paulsen et al. 2003, Selvaraj et al. 2005, Kumari and Sahoo 2006, Kim et al. 2009).

Methisoprinol is a well-known immunostimulator that has been used for years, the active ingredient of which is isoprinosine (a compound of inosine and 1-(dimethylamino/2-propanol/4-acetamidobenzene at a ratio of 1:3). It has a stimulatory effect on cellular and humoral defense mechanisms in both humans and animals. It activates T and B lymphocytes increasing the capacity of their proliferative response to antigens and mitogens, particularly in individuals with lowered resistance. Methisoprinol also increases the proliferation of macrophages and their phagocyte activity. Additionally, it induces the excretion of interferon and corrects the ability of cells under the influence of immunosuppresants to synthesize it (Campoli-Richards et al. 1986, Kowalski 1989). Studies of the effectiveness of the antiviral properties of methisoprinol have been conducted on various fish species including carp, *Cyprinus carpio* L.; black bullhead, *Ameiurus melas* (Rafinesque); European catfish, *Silurus glanis* L.; and rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Siwicki et al. 2002a, 2002b, 2003).

Glucans have also long been used in aquaculture, and among the wide range of them, those obtained from yeast have been studied the most thoroughly (Sakai 1999). The origin from which glucans are derived is extremely important because these differences have been confirmed to impact animal immunity mechanisms (Nikl et al. 1991, Wang and Wang 1997). As weak antigens, they exhibit an ability to stimulate non-specific immunity powerfully. Reports can be found in the literature regarding the impact of glucans on the non-specific cellular and humoral defense mechanisms in various fish species, including: Atlantic salmon, *Salmo salar* L.; rainbow trout; turbot, *Scophthalmus maximus* (L.); African catfish, *Clarias gariepinus* (Burchell); carp; channel catfish, *Ictalurus punctatus* (Rafinesque); tilapia, *Oreochromis niloticus* (L.); and zebra fish, *Danio rerio* (Hamilton) (Yano et al. 1991, Chen and Ainsworth 1992, Jørgensen et al. 1993, Santarem et al. 1997, Paulsen et al. 2003, Rodriguez et al. 2009, El-Boshy et al. 2010). To date, studies addressing the effectiveness of glucans in modeling immunological parameters have focused primarily on the impact on non-specific immunity to bacteria, while reports regarding its antiviral activity are not as numerous. When used both *in vitro* and *in vivo*, β-glucan stimulates the excretion of cytokines (IFN-γ, Il-1, Il-6, Il-10, Il-12, TNF-α), increases the production of mononuclear cells, stimulates lymphocyte activity, and activates the complementary system (Kakumu et al. 1991, Jagodzinsky et al. 1994, Wójcik et al. 2007). The aim of the current experiment was to determine the impact Bioimmuno with methisoprinol and glucans have on the non-specific cellular and humoral defense mechanisms of African catfish. Simultaneously, the impact of this agent on the anti-infective resistance of these fish following experimental infection with iridovirus was also determined.

Materials and methods

The experiment was conducted at the Department of Fish Pathology and Immunology, Żabieniec (IFI Żabieniec), Inland Fisheries Institute in Olsztyn and at the Department of Clinical Microbiology and Immunology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn. The catfish used in the experiment, which were in good condition and clinically healthy, were obtained from the Department of Ichthyobiology and Fisheries Management PAS in Gołysz. The fish were fed manually three times daily, and the daily ration of Aller Möele feed (2% of stocking biomass) was divided so that the last portion of feed the fish received daily was the largest. The basic water quality parameters in the fish tanks were monitored during the experiment: water temperature was 27±1°C; dissolved oxygen content was 4 mg dm^{-3}; water pH was in the 6.5-7.0 range. The concentration of ammonia nitrogen (NH_4), nitrite nitrogen (NO_2), and nitrate nitrogen (NO_3) oscillated around values of approximately 8 mg dm^{-3}, 0.25 mg dm^{-3}, and 250 mg dm^{-3}, respectively. The

fish for the experiment were anesthetized with Propiscin (IFI Żabieniec) at a dose of 0.5 ml dm^{-3} water, and then the cephalic kidney and spleen were collected aseptically.

Bioimmuno (IFI Żabieniec), comprising β-1.3/1.6 glucan (Biolex-Beta HP with 85% content of β-1.3/1.6 glucan, Leiber, Germany), at quantities of 100 g kg^{-1} of preparation; methisoprinol (Polfa Grodziskie Pharmaceutical Company, Poland) at quantities of 40 g kg^{-1} preparation, and *Saccharomyces cerevisiae* yeast per 1 kg. Lymphocytes were infected experimentally with iridovirus 59.90 at a dose of 10^4 $TCID_{50}$ ml^{-1}, which was obtained from the Laboratoire vétérinaire départemental (LVD 39) in Lons Le Sounier, France. This virus is from the genus *Ranavirus* and is included in the group of viruses causing epizootic hematopoietic necrosis (EHN) (Whittington et al. 2010).

