Influence of VitaSil® Additive to Carp Fish Natural Immune Factors

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Abstract

Common carp is one of the most important commercially produced fish all around the globe. Even not essential to fish, pro-nutrients could benefit animals' health in many ways. Key role in stock animals' protection against pathogens is played by the complement system. The aim of this study was to investigate the impact of the feed supplement VitaSil to the two major pathways of complement activation – classical and alternative, in extensively grown carp. The initial level for the Alternative Pathway of Complement Activation (APCA) among both groups was 579.37 CH50. During the first month of treatment both groups showed significant decrease in the levels of this parameter. The obtained values on the 30th day of challenge were 498.05 CH50 for the control and 448.06 CH50 for the treated group. On the 60th day of the trial, the APCA levels of the control group decreased to 417.83 CH50, while the challenged fish exhibited dramatic increase: 801.28 CH50. The results obtained for the Classical Pathway of Complement Activation (CPCA) were almost identical. The initial value for both groups was about 215.70 CH50. On the 30th day control group showed slightly higher levels compared to the challenged fish, 201.45 CH50, and 199.34 CH50, respectively. On the 60th day experimental carp fish exhibited significantly higher level of complement activity than control group, 229.55 CH50 and 167.91 CH50, respectively. The obtained data for both pathways of complement activation, unambiguously show the immunostimulative effect of VitaSil to carp fish, grown by extensive technology.

Keywords: *Complement system, Cyprinus carpio, VitaSil*

INTRODUCTION

Intensive aquaculture requires optimization in management, integration of good breeding program and improved nutrition. The relatively easy way of growing and low living requirements, makes common carp (*Cyprinus carpio* L.) one of the most desirable fish to any farmer in the world. It is mostly reared using semi-intensive or even extensive technology (Heydarnejad, 2012). Due to the dramatic economic growth in aquaculture (Lall, 2000), fish nutrition programs became a globally discussed subject. The use of additives in carp culture has become inevitable for successful growing of this fish type (Shahzadi et al., 2006).

Even not essential to fish, pro-nutrients could benefit animal health in many ways. The impact of other nutritional supplements has been used to improve growth rate, feed conversion ratio (FCR) and mortality rates in fish (Staykov et. al., 2007).

VitaSil feed supplement is made from the seeds of *Carduus marianus* L, Asteraceae. The active component of this plant is silymarin, in the form of a standardized extract in VitaSil, obtained from the seeds, containing approximately 70 - 80% aromatic flavonoids and approximately 20 - 30% dry uncertain components, including polymeric and oxyphenols compounds. Main features of VitaSil are the stimulation of hepatocyte

regeneration and reduction of the toxic effect caused by some chemicals or drugs (Atanasov *et al.*, 2010).

Animals' health state is crucial for the success of any nutrition program. Complement system is known to play significant role in both innate and adaptive immune response, which makes it an indispensable defender of invertebrates as well as vertebrates. Some pathogenic microorganisms induce activation of complement response directly by activating the alternative pathway, while others need specific antibodies to activate the function of the classical pathway of complement cascade (Fujita, 2002; Mueller-Ortiz *et al.*, 2004).

Breed, species, age and sex have been evaluated as factors with huge impacts on the levels of serum lysozyme and Alternative Pathway of Complement Activation (APCA) activity (Koynarski and Sotirov, 2012; Sotirov *et al.*, 2011).

Taking into account the aforementioned aspects and the importance of the complement system to animals' health, we decided to investigate the impact of the food supplement VitaSil on the two major pathways of complement activation in extensively grown carp fish.

MATERIALS AND METHODS

The experiment was conducted at Trakia University's fish facility using the eight cage production system. Two replicate cages measuring 3 x 3 x 2.5 m were used. Each cage was housing 15 carps with an average initial weight of 200 g. The diets of both groups (control and experimental) were identical, based on a commercial formulas, with the exception of VitaSil supplement. The trial lasted for 60 days, and both parameters of interest were analyzed on the first, 30th and 60th day of challenge. Blood samples for analysis were obtained aseptically from the heart.

The levels of the alternative pathway of complement activation (APCA) was assessed by the method of Sotirov (1991). Each serum sample was first diluted by mixing 100 µl serum with 350 µl veronal-veronal Na buffer (final concentrations: 146 mM NaCl, 1.8 mM 5.5-diethylbarbituric acid sodium salt; 3.2 mM 5.5-diethylbarbituric acid; 1 mM EGTA and 0.8 mM MgCl₂), in U bottomed plates (Flow Laboratories, UK). From each diluted serum 7 serial dilutions were again prepared in veronal-veronal Na buffer: 80 µl diluted serum + 20 µl buffer, 70 µl diluted serum + 30 µl buffer, 60 µl diluted serum +

40 µl buffer, 50 µl diluted serum + 50 µl buffer, 40 µl diluted serum + 60 µl buffer, 30 µl diluted serum + 70 µl buffer and 20 µl diluted serum + 80 µl buffer. The final serum dilutions were, 8/45, 7/45, 6/45, 5/45, 4/45, 3/45 and 2/45, respectively. Then 50 µl buffer and 100 µl of 1% rabbit erythrocyte suspension were added to each well. After 1 hour incubation at 37°C the samples were centrifuged at 150 G for 3 minutes at room temperature (23°C). Thereafter, 150 µl of supernatant was removed from each well and placed in flat bottomed plates for measurement of optical density at 540 nm using "Sumal-PE2" ELISA reader (Karl Zeiss, Germany).

