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Study on Growth Performance, Survival Rate, Hematological Parameters in Rainbow Trout (*Oncorhynchos mykiss*) in Mazandaran Province of Iran

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Abstract: The aim of this study was the evaluation of the immunostimulatory effects of alginic acid and anti-streptococcus vaccine on the Growth Rate (GR), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Condition Factor (KF) and Survival Rate (SR%). The weight range of fish were 5-8 g and the trials were divided to 4 treatments with triplicate groups including control, vaccine, vaccine+alginic acid and alginic acid and each treatment included 3000 fishes. The period of this study was 4 month and biometry was done 6 times (every 20 days intervals) at the end of experimental period the blood samples were taken from all treatments and hematological parameters were evaluated. The results showed that GR had a significant increase in vaccine+alginic acid treatment when compared with the control group ($p<0.05$). SR% showed a significant increase in vaccine+alginic acid and alginic acid treatment in compared with control group ($p<0.05$). Significant elevation in the percentage of lymphocyte observed in vaccine, vaccine+alginic acid and alginic acid treatment when compared with control group have observed. According to the results, it seems that prescription of vaccine and alginic acid were stimulated lymphocyte proliferation in all treated groups and therefore fish resistance increase to environmental stress and pathogen.

Key words: Rainbow trout, hematological parameters, growth, alginic acid

INTRODUCTION

The characteristic that caused interest to rainbow trout fish is its adaptation with density of culture conditions, resistance to in undesirable environments, good adaptation to handle feeding and having high speed development (Vosoghi and Mostajir, 1998). Streptococcusis is the disease resulted from different species of *Streptococcus* bacteria.

This disease was initially described among population of rainbow trout farmed in Japan. Then, the disease has assumed importance of rainbow trout farms of within South Africa, USA, Great Britain and Norway (Austin and Austin, 1987). Streptococcusis segregated in Iran from sea bass in Khozestan Province in 1996 (Mazlomi, 2003), in rainbow trout farms from Mazandaran Province in 2000 (Ghiasi *et al*., 2000) and in rainbow trout farms from Fars Province in 2003 (Mazlomi, 2003) and cnidaria (*Beroe ovata*) digestive system in Caspian sea in 2002 (Zahedi, 2002). Because of high losses for this disease (that sometimes estimated to 80-90%) from 1995, using vaccine against this disease started that had high effects on this disease reduction (Bachrach *et al*., 2001). Immunostimulants can reduced the losses caused by disease in aquaculture, however, they may not be effective against all disease (Sakai, 1998). Alginic acid is indicated as immunostimulant. Alginic acid is composed of 0.002% unspecified plant extract, 1% alginic acid from *Laminaria digitata* and 98.998% algae based carrier (Peddie *et al*., 2002). That result in oxygen metabolism increase and so improving in free radicals production of oxygen in macrophages. Immunostimulants are activators of white blood cell

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(Raa, 2000). Lymphocytes are one of most important protective factors against microbial factors. This group of the cells has a phagocytosis and antibody production availability and measuring the numbers and percentages are one of main factors deciding immune system (George and Teroki, 1999).

MATERIALS AND METHODS

Adaptation

In this research that conducted in Ecological Institute of Caspian sea. In 2007, 12000 rainbow trout fingerlings weighing 5-8 g were used, that were signified non-polluting to streptococcus by assured clients. Fish were kept in 1000 sets in 12 round pools (3 m diameter and 80 cm depth with the volume of 2 m^3).

Fish Feeding

The fingerlings were introduced in pond for adapting in new conditions and fed them 3 times per days. At the primary stages of culturing SFT and during growth FFT1 were provided by a well-known manufacturer of rainbow trout food.

Biometry

During the course of examination 30 specimens from each treatment were sampled for biometrical parameters in 20 days intervals. During the final sampling, at the end of the farm trial, all the fish were counted and weighed, in order to indicate the group mortality and survival rate and analyzed.

Vaccination

The used vaccine (Aquavac Garvetil) contained two important pathogenic varieties responsible for fish streptococcosis that include inactive vaccine of pathogenic bacteria *Streptococcus iniae* (1×10^9 jerm mL^{-1}) and *Loctococcus garviea* (1×10^9 jerm mL^{-1}) maintaining vector antigen profile and include two stages of vaccination. The primary vaccinated with immersion in 9 L pond water and 1 L vaccine at 60 sec for vaccination of 100 kg of fingerlings. Eighty days after the primary vaccination, the level of safety, the second stage vaccine was repeated, as oral vaccine, during 5 days, 0.01 mL to each fish in every day.

Alginic Acid

After adaptation, 0.5%, the alginic acid was mixed with the food and prescribed to vaccine+alginic acid and alginic acid treatments. Alginic acid prescribing method combined to the list: 10 days alginic acid for 0.5% with food, 10 days common food, 10 days alginic acid for 0.5% with food, 10 days alginic acid for 0.5%, 20 days common food and 10 days alginic acid for 0.5%.