In the first phase of the experiment, the impact of different doses of Bioimmuno administered with the feed on selected hematological parameters and non-specific cellular and humoral defense mechanisms in African catfish were evaluated, including hematocrit – Ht (%) determined with the macrohematocrit method (Pawelski 1983); hemoglobin concentration – Hb (g l^{-1}) determined with the cyanmethemoglobin method using Drabkin's reagent (BIOMED Kraków, Poland) and a standard solution of cyanmethemoglobin (Pawelski 1983); the number of erythrocytes (ERYs) (thousand ml^{-1}) determined with spectrophotometry using Pawiński's solution (Stankiewicz 1973) and modified for fish (Svobodova et al. 1985). Total protein levels (g l^{-1}) was determined with spectrophotometry as described by Lowry et al. (1951) using a total protein determination kit (total protein reagent, Sigma Diagnostics St. Louis, USA); lysozyme activity in the serum and kidney and spleen (mg l^{-1}) determined with the spectrophotometric method described by Siwicki and Anderson (1993) using a *Micrococcus lysodeiticcus* (Sigma, USA) bacteria suspension and standard hen egg white lysozyme protein (Sigma, USA); ceruloplasmin (IU) was determined in the serum with spectrophotometry as described by Rice et al. (1986) using an acetate buffer (pH 5.2) and sodium azide as the inhibiting agent; levels of gammaglobulin (g l^{-1}) were determined with the method described by Siwicki and Anderson (1993) with a kit from (Sigma, USA).

The fish were from four groups that had been fed feed manufactured by Aller Aqua (Poland) with various amounts of the supplement Bioimmuno for four weeks. Group I received Bioimmuno at a dose of 1 kg of the supplement per 100 kg feed; group II – 1 kg of the supplement per 50 kg feed; group III – 1 kg of the supplement per 25 kg feed. Control group K received feed without supplementation with Bioimmuno. The initial mean body weights of the fish were about 100 g, and the age was 4 months. The combined number of fish used in this phase of the experiment was 320 individuals. Groups of 80 fish each were stocked into tanks with volumes of 1000 l, and with a continuous supply of aerated water at a temperature of 25-27°C. After an acclimation period, the fish were infected experimentally with iridovirus 59.90 at a dose of 10^4 $TCID_{50}$ ml^{-1}, using 0.4 ml of solution per individual. On days 3, 7, 14 and 21 following infection, the fish were anesthetized with Propiscin (IFI Żabieniec) and blood samples were collected *in vivo* from the heart using a heparin needle and drawn into an Eppendorf tube for hematological and immunological tests. Simultaneously, kidney and spleen samples were collected for lysozyme activity determinations. Clinical and anatomical pathology examinations with the aim of describing any changes caused by viral activity.

In the second stage of the experiment, African catfish with an initial mean body weight of about 30 g were used. The total number of fish used in this phase was 760 individuals. The fish were divided into two groups. After the acclimation period, one group was fed Aller Aqua (Poland) feed, and the second was fed the same feed supplemented with Bioimmuno. The fish were fed for 14, 30, and 60 days. After the feeding period, the fish were infected experimentally with iridovirus 59.90 at a dose of 10^4 $TCID_{50}$ ml^{-1}, using 0.4 ml of suspension per individual, and then the fish were observed for 21 days and evaluated according to the following criteria: the fish were examined daily for characteristic symptoms of iridovirus infection; any anatomical pathology confirming viral activity was noted; cumulative mortality was calculated based on

the number of dead fish. After experimental infection, the virus was re-isolated from the organ exhibiting pathology in order to confirm that the pathology had been caused by iridovirus.

The third phase of the experiment was performed on African catfish with mean body weights of about 200 g which received Aller Aqua (Poland) feed supplemented with Bioimmuno at a dose of 1 kg of the supplement per 50 kg feed for 14 days. These fish were then infected intraperitoneally with iridovirus 59.90 at a dose of 10^4 $TCID_{50}$ ml^{-1}, using from 0.3 to 0.4 ml of suspension per individual. The proliferative response of the lymphocytes isolated from the cephalic kidneys to the mitogens was evaluated with spectrophotometric measurements of the reduction degree of MTT tetrazolium salts (3-4,5 dimethythiazol-2-yl/2,5-diphenyltetrazolium bromide) by living leukocytes as described by Mossmann (1983) and modified by Siwicki et al. (1996). The respiratory burst activity (RBA) method described by Secombes (1990) with modifications by Siwicki et al. (1996) was used to evaluate the metabolic activity of neutrophils and macrophages isolated from the spleen. During the RBA test, the phagocytes were stimulated with phorbol miristate acetate (PMA, Sigma-Aldrich) in a 0.1% solution of nitrotetrazolium blue chloride (NBC, Sigma-Aldrich). The potential killing activity (PKA) method described by Rook et al. (1985) with modifications by Siwicki and Anderson (1993) was applied to evaluate the phagocytic capabilities of macrophages and neutrophils isolated from the spleen. In the PKA test, the bacteria *Aeromonas hydrophila* in a 0.1% NBT solution was added to appropriately prepared cells. The lymphocytes obtained from the experimental fish, i.e., those fed feed supplemented with Bioimmuno, were divided into two groups: control group B of lymphocytes isolated from fish that had not been infected, and group B+W of lymphocytes isolated from fish that had been infected with the virus.The lymphocytes from each group were divided into three sub-groups comprising the controls that were not stimulated with mitogens, cells stimulated with ConA, and cells stimulated with LPS. The phagocytes were divided into groups analogous to those of the lymphocytes.

Statistical analysis

The results analyzed statistically using the ANOVA test for multiple comparisons of mean (LSD test) at $P<0.05$ and $P<0.01$ and standard deviations were determined.

