

Effect of Garlic (*Allium sativum*) on Hematological Parameters and Plasma Activities of ALT and AST of *Rainbow trout* in Temperature Stress

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Abstract: The pathogenic factors (infectious or non infectious) causes to reduce in production, especially in cold water fish in the aquaculture industry. To solve this problem many approaches such as disease resistant fish, an enriching diet, vaccinations, antibiotics and immunostimulants must be utilized. The garlic has wide variety characteristics, including immunostimulant and also increase the immunocompetence due to binding s-o-s and polar components. To evaluation these effects, a short study was carried out for 58 days. In this study, the effects of garlic, with three different doses 0.3, 0.45, and 0.6 g powder of garlic per kg dry diet consumption, were surveyed in two interrupted periods of time in heat stress condition on 120 *Rainbow trout* species and some hematological parameters related to nonspecific immunity in three treatment groups and one control group were studied. The results indicated that lymphocyte count increased significantly in fish fed on diets containing all garlic doses compared with the control group. Lymphocyte and erythrocytes count increased significantly in fish fed on diets containing 0.45, and 0.6 g/kg doses compared with in fish fed on diets containing 0.3 g/kg doses ($P < 0.05$). The erythrocytes count in all treatment groups in comparison with the control group showed no significant difference ($P > 0.05$). There was a significant increase in the Leukocyte count in fish fed a diet containing 0.6g/kg garlic compared with the control group and the diets containing 0.3, 0.45g/kg. The mean leukocyte count in all treatment groups were more than the control group. The mean percent of neutrophils decreased significantly in fish fed on diets containing 0.45, and 0.6 g/kg doses compared with in fish fed on diets containing 0.3 g/kg doses. A statistically non significant change in hemoglobin content and hematocrit percent in fish fed on garlic was seen when compared with the control group. During the experiment, a non-significantly different in the mean corpuscular hemoglobin concentration (MCHC) and mean concentration of hemoglobin (MCH) was observed in all treatment groups in comparison with the control group, but mean of MCH decreased significantly in fish fed on diets containing 0.45g/kg in comparison with in fish fed on diets containing 0.3g/kg. Mean of mean corpuscular volume (MCV) of fish fed on diets containing 0.45 and 0.6g/kg decreased significantly compared with in fish fed on diets containing 0.3g/kg. In evaluation of the plasma parameters (ALT and AST) revealed a non-significant difference in all treatments compared to the control. In conclusion, use of garlic balanced and formulated in suitable doses can decrease mortality rate and increase immunity.

Key words: Garlic, Temperature stress, Hematological parameters, ALT, AST, *Rainbow trout*

INTRODUCTION

Fish culture is an important industry in which the production of fish worldwide increases every year. Intensification of fish adversely affects fish health and tends to produce a poor environment for fish, increasing their susceptibility to infections (Sakai, 1999). Disease outbreaks were recently identified as a major constraint to aquaculture production and trade, with a consequent effect on the industry's economic development (Yunxia *et al.*, 2001). Over the past 20 years various chemotherapeutics have been used to treat bacterial infections in

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cultured fish (Aoki). The use of disinfectants and antimicrobials has shown limited success in preventing or curing aquatic diseases (Subasinghe, 1997). However, the use and abuse of antimicrobials in aquaculture can help to growing, but they result in the selective pressure exerted on the microbes and encourage the emergence of resistant bacteria by transferring resistance-genes to bacteria not exposed to antibiotics. In addition, the antimicrobial besides having a negative impact on the environment, result in drug residues in the treated fish (FAO/WHO/OIE 2006). Antimicrobials can generate cross-resistance against human antimicrobials, which could pose a hazard (Witte *et al.*, 1999). Vaccination may prevent fish disease outbreaks, but the development of vaccines against many intracellular pathogens has not yet been successful. Therefore, the immediate control of all fish diseases using only vaccines is impossible, and commercial vaccines are expensive for fish producers, and may not be available against the encountered and emerging diseases (Raa *et al.*, 1992). Not only application of immunostimulants have more effective approach to controlling disease in aquaculture through the enhancement of natural disease resistance, but also contribute to health management in aquaculture through the enhancement of immunocompetence and disease resistance in fish (Sakai, 1999). The immunostimulants result in enhancing the activity of the non-specific defense mechanisms and increasing disease resistance (Raa, 1996). Garlic increases the welfare of fish, and it can help in the control of pathogens, especially bacteria and fungi (Corzo-Martinez *et al.*, 2007). The garlic has several beneficial effects including antioxidant, antihypertensive, and antimicrobial properties (Sivam, 2001). The present study was aimed at evaluating the effect of *Allium sativum*, an immunostimulant, on the hematological parameters and plasma activities of ALT and AST of *Rainbow trout* in thermal stress conditions through field experiments.