The classical pathway of complement activation (CPCA) was determined by the method of Stelzner and Stain (1971). Each serum sample was first diluted by mixing 30 µl serum with 170 µl veronal-veronal Na buffer (in final concentrations: 146 mM NaCl; 1.8 mM 5,5-diethylbarbituric acid sodium salt; 3,2 mM 5,5-diethylbarbituric acid; 15 mM CaCl₂ and 0.8 mM MgCl₂) in U bottomed plates (Flow Laboratories, UK). From each diluted serum 5 other serial dilutions were again prepared in veronal-veronal Na buffer: 3/20, 3/80, 3/160, 3/320 and 3/640. Then, 100 µl buffer and 100 µl of 1% sheep erythrocyte suspension sensitized with haemolytic antibodies were added to each well. After 1 hour incubation at 37°C the samples were centrifuged at 150 G for 3 minutes at room temperature (23°C).

The last step for both pathways was to pipette out 150 µl of each supernatant in flat bottomed plates and measuring the optic density at 540 nm using a "Sumal-PE2" ELISA reader (Karl Zeiss, Germany). The final APCA and CPCA activity was calculated using a special computer software developed at Trakia University and expressed as CH50 units (CH50 units correspond to 50% of complement-induced haemolysis of applied erythrocytes).

The obtained data was subjected to ANOVA with confidence limits set at 95%, using the data analysis tool pack (Microsoft Excel 2010, Microsoft Corporation Ltd.).

RESULTS AND DISCUSSION

The obtained data for both parameters of interest exhibited similar tendencies. The initial value of the APCA among both groups was of 579.37±41.47 CH50. During the first month of treatment both groups showed significant decrea-

se in the levels of this parameter. The values obtained on the 30th day from the beginning were 498.05 ± 21.43 CH50 for the control group and 448.06 ± 8.73 CH50 for the challenge group. Statistically processed data showed significant difference between the two groups in favor of the control one ($P < 0.05$). On the 60th day of the trail we found dramatical change in the APCA values between the two groups. The levels of this parameter among the control group continued to decrease (417.83 ± 22.37), while the challenged fish exhibit almost twice as high result (801.28 ± 29.35). The huge difference between the two groups was statistically significant at $P < 0.01$. The variation in the levels of this parameter is presented in Fig. 1.

The results for the classical pathway of complement activation were almost identical. The initial values for both groups were about 215.70 ± 9.68 . Although not significant, on the 30th day of challenge experimental fish showed lower CPCA activity compared with the controls, 199.34 ± 10.11 and 201.45 ± 9.51 , respectively. On the 60th day the group of experimental carp fish exhibited significantly higher level ($P < 0.01$) of complement activity than the control group, 229.55 ± 14.05 and 167.91 ± 7.77 , respectively. The fluctuation in the CPCA activity within both groups is presented in Fig. 2.

Commercial production of fish requires advanced research in all aspects of aquaculture

Fig. 1. Impact of VitaSil food additive on APCA activity (CH50) in carp fish, $^{acdefg} - P < 0.01$; $^b - P < 0.05$.

Fig. 2. Impact of VitaSil food additive on CPCA activity (CH50) in carp fish, $^{a-b} - P < 0.01$.

science. Modern fish farming is mostly based on innovations in growing technology and dietary programs. To maximize performance traits we should focus on fish health state and condition. The presently adopted way to improve health parameters in livestock is the integration of a good breeding program, where the genetic potential of individuals is used to achieve better health traits in their offspring. Although this strategy is proved to have great results in other species, it is difficult to apply it in aquaculture. Another key factor is the usage of feed additives or pro-nutrients to boost the immune responses of the animals against pathogens. Supplementation of the diet with immunostimulative substances can easily improve both performance and health traits of animals. In this study we observed interesting fluctuations in the levels of the two pathways of complement activation. Both parameters exhibited relatively low decrease during the first month of the experiment. This phenomenon was observed among both experimental and control groups. Possible explanation of this tendency is the accommodation process of fish to the new environment and the need for accumulation of the tested additive. Even though the activity of both pathways was decreased, we should clarify that the observed levels were within the normal range for common carp. The results for the second period were diametrically opposed. Activity levels for both parameters of interest were almost twice as high among treated fish, whereas the values for the control fish continued the decreasing trend. The statistically processed data unambiguously showed the immunostimulative effect of the VitaSil additive to both pathways of complement activation, hence better protection of fish against pathogens. Presumably this effect is due to the hepatoprotective effect of the used feed supplement and its antioxidant properties. The liver is the main body part where complement cascade operates, therefore the better performance of its cells leads to higher complement activity.

In a similar experiment Staykov *et al.* (2007) determined much higher levels of both APCA and CPCA activity among rainbow trout treated with the Bio-Mos® supplement. In addition authors established better performance traits of the treated fish in both net cage and raceway systems.

Samrongpan *et al.* (2008) report significant impact of mannan-oligosaccharides (MOS) on

growth performance of Nile tilapia (*Oreochromis niloticus* Linnaeus). The authors determined significantly higher resistance of treated fish against *Streptococcus agalactiae* infection. Although not investigated by the authors, we may suppose that this phenomenon is partially due to the higher levels of complement activity, induced by the used MOS supplement.

Shahzadi *et al.* (2006) investigated the influence of cottonseed supplement for the growth performance and feed conversion ratio of hybrid fingerlings (*Catla catla* X *Labeo rohita*). The authors determined significantly higher indexes for both parameters of interest in the treated fish, compared with the controls.

The effect of antioxidants on the levels of complement activity had been described among other species also. Sotirov *et al.* (2007) determined higher blood serum lysozyme and complement activity in sows and their progeny treated with the SelPlex feed supplement. The authors claim for better innate immunity and performance traits of the treated animals.

CONCLUSION

The obtained data for both pathways of complement activation, unambiguously show the immunostimulative effect of VitaSil to extensively grown carp fish. Therefore we strongly recommend introduction of this feed supplement to fish diet.

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