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Evaluation of Resistance in *Acipenser percicus* Larvae Fed with Bioencapsulated *Daphnia magna* via *Saccharomyces cerevisiae* Product (Amax) Against Challenge Test

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Abstract: The aim of this study was to estimate resistance of *Acipencer percicus* larvae against salinity, temperature, ammonia and pH stress at the end of experimental period. Experimental treatments Persian sturgeon larvae were fed by bioencapsulated Daphnia with *Saccharomyces cerevisiae* product (Amax) in three concentrations (50, 100 and 150 mg/l Amax) for four consecutive weeks also control larvae were fed by unbioencapsulated Daphnia. They were acclimatized to the laboratory conditions for 10 days before the start of the experiment. Moreover, a 12 h dark: 12 h light photoperiod was provided. We prepared five stressor factors to challenge *Acipencer percicus* after 28 days feeding trials that are included: pH (2 and 13), salinity (120 ppm/l), temperature (35°C) and ammonia (2.5mg/l). Experimental larvae after feeding via bioencapsulated Daphnia by *Saccharomyces cerevisiae* extract (Amax) gave a relative percentage survival rate in comparison with control treatment and also during of experiment survival rate of experimental groups was significantly higher than control ($P<0.05$). The analyses were based on challenge test data (in seconds) from four treatments. In conclusion, bioencapsulated *Daphnia* with yeast product could be enhances the resistance against stress and disease in *Acipencer percicus*.

Key words: Persian sturgeon