MATERIALS AND METHODS

Animals:

120 juvenile male and female *Rainbow trout* with no clinical signs or abnormal behavior were obtained from the nearest of fish farms in Sari and transported to Ecology Research of Khazar Sea (ERKS) by a fiberglass. The fish initial weight was 50 ± 5 g (mean \pm SD) with no significant size difference. In ERKS these fish were divided into four groups, and were held in four fiberglass tanks, that had been cleaned and disinfected, previously. There were 30 fish in each group. These tanks were filled with river water and placed in a room containing an air conditioner, and the water in the tanks was changed one or two times daily.

Experimental Diets:

Three experimental diets were formulated to contain different levels of *Allium sativum* powder (group 1, 2, 3 added 20, 30, and 40g/kg diet, respectively), which was equal to 0.3, 0.45, and 0.6 g per total daily diet. The control diet was free from *Allium sativum* (group 4).

Experimental Design:

The fish were held in adaptation period for 15 days and received only routine food. The basal diet was SFT. The daily diet was calculated based on the body mass weight of the fish and the environmental temperature, and twice a day the fish were given manual feeding, carefully. Garlic without skin was purchased from the local market and dried at 35 °C for 72 hours, then ground to become powder. The dried diet was packaged into a plastic bag and stored frozen at -20 °C until use. The level of garlic powder determined was that described by Shslaby. After the adaptation period (15 days), parallel to the starting of thermal stress (22 °C), 3 ml vegetable oil was added and mixed with SFT and garlic, with the dosage the same as that described previously, to assure the homogeneity of the ingredients period. The fish were then given manual feeding for 15 days. One day later, on day 16, the garlic was omitted from the diet, and following this day, 15 fish from each group were anesthetized by extraction of clove and blood samples collected from their caudal vein using sterile syringes with EDTA. Subsequently, fishes were grazed of SFT, without garlic, for 10 days. After that, garlic was again added to feed for 15 days. One day later, the blood samples of 5 fish from each group were collected as described. During this study, we recorded the mortality rate in all groups. The death percentage of the fish in the treatment groups was 40, 30, 20, respectively, in comparison to the control group. So, we observed a lower mortality rate with the increasing dose of garlic.

Hematological Parameters and Plasma Activities of ALT and AST:

At the end of the experiment, blood samples were collected from the fish caudal vein by a sterile syringe containing EDTA as an anticoagulant. Haematological parameters including erythrocytes count, haemoglobin concentration, hematocrit value, leukocytes count, mean corpuscular volume (MCV), mean corpuscular

haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were measured using the standard routine techniques (Feldman *et al.* 2000). Plasma was obtained by centrifugation at 750 g for 15 min and the non-haemolysed plasma was stored in a freezer at -20°C until analysis. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically using kits supplied by Diamond Diagnostics, according to the Reitman and Frankel method (Coles, 1986).

Statistical Analysis:

One way analysis of variance (ANOVA) and Tukey were used to determine the differences among treatments (mean at a significant level of $P < 0.05$), and standard errors were also estimated. All analysis was run on the computer using the SPSS program.