Hematology Experiments

Fifteen days after the second stage of vaccination, the samples of blood were gathered. Of each treatment 15 were bleed averaging 110 g. Then counting Red Blood Cells (RBC), White Blood Cells (WBC), hematocrit (PCV or HCT), hemoglobin (HB), (MCV), (MCH), (MCHC), differential counting of white blood cell (Simons, 1997; Tabarestani, 1985).

Statistical Analysis

Statistical method based on complete random design. Data analysis was performed using the SPSS software statistical package. One-way ANOVA was applied to detect any difference due to immunostimulant and vaccine at different sampling times. When variance was found not homogeneous, the Duncan test was used for multiple comparisons. The level for accepted statistical significance was $p<0.05$.

RESULTS AND DISCUSSION

The result of the analysis, after 4 months, in the Table 1, show that the growth rate in the treatment group vaccine+alginic acid has a meaningful difference with the control group ($p<0.05$). The other growth performance, despite the numerical nobility, there was no meaningful statistical difference ($p>0.05$). The survival rate in the treatment groups vaccine+alginic acid, alginic acid have a meaningful difference compared with the control group ($p<0.05$), while the vaccine treatment has not shown any meaningful difference compared with the control group ($p>0.05$). In blood parameters the RBC, HCT or PCV, MCV, HB, MCH, MCHC shown no meaningful difference compared with the control group ($p>0.05$). The WBC of treatment group compared with the control group showed a numerical increase but there was no statistical difference ($p>0.05$). In differential counting of WBC, the number of lymphocyte in the treatment groups of vaccine, vaccine+alginic acid, alginic acid showed a meaningful difference compared with the control group ($p<0.05$).

Fish growth performance is affected by different factors including water quality, stresses and diseases, diet quality and quantity and etc. In addition, growth promoting activity has been noted in fish or shrimp treated with Glucan and Lactoferin (Sakai, 1998). Boonyaratpalin *et al.* (1995) reported that black tiger shrimp fed with peptidoglycan-supplemented feed showed better growth and feed conversion rates than those fed a normal diet. The Levamizole hydrochloride is an immunostimulant enhance the metabolic in mammals (Sakai, 1998). The oral administration of Levamizole hydrochloride causes 20% increase biomass in Russian sturgeon (*Acipencer baeri*) and Bester (Vahabzadeh, 2003).

In addition, adding 100 mg Levamizole for each kg of food to stripped bass diet showed a meaningful increase in growth compared with control group (Li *et al*., 2006). Alginic acid also is known as an immunomodulatory (Montero-Rocha *et al*., 2006).

In one survey Montero-Rocha *et al*. (2006) reported that alginic acid was administrated orally to intermolt adult white shrimp after 15 days. Analysis of data concerning relative and absolute growth in weight and length showed that incorporation of alginic acid in to diets at the concentration of 0.5% (w/w) enhanced the growth of the shrimp over the 15 days experimental period. So, the use of alginic acid as a dietary supplement was observed to exert positive physiology in white shrimp. The long-time and short-time effects of a dietary yeast β-glucan (0.1%) and alginic acid (0.5%) in sea bass showed that the growth performance did not significantly in the treatment fish compared with control. While during the coldest seasons fish reduce feeding and in such conditions administration of glucan and alginic acid induce a positive effect on the lowest temperature (Bagni *et al*., 2004). Also the researches showed that using the vaccine Aquavac Garvetil improve the growth performance.

Inspiriting these, this theory about rainbow trout was done and growth performance was surveyed in different treatments. According aforementioned results in Table 1 between surveyed growth performance, the special growth rate between vaccine+alginic acid treatment compared with the control group showed a meaningful increase ($p<0.05$). This explains that fish along with the environment stresses also had good appetite for feeding (Montero-Rocha *et al*., 2006). The oral administration of 1, 3-β-glucan, Levazmizol, vitamin C and E as immunomodulatory in Indian carp fish

Table 1: Comparing the growth performance and survival rate in treatments with control group using Duncan test ($p<0.05$)

	Treatments			
Parameters	Control	Vaccine	Vaccine+alginic acid	Alginic acid
Growth rate	68.01±4.36^{b}	71.41±4.98^{ab}	75.62±1.88^{a}	72.01±1.72^{ab}
Specific growth rate	1.05±0.03^{a}	1.08±0.06^{a}	1.13±0.01^{a}	1.09±0.00
Feed conversion ratio	1.30±0.27^{a}	1.27±0.09^{a}	1.16±0.09^{a}	1.23±0.09^{a}
Condition factor	1.73±0.03^{a}	1.35±0.03^{a}	1.38±0.03^{a}	1.33±0.10
Survival rate (%)	84.20±4.70^{c}	89.10±1.32^{bc}	96.03±0.90	94.53±3.81^{ab}