Results

No statistically significant differences among groups were noted in the hematocrit values, hemoglobin concentrations, or erythrocyte numbers in the blood of the control fish or that of the fish fed feed supplemented with Bioimmuno. The levels of total protein in the serum prior to infection in all the fish groups fed different doses of Bioimmuno were of similar values that were higher than those in the control group. Three days following infection (DFE) with iridovirus, there were increases in total protein in all groups. At 7 DFE, the value of this parameter in the experimental groups decreased and then remained similar levels until the conclusion of the experiment. However, the protein level in the control group gradually decreased, and by 21 DFE it had reached a level similar to that prior to infection (Fig. 1).

The lysozyme activity in the serum of the experimental groups prior to infection was higher than that in the control group. At 3 DFE, the lysozyme activity in all of the groups increased, and it was most pronounced in group II, but at 7 DFE, the value of this parameter in all of the groups had decreased and remained stable until 14 DFE. On day 21 of the experiment, an increase in this parameter was noted in the experimental groups, while in control group K the value of it was similar to the initial one (Fig. 2).

Ceruloplasmin activity in the serum prior to infection was similar in all groups. At 3 DFE, there was a significant increase in ceruloplasmin activity in groups I and II, while at 7 DFE, a decrease in the value of this parameter was observed, and by 14 DFE it was similar to that prior to infection. Ceruloplasmin activity at 21 DFE was the highest in

groups K and I, while in groups II and III it was significantly lower in comparison to the control group at values that were lower than those prior to infection (Fig. 3).

The lowest levels of serum gammaglobulin prior to infection were noted in the control group (Fig. 4). Increased gammaglobulin levels were noted at 3 DFE in all groups, with significantly higher values in the experimental groups in comparison to the control group. At 7, 14, and 21 DFE, gradual decreases in gammaglobulin levels were noted, and in group II the value of this parameter was always at a higher level in comparison to the other groups. At 21 DFE the values of this parameter in all groups were higher than those determined prior to infection.

The kidney (Fig. 5) and spleen (Fig. 6) lysozyme activities exhibited increasing trends in all the experimental groups. The highest kidney

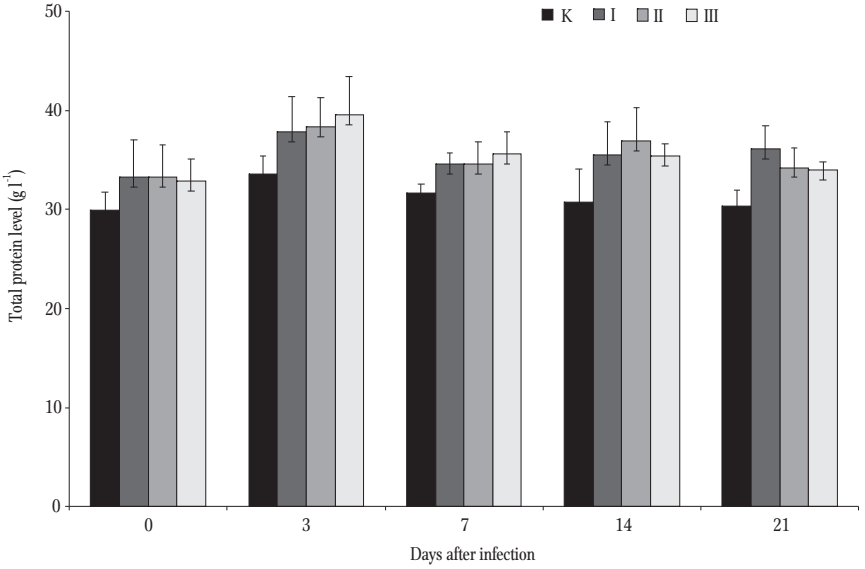

Figure 1. Total protein levels in the serum of African catfish fed feed supplemented with Bioimmuno after experimental infection with iridovirus (n=20, mean ± SD). K – control group; I – group receiving Bioimmuno at a dose of 1 kg × 100 kg^{-1} feed; II – group receiving Bioimmuno at a dose of 1 kg × 50 kg^{-1} feed; III – group receiving Bioimmuno at a dose of 1 kg × 25 kg^{-1} feed.

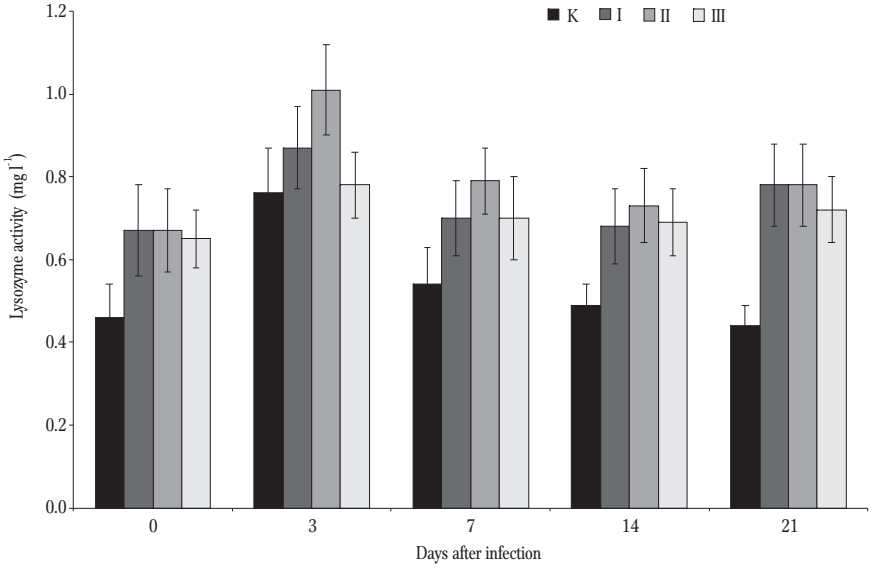

Figure 2. Lysozyme activity in the serum of African catfish fed feed supplemented with Bioimmuno after experimental infection with iridovirus (n=20, mean ± SD).