RESULTS AND DISCUSSION

Results of hemoglobin concentration, hematocrit value, and erythrocytes count (RBCs) are summarized in Table. 1. It shows that diets containing 0.3g/kg, 0.45g/kg, and 0.6g/kg diet of garlic increased some of the examined blood parameters, which were significantly different from those of the control. Lymphocyte count increased significantly in fish fed on diets containing all garlic doses compared with the control group. Lymphocyte count increased significantly in fish fed on diets containing 0.45, and 0.6 g/kg doses compared with in fish fed on diets containing 0.3 g/kg doses ($P < 0.05$). The erythrocytes count in all treatment groups in comparison with the control group showed no significant difference ($P > 0.05$), but among the treatment group, in 0.45 and 0.6 g/kg doses, in comparison with the 0.3 g/kg doses there was a significant increase difference. There was a significant increase in the Leukocyte count in fish fed a diet containing 0.6g/kg garlic compared with the control group and the diets containing 0.3, 0.45g/kg. The mean leukocyte count in all treatment groups were more than the control group. The mean percent of neutrophils decreased significantly in fish fed on diets containing 0.45, and 0.6 g/kg doses compared with in fish fed on diets containing 0.3 g/kg doses ($P < 0.05$). A statistically non significant change in hemoglobin content and hematocrit values in fish fed on garlic was seen when compared with the control group. During the experiment, a non-significantly different mean of MCHC and MCH was observed in all treatment groups in comparison with the control group, but mean of MCH decreased significantly in fish fed on diets containing 0.45g/kg in comparison with in fish fed on diets containing 0.3g/kg. Mean of MCV of fish fed on diets containing 0.45 and 0.6g/kg decreased significantly compared with in fish fed on diets containing 0.3g/kg. An examination of the plasma parameters (ALT and AST) revealed a nonsignificant difference in all treatments compared to the control.

Table 1: Haematological parameters and plasma activities of ALT and AST of *Rainbow trout* fed on diets containing different levels of *Allium sativum*

Treatment	<i>Allium sativum</i> levels (g/kg diet)		Control		<i>Allium sativum</i> levels (g/kg diet)		Control	
Parameters	Stage 1		Stage1		Stage 2		Stage2	
	20	30	40	(0)	20	30	40	(0)
RBC ($\times 10^{12}/L$)	0.99 \pm 0.17	1.36 \pm 0.26	1.19 \pm 0.3	1.02 \pm 0.21	0.84 \pm 0.12	1.20 \pm 0.229	1.28 \pm 0.396	1.13 \pm 0.420
Hemoglobin (g/ L)	110.6 \pm 7.3	132.2 \pm 13.9	123.2 \pm 19	119.8 \pm 13	93.6 \pm 12	117.8 \pm 13.9	119 \pm 23	107.6 \pm 24
PCV (L / L)	0.382 \pm 0.03	0.428 \pm 0.03	0.404 \pm 0.06	0.364 \pm 0.02	0.382 \pm 0.03	0.392 \pm 0.03	0.376 \pm 0.07	0.362 \pm 0.07
MCV(fl)	382.62 \pm 41.47	319.9 \pm 33.5	348.46 \pm 44.1	375.26 \pm 55.5	382.24 \pm 36.28	312.52 \pm 20.69	312.72 \pm 44.08	335.28 \pm 55.3
MCH (pg)	119.68 \pm 10.9	96.54 \pm 13.4	105.9 \pm 11.9	111.38 \pm 11.8	111.52 \pm 11.6	99.52 \pm 10.05	105.62 \pm 22.75	99.04 \pm 6.5
MCHC (g/ L)	314.20 \pm 6.94	308.60 \pm 5.97	305.40 \pm 12.6	304 \pm 5.0	290.80 \pm 12.0	299.80 \pm 9.6	306.20 \pm 9.2	295.80 \pm 6.2
WBC ($\times 10^9/ L$)	4.96 \pm 1.11	5.12 \pm 0.72	7.46 \pm 1.08	4.30 \pm 0.77	6.64 \pm 0.93	10.50 \pm 1.23	17.90 \pm 1.08	4.92 \pm 1.126
Lymphocyte ($\times 10^9/L$)	4.41 \pm 0.05	4.81 \pm 0.05	7.18 \pm 0.09	3.80 \pm 0.10	6.18 \pm 0.10	10.06 \pm 0.17	17.58 \pm 0.23	4.38 \pm 0.07
Neutrophil ($\times 10^9/ L$)	0.54 \pm 0.07	0.31 \pm 0.05	0.28 \pm 0.09	0.50 \pm 0.10	0.46 \pm 0.10	0.44 \pm 0.13	0.32 \pm 0.23	0.54 \pm 0.07
ALT (U/L)	2.56 \pm 0.63	2.94 \pm 0.78	3.75 \pm 0.91	3.30 \pm 0.82	7.10 \pm 1.86	5.38 \pm 1.23	7.01 \pm 1.97	2.60 \pm 0.59
AST (U/L)	122.80 \pm 11.6	146.74 \pm 17.1	154.50 \pm 19.4	150.94 \pm 18.8	171.86 \pm 20.13	202.72 \pm 25.94	271.36 \pm 28.79	194.58 \pm 21.24

Discussion:

The contribution of aquaculture to fish production is steadily increasing. Intensive fish culture creates a highly stressful environment for fish that further suppresses the immune response and outbreak of infection occurs. (Kumari and Sahoo, 2005). The use of immunostimulants is being introduced into fish farming routine procedures as a prophylactic measure. These substances haven't any negative side effects that live vaccines and antibiotics may have on consumers and on the environment, and are generally classified as biological response

modifiers (Anderson, 1992; Secombes, 1994). Immunostimulants can activate fish immune functions, even in immunosuppressive conditions caused by any form of stress or toxic situations. They reverse the deleterious effects mediated by stress (Anderson, 1992; Sakai, 1999; Sahoo and Mukherjee, 2002, 2003).

Immunostimulants may be useful in fish culture and seem to be valuable for the control of fish diseases. It includes polysaccharides, nutritional factors, chemical agents, bacterial components, cytokines, and animal or plant extract. Not only, these immunostimulants facilitate the phagocytosis by phagocytic cells, but also increase their bactericidal activities. The immunostimulants also can stimulate lysosome, natural killer cells, and humoral responses of fish (Lau *et al.*, 1991; Sakai, 1999). The innate immunity in fish can be stimulated by many immunostimulants such as glucan (Jorgensen and Robertsen, 1995; Jeney *et al.*, 1997), kitosan (Siwicki *et al.*, 1994), glucan plus vitamin C (Verlhac *et al.*, 1996), levamisole (Jeney and Anderson, 1993), lipopolisaccharide (Dalmo and Seljelid, 1995), and yeast RNA (Sakai *et al.*, 2001).

Some of the immunostimulants have various disadvantages, such as high cost and limited effectiveness, so cannot use them. There are large numbers of plants which have been used in traditional medicine for the control and treatment of diseases (Duke, 1987). Garlic, an important medicinal plant, has a wide spectrum of actions; not only antiviral, antibacterial, antiprotozoal, and antifungal but also has beneficial effects on the immune and cardiovascular systems (Harris *et al.*, 2001). Garlic contains a therapeutic factor, Germanium, which enhance natural kill cell and macrophage activity in experimental animals (Aso *et al.*, 1985). Humoral innate factors such as lysozyme were higher in garlic treated fish groups compared with the control fish group (Sahu *et al.*, 2006). Ndong and Fall (2006) reported that Garlic at a concentration of 0.5 % over a 2-4 week period in juvenile hybrid tilapia improved lysozyme activity. They also reported that 0.5 % supplementation of garlic had significantly improved respiratory burst, leukocyte count, phagocytic index, and phagocytic activity, indicating the immunostimulant properties of garlic in juvenile hybrid tilapia. Whereas, Juvenile hybrid tilapia fed garlic 1 % showed no improvement in phagocytic activity, lysozyme activity, and phagocytic index, which indicates that the immunostimulant properties of garlic seem to disappear at high concentration (Ndong and Fall, 2006). Chemical complexity and broad-spectrum action do not promote acquisition of antibiotic resistance. In addition, direct intragastric effects are feasible because *Allium sativum* antimicrobials are not affected by acid environments (Lawson, 1996); otherwise the gastric juice enhances the antimicrobial activity of *Allium sativum* constituents (Fortunator, 1995).

Immunostimulants attach to specific receptors on the cell surface of the phagocytes and lymphocytes activating this cell to produce some enzymes that can destroy pathogens. Moreover, they can increase the production of some chemical messengers (interferon, interleukins and complement proteins) that stimulate other arms of the immune system and increase the activity of T and B lymphocytes (Raa *et al.*, 1992). Garlic (*Allium sativum*) has traditionally had dietary and medicinal applications as an anti-infective agent against many bacteria (Ress *et al.*, 1993), fungi (Adetumbi *et al.*, 1986) and viruses (Weber *et al.*, 1992). Its antibacterial action depends on allicin and is thought to be due to multiple inhibitory effects on various thiol-dependent enzymatic systems (Ankri and Mirelman, 1999). Allicin is formed catalytically by crushing raw garlic or adding water to dried garlic when the enzyme alliinase comes into contact with alliin. Steam distillation of mashed garlic produces garlic oil containing methyl and allyl sulphides of allicin, having the practical advantage of being more stable than allicin itself. Individual constituents of garlic oil and garlic powder have shown a range of potencies when tested *in vitro* against human enteric bacteria including *H. pylori* (O'Gara *et al.*, 2000; Ross *et al.*, 2001). The role of some immunostimulants in the anabolism after a prolonged period of administration was investigated. Garlic contains allicin, which promotes biogenic performance due to its positive effect on the intestinal flora, thereby improving digestion, availability of natural feed, supply of nutrients and utilization of energy which influences the growth of fish (Khalil *et al.*, 2001). Extracts of fresh garlic contain antioxidant phytochemicals that prevent oxidant damage. These include unique water-soluble organosulfer compounds, lipid-soluble organosulfer components and flavonoids, notably alilixin and selenium. These extracts enhance the cellular oxidant enzymes superoxide dismutase, catalase and glutathione peroxidase (Borek, 2001).