Unlike symbolic letter(s) are signs of meaningful difference

(*Labeo rohita*) decreased mortality rate meaningfully (Sahoo and Mukherjce, 2001). The immersion administration of Levamizole hydrochloride results in decreasing loss in larvae of two species: Siberian and Persian sturgeon in ponds (Siwicki, 1989) and also oral administration of this drug had the same results in sea bass. Alginic acid, as an immunostimulatory, reinforces fish defensive system. This action results in increase of fish resistance against infections and increases their ability for rehabilitation of affected tissues. Also results in improving mucus producing cells actions and skin covering cells that so decrease the possibility of pathogenic factors contact that cause diseases. The studies showed that alginic acid consumption results in mortality of fish that is the result of season changes, temperature fluctuations, water quality, high density and fish handling (fishing, sorting, fish transportation). Basically, stress is known as one of the weakening factors of fish immune system which finally results in decreasing survival rate (George and Teroki, 1999). Also showed that using vaccine anti-streptococcusis (Aquavac Garvetil) result in increase in survival rate. According to studies done, survival rate in alginic acid+vaccine treatment compared with control group had meaningful increase ($p<0.05$). It is necessary to mention that in vaccine treatment, survival rate compared with control group is more but did not meaningful. Maybe, the use of alginic acid as immunostimulant results in resistance to environmental stimulus and stress, so increasing the function of metabolism and finally growth performance improvement and survival rate increases.

The results in Table 1 shows that alginic acid has not much effect on RBC, HCT or PCV, MCV, HB, MCH, MCHC. Nowadays materials called immunostimulant that result in the number and the power of phagocytosis in lymphocytes (Raa, 2000). Materials like Levamizole hydrochloride, alginic acid, bovine Lactoferine, vitamin C and E nowadays are known as immunostimulatories of fish and shrimp (Anderson and Siwicki, 1989; Mulero *et al*., 1995; Montero-Rocha *et al*., 2006; Sakai, 1998). The oral administration bovine lactoferin showed increased number of lymphocytes in the blood in sea bass (*P. major*) (Sakai, 1998). The using of high doses vitamin C results in proliferation of rainbow trout lymphocytes. Furthermore, cat fish fed high dose of vitamin E had an increasing the power of lymphocytes phagocytosis (Sakai, 1998). In a study, following intraperitoneal administration of alginic acid to rainbow trout (100-500 g) in dosage 1 mg stimulated immune system (Peddie *et al*., 2002). Immune modulation of both rainbow trout (*Oncorhynchos mykiss*) and stripped snaked (*Channa striata*) has been observed following intraperitoneal administration alginic acid (Montero-Rocha *et al*., 2006). Alginic acid increases lymphocytes and macrophages reproduction and production of cytokinin and lysozyme and also reacting to vaccination in fish is enhanced. During done surveys by Alishahi (2006) using vaccine anti-streptococcus results increasing white blood cell and lymphocytes population. The aforementioned results in Table 2 show the accounted white blood cell in vaccine, alginic acid+vaccine and alginic acid compared with control group, in addition to numerical nobility, do not show meaningful difference. In vaccinated treatments because of strain of streptococcus inactive bacteries, possibility specific immune (the antibody titr level) raised that

Table 2: Comparing the blood parameters in treatments using Duncan test ($p<0.05$)

	Treatments			
Parameters	Control	Vaccine	Vaccine+alginic acid	Alginic acid
Red blood cell$\times10^5$ (n mm^{-3})	9.49±0.71^a	9.63±3.33^a	10.10±1.18^a	10.45±0.97^a
White blood cell$\times10^2$ (n mm^{-3})	11.30±2.05^a	12.50±0.72^a	13.10±2.09^a	13.20±1.72^a
Hematocrit or packed cell volume (%)	21.10±0.65^a	21.40±3.85^a	23.63±2.55^a	24.30±6.20^a
Hemoglobin (g dL^{-1})	5.23±0.64^a	5.54±1.20^a	5.50±0.81^a	5.68±0.50^a
Mean corpuscular volume (fl)	222.66±9.29^a	221.00±44.84^a	238.33±33.3^a	228.58±51.01^a
Mean corpuscular hemoglobin (pg)	55.33±6.50^a	57.66±14.57^a	54.00±1.73^a	56.66±3.21^a
Mean corpuscular hemoglobin concentration (g dL^{-1})	26.66±3.21^a	23.33±5.13^a	23.33±4.10^a	25.00±5.00^a
Lymphocyte (%)	82.00±5.10^b	97.00±2.00^a	95.00±2.60^a	96.00±4.10^a

Unlike symbolic letter(s) are signs of meaningful difference

weren't surveyed. The results of Table 1 shows that the lymphocyte average percentage between control group with three test treatments showed meaningful difference ($p<0.05$). Maybe the increase of the percentage of lymphocytes in this survey along with increasing the white blood cell rate results in increase resistance to environment motivation and stress cause increase in metabolism and finally growth performance improvement and survival rate enhanced.

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