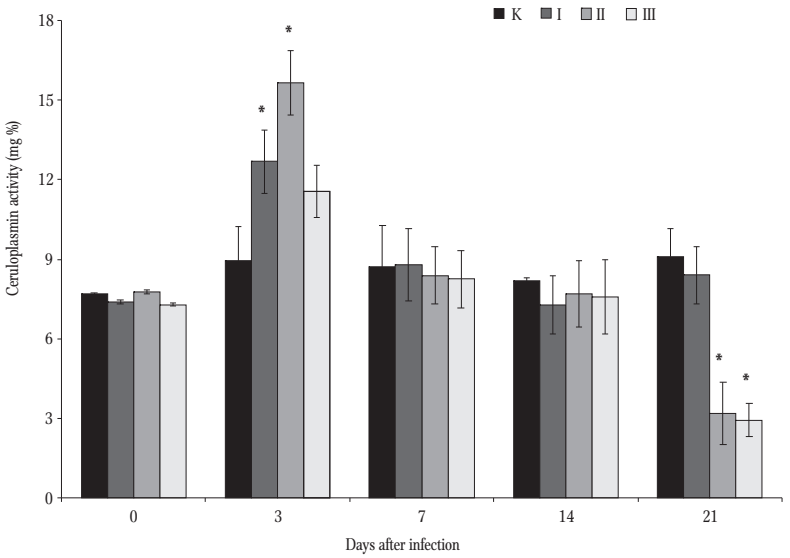

Figure 3. Ceruloplasmin activity in the serum of African catfish fed feed supplemented with Bioimmuno after experimental infection with iridovirus (n=20, mean ± SD, *statistically significant at P < 0.05).

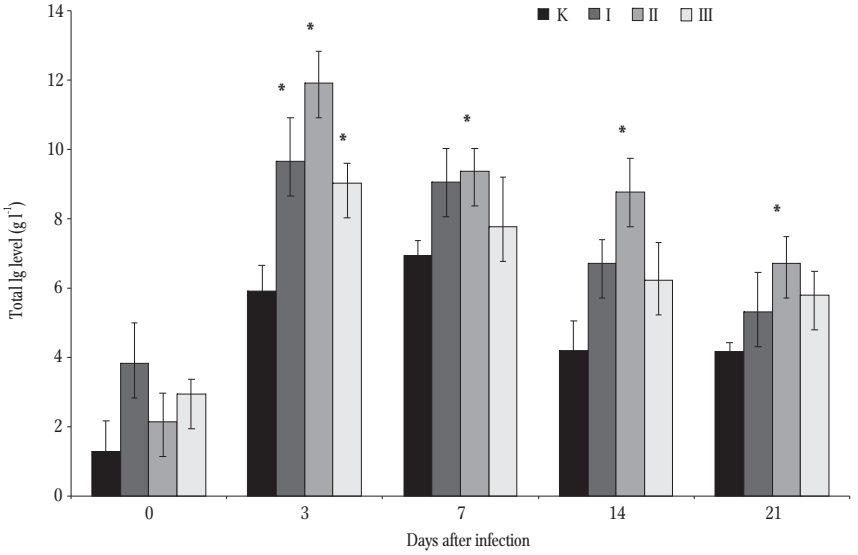

Figure 4. Gammaglobulin levels in the serum of African catfish fed feed supplemented with Bioimmuno after experimental infection with iridovirus (n=20, mean ± SD, *statistically significant at P < 0.05).

lysozyme values were obtained at 21 DFE, when more than two-fold higher values were noted in groups I and II and three-fold higher in group II in comparison to the results obtained prior to infection. However, the highest values of this parameter for the spleen were noted in groups I and II from 7 to 21 DFE. From 14 DFE, a gradual decrease was noted in group I spleen lysozyme activity, while at the same time in group II the value of this parameter remained at a stable level.

Typical clinical symptoms and anatomical pathology were noted in all the fish following experimental infection with iridovirus 59.90. The cumulative mortality among the fish that had received feed supplemented with Bioimmuno for 14 days prior to infection increased to 10% 13 DFE and remained at this level until the end of the experiment (Fig. 7). At 9 DFE, this same parameter among fish that were fed correspondingly for 30 days was 5% and it increased gradually to 35% on day 14 of the