Immunostimulants can increase non-specific immunity by either increasing the number of phagocytes or activating phagocytosis (Shoemaker *et al.*, 1997). Many defense mechanisms activated by garlic counteract the challenge infection including the production of superoxide anions against the *A. hydrophila* infection. It has been found that the aqueous extract of raw garlic and dried powder scavenge hydroxyl radicals (Yang *et al.*, 1993; Kim *et al.*, 2001), and superoxide anions (Kim *et al.*, 2001). The percent volume of erythrocytes in fish blood gives a clue to the health status of fish and can be helpful in determining any abnormalities arising from the use of immunostimulants. Anemic fish may have hematocrit as low as 10 %. Reduced hematocrit may indicate that fish are not eating or are suffering infections (Blaxhall, 1972). The employment of hematological techniques, including evaluation of erythrocyte count, haemoglobin concentration, hematocrit value and leukocyte

count, has provided valuable knowledge for fishery biologists in the assessment of fish health (Blaxhall, 1972). These results reflect the health status of fish cultured with all treatments. Addition of *Allium sativum* to fish diets increased the erythrocyte number, haemoglobin concentration, hematocrit value, leucocytes, and thrombocytes (Martins *et al.*, 2002). *Allium sativum* has some constituents that may play a role in the immune system stimulation and in the function of organs related to blood cell formation such as thymus, spleen, and bone marrow (Jeorg and Lee, 1998). Also, Faisal (2003) reported significantly increased values of erythrocyte count, haemoglobin concentration, and hematocrit value in catfish (*Clarias farias*) at the 1st and 3rd days after administration of ciprofloxacin, amoxycillin and ampicillin. Blood indices (MCV, MCH and MCHC) are particularly important for the diagnosis of anemia in most animals (Coles, 1986). This study showed a significant decrease of MCH and MCV in fish fed on the highest level of *Allium sativum*. So, it is assumed that the decrease or increase of blood indices may be attributed to a defence reaction against *Allium sativum*, which occurs by stimulation of erythropoiesis. Changes in the physiological state often reflect alteration of hematological and biochemical values. Clinical chemical analysis is a fundamental tool used to diagnose and predict the outcome of diseases and to monitor the effects of therapeutic, nutritional and environmental management in human and veterinary medicine. Blood biochemical values are not commonly used as a diagnostic tool in fish medicine, partly because of the lack of reference intervals for various fish species, and also because changes in blood analysis associated with specific diseases and metabolic disorders are not well characterized with sufficient background data; thus, clinical biochemical analysis could be developed to detect metabolic disorders and sublethal diseases that affect the production efficiency (Shalaby *et al.*, 2006). Results of one study showed that serum AST and ALT activities decreased significantly in the fish group fed on all levels of *Allium sativum* and chloramphenicol (Shalaby *et al.*, 2006). These data agree with those reported by El-Shater *et al.* (1997) and Augusti *et al.* (2001), who found that the lipid parameters and enzyme activities (AST, ALT, and ALP) in rat serum decreased significantly when they were fed a diet containing 5% *Allium sativum*. Also, Faisal (2003) mentioned reduced AST in the serum of catfish after ampicillin administration, but no different was observed in the serum AST and ALT in the treatment groups in comparison with the control group.

These results can be attributed to *Allium sativum*, which may cause stabilization cell membrane and protect the liver against deleterious agents and free radical-mediated toxic damages to the liver cells. This is reflected in the reduction of liver enzymes. *Allium sativum* helps the liver to maintain its normal function by accelerating the regenerative capacity of its cells. In conclusion, utilization of garlic, balanced and formulated in a suitable dose can decrease mortality rate and increase immunity.

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