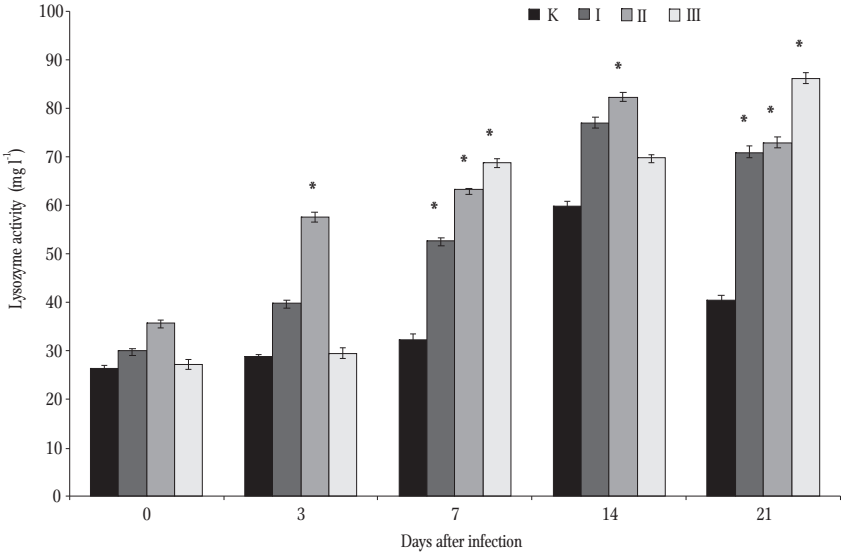

Figure 5. Lysozyme activity in the kidneys of African catfish fed feed supplemented with Bioimmuno after experimental infection with iridovirus (n=20, mean ± SD, *statistically significant at P < 0.05).

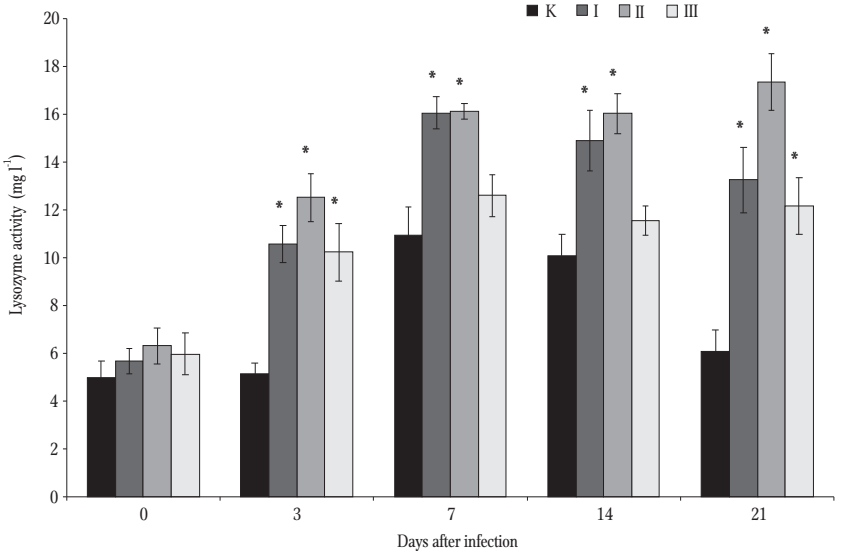

Figure 6. Lysozyme activity in the spleens of African catfish fed feed supplemented with Bioimmuno after experimental infection with iridovirus (n=20, mean ± SD, *statistically significant at P < 0.05).

experiment (Fig. 8), but among fish fed for 60 days, mortality was confirmed 2 DFE, following which it increased gradually until the end of the experiment and reached 60% (Fig. 9). Simultaneously, the cumulative mortality in the control groups after 14 days was 40% and after 30 days it was 80%, but after 60 days it was 65%.

The lymphocytes obtained from the fish in group B exhibited high values of proliferative activity under the influence of ConA (MTT assay) in comparison to that in the control group K. Increases in lymphocyte proliferative activity in group B+W were observed in comparison to those in group K+W. The tendencies of changes in the lymphocytes stimulated with LPS mitogens in the different groups was similar to that when ConA was used; however, the differences among the respective groups were not as distinct (Fig. 10). Higher phagocyte metabolic activity (RBA assay) was confirmed in experimental group B in comparison to that in

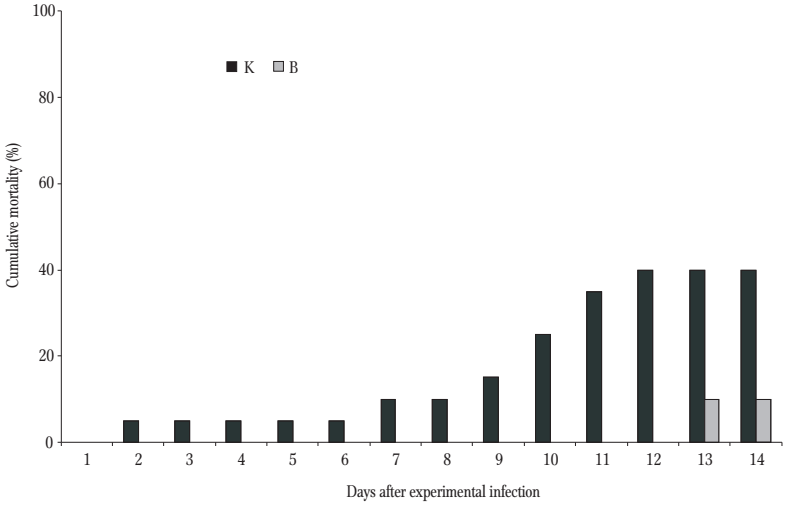

Figure 7. Mortality of African catfish fed feed supplemented with Bioimmuno for 14 days after experimental infection with iridovirus (n=20).

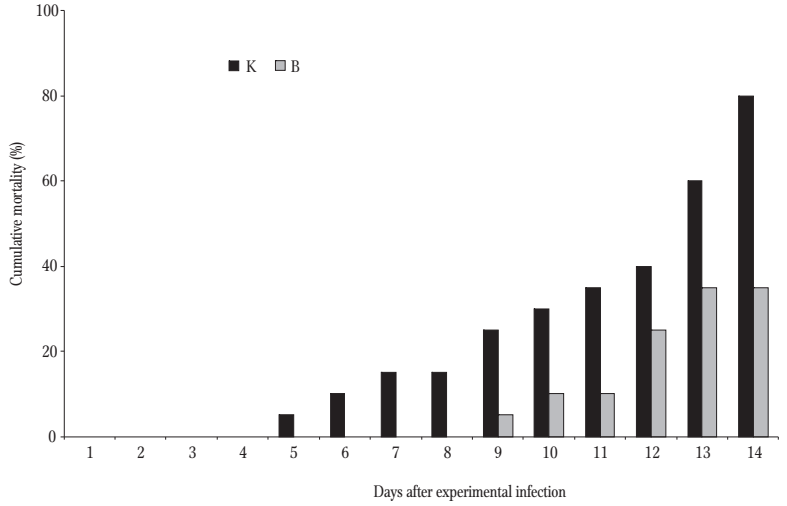

Figure 8. Mortality of African catfish fed feed supplemented with Bioimmuno for 30 days after experimental infection with iridovirus (n=20).

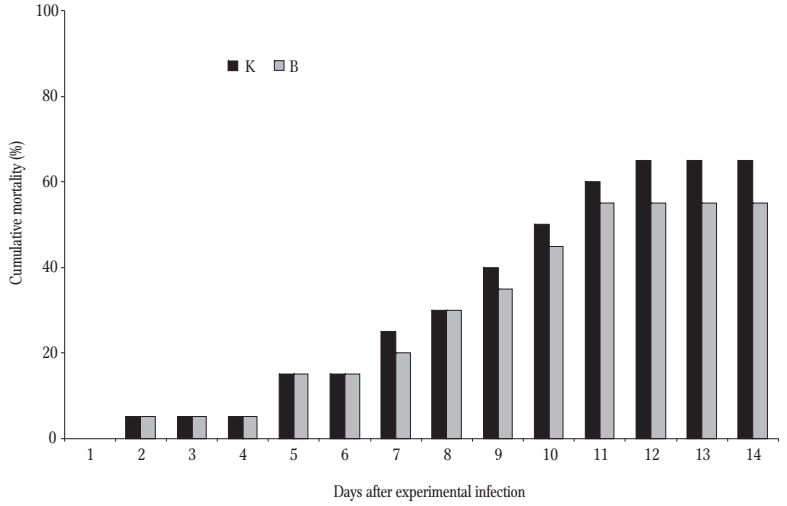

Figure 9. Mortality of African catfish fed feed supplemented with Bioimmuno for 60 days after experimental infection with iridovirus (n=20).

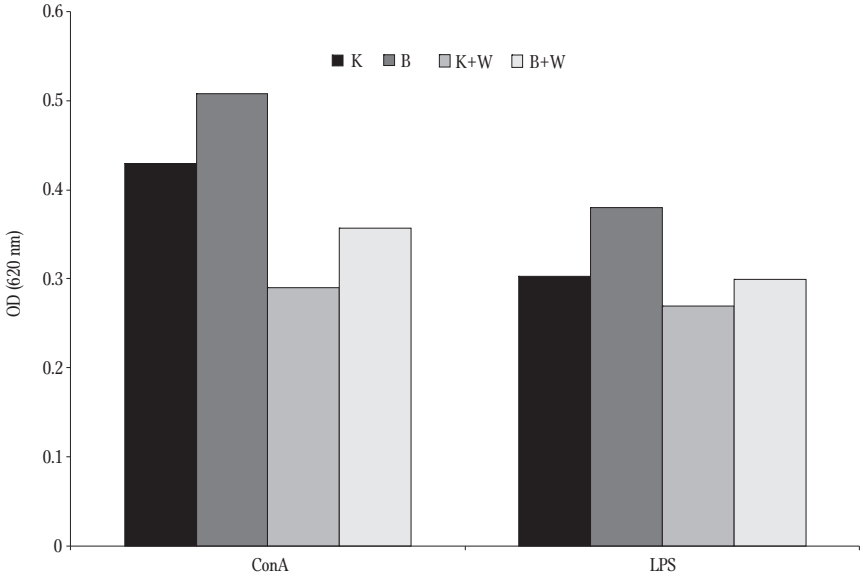

Figure 10. Impact of Bioimmuno on the lymphocyte proliferative response of African catfish experimentally infected with iridovirus and stimulated with mitogens ConA and LPS (mean, n=10, SD < 10%). K – lymphocytes isolated from fish fed feed not supplemented with Bioimmuno; B – lymphocytes isolated from fish fed feed supplemented with Bioimmuno; K+W – lymphocytes isolated from fish fed feed not supplemented with Bioimmuno and infected with iridovirus at a dose of 10^4 $TCID_{50}$; B+W – lymphocytes isolated from fish fed feed supplemented with Bioimmuno and infected with iridovirus at a dose of 10^4 $TCID_{50}$.

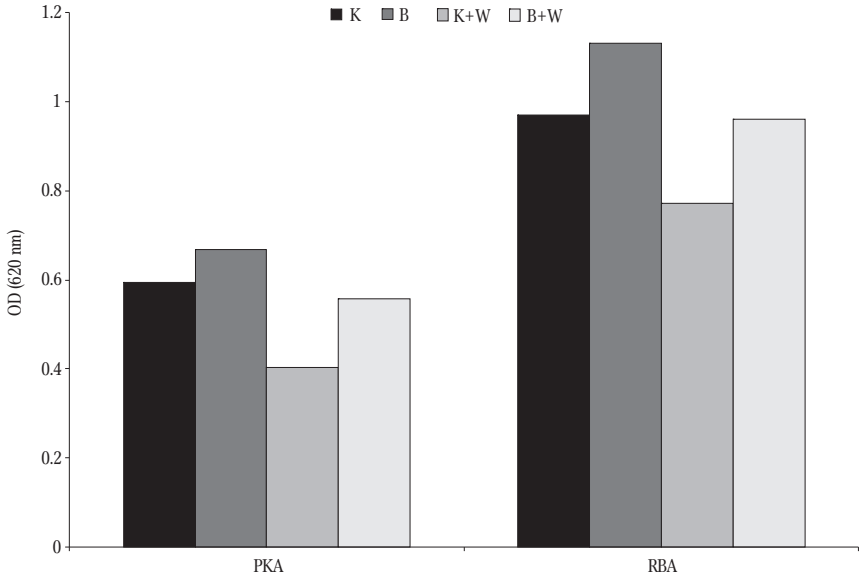

Figure 11. Impact of Bioimmuno on phagocyte metabolic activity and phagocytic activity of macrophages and neutrophils of African catfish experimentally infected with iridovirus (mean, n=10, SD < 10%). PKA – potential killing activity; RBA – respiratory burst activity; K – phagocytes isolated from fish fed feed not supplemented with Bioimmuno; B – phagocytes isolated from fish fed feed supplemented with Bioimmuno; K+W – phagocytes isolated from fish fed feed not supplemented with Bioimmuno and infected with iridovirus at a dose of 10^4 $TCID_{50}$; B+W – phagocytes isolated from fish fed feed supplemented with Bioimmuno and infected with iridovirus at a dose of 10^4 $TCID_{50}$.

control group K. Higher RBA values were also noted in group B+W in comparison to those in group K+W (Fig. 11). Higher phagocyte activity values (PKA assay) were noted in experimental group B in comparison to those in control group K, while higher PKA values were noted in group B+W in comparison to those in K+W (Fig. 11).

Discussion

The results of the current study indicate that providing African catfish with the supplement Bioimmuno prior to experimental infection with iridovirus resulted in a statistically significant increase in the values of the tested parameters of non-specific cellular immunity of this species. The supplement Bioimmuno provided to African catfish at a dose of 1 kg of the supplement per 50 kg of feed for 14 days cause increased proliferative activity in both T and B lymphocytes in comparison to that in the control groups and increased metabolic and phagocytic activity. Similar tendencies were observed in the fish infected with iridovirus. Undoubtedly, the two immunostimulators administered simultaneously, isoprinosine and glucan, also had an impact on the results obtained.

Earlier studies by the present authors also indicated that methisoprinol can cause increased lymphocyte T and B proliferative activity in response to mitogens in various fish species, including carp, black bullhead, wels catfish, and rainbow trout (Siwicki et al. 2002a, 2002b, 2003). It was confirmed that, above all, methisoprinol stimulates the cellular immune mechanisms linked to T lymphocytes, which could translate as the antiviral activity of this supplement following its application *in vivo*. Research conducted on fish phagocytes indicated increases in both respiratory burst activity (RBA) and potential killing activity (PKA). This is confirmed by the results of studies by other authors that are reported in the available literature (Ohnishi et al. 1983, Kowalski 1989).

Numerous scientific articles also report the stimulatory impact of glucans on the cellular immunity mechanisms of animals. The *in vitro* impact of glucan on the proliferative ability of lymphocytes obtained from fish are described (Kamilya et al. 2006), and from these, the present authors learned that glucan substantially stimulates lymphocyte proliferative activity under the influence of ConA. Significant increases in metabolic (RBA) and intracellular blood phagocyte killing (PKA) activities were also observed in lambs fed feed supplemented with β-1,3/1,6-D-glucan. The positive impact of β-glucan was also expressed in the higher proliferative response of blood lymphocytes (MTT) in lambs stimulated with ConA and LPS (Wójcik et al. 2007). However, Verlhac et al. (1998) demonstrated that the stimulatory effects of glucan indirectly. These authors provided rainbow trout with feed supplemented with β-1,3/1,6 glucan obtained from yeast *S. cerevisiae* together with vitamin C and with vitamin C alone. Based on the different results obtained, they proposed that glucan results in increased proliferative activity in lymphocytes that are stimulated with ConA. Other authors also concluded that β-glucan had a modulatory impact on the proliferative response of lymphocytes stimulated with various mitogens (ConA, PHA, LPS) in mice and rats in *in vitro* and *in vivo* studies (Suzuki et al. 1989, Rios-Hernandez et al. 1994).

Since non-specific immune mechanisms play an important role in protecting fish from many infectious agents, it was essential in the current study to test immunological parameters such as the lysozyme activity, gammaglobulin levels, and ceruloplasmin activity. The dynamics of change in the various parameters following experimental infection with iridovirus were similar, and in all instances, statistically significant increases in the studied parameters were observed three days following infection.

The serum lysozyme activity in all the experimental groups was similar prior to infection, and three days after infection significant increases in this parameter were noted in all the experimental groups. Lysozyme activity decreased significantly beginning on day seven following infection; however, this parameter was at a higher level in the group that received the Bioimmuno supplement 21 days following infection than it was in the control group that did not receive any immunostimulator. The dynamics of change in lysozyme activity in the kidney and spleen were similar; lysozyme activity increased until day 14, but on day 21 a slight decrease in this parameter was observed in most of the experimental groups as was a significant decrease in the control group. Both the dynamics of change in lysozyme activity and

statistically significant differences among groups suggest that methisoprinol induces a strong immunological response. This could be linked to the direct impact methisoprinol has on cellular response through the activation of macrophages, as is reported in the literature (Kowalski 1989). There are also similar reports regarding the impact β-glucan has on lysozyme production in Atlantic salmon, rainbow trout, and turbot (Engstad et al. 1992, Jørgensen et al. 1993, Baulny et al. 1996, Santarem et al. 1997, Paulsen et al. 2003). Additionally, when provided to pigs, this supplement had a significant impact on increased lysozyme activity, gammaglobulin levels, and total serum protein (Li et al. 2005).

Acute phase protein plays an important role in removing infectious agents from bodies and reestablishing homeostasis. In their own studies, the present authors have determined the activity of ceruloplasmin, which is primarily produced in the liver. While there are no reports in the available literature on how ceruloplasmin activity is influenced in fish following infection with viruses, there are publications regrading this topic in reference to other animals. According to information in the literature, the concentration of this protein increases once infectious agents are active, and it reaches its highest levels after 48-72 hours (Kostro et al. 1996). The current experiment deomstrates that three days following infection there are highly statistically significant increases in ceruloplasmin activity in groups I, II, and III, while the differences are statistically significant in group K. Decreases in this parameter were noted on subsequent days until day 14 of the experiment when the level of it was the same as it had been prior to infection. The results of the current study correlate with those from similar studies performed on other animals.

Natural gammaglobulins of low specificity occur in the serum of unimmunized fish, and these protect the body at a time when the elements of specific humoral immunity are not yet developed. The lowest levels of gammaglobulin were noted in the present study in the control group (K) prior to infection. A statistically significant increase in this parameter was noted three days following infection in all of the groups, i.e., K, I, II, and III, while seven and 14 days after infection gradual decreases in gammaglobulin levels was noted; however, in group II the level of this parameter always remained at a higher level in comparison to that in all the other groups. On day 21 following infection, higher gammaglobulin levels were noted in groups I, II, and III in comparison to the control group.

The results of the current work, similarly to those available in the literature, indicate there is a strict correlation between fish gammaglobulin levels and lysozyme activity (Stosik and Deptuła 1990). The greatest activities of lysozyme and ceruloplasmin and the highest gammaglobulin levels were noted in the group of fish that received a Bioimmuno dose of 1 kg per 50 kg feed.

In the next phase of the study, an attempt was made to assess the impact providing the fish the supplement Bioimmuno had on the survival of fish infected experimentally with iridovirus. Conflicting reports on the topic of using methisoprinol immediately before exposing animals can be found in the literature. According to Ginsberg and Glasky (1977), it has the most effective impact on the immune system when the lymphocytes are already in the proliferative phase by supporting this process. Rumińska-Groda (2002) observed that providing turkeys isoprinosine in feed at a dose of 50 mg kg^{-1} for three days prior infection with hemorrhagic enteritis virus (HEV) did not result in reduced mortality. However, it is important to remember that fish cease to feed the moment disease occurs. This is also why in the current study the fish received feed supplemented with Bioimmuno at a dose of 1 kg of supplement per 50 kg feed for 14, 30, and 60 days, and then they were infected with iridovirus intraperitoneally. The comparison of mortality percentages among these three groups of fish led to the conclusion that the best effects were obtained by providing feed supplemented with Bioimmuno for a period of 30 days; mortality in the experimental group was barely 35%, while in the control group it was 80%. It is possible that the results were because isoprinosine boosts interferon activity (Werner 1979), which, in turn, halts the proliferation of iridovirus. Data from the literature

indicate that although isoprinosine can halt the proliferation of some viruses such as herpes simplex and herpes zoster (Gordon et al. 1974, Kowalski 1989), it does not provide positive results with other viruses such as HIV (Teglbjaerg et al. 1992). This likely stems from the differences in proliferation structures and models of individual viruses. It is also possible that the halted proliferation of iridovirus observed in the current study and the corresponding reduced mortality of African catfish in the groups that received Bioimmuno supplementation was linked to such differences.

The administration of isoprinosine with glucans in the current study also certainly had a significant impact on limiting mortality among infected fish. As Lapatra et al. (1998) demonstrated, administering glucans to rainbow trout substantially reduced fish mortality following infection with the IHN virus. Intensive fish culture is inextricably linked with the cyclic occurrence of numerous stressors associated with transport, sorting, etc., which is why attempts have been made for years to use various immunostimulators to mitigate culture stress. The results indicate that the most advantageous results can be obtained by providing the fish with Bioimmuno at a dose of 1 kg of the supplement per 50 kg feed from 14 to 30 days prior to periods in which the threat of stressors occurring is the highest, including planned manipulations, as well as before periods when the fish are most at risk from pathogens. The analysis of the results from the present study permit concluding that using Bioimmuno could help reduce viral infections in African catfish, and thus foster better economic results from the cultivation of this species without having to resort to expensive chemotherapeutic agents that could be dangerous to the environment. Currently, the best method for fighting viral diseases in fish is to prevent introducing pathogens to culture facilities. If prophylactic measures fail and disease breaks out, contemporary veterinary medicine does not have effective chemotherapeutic agents for fighting viruses. This is why immunoprophylactics that aim at limiting or eliminating pathogens in animals are of such significance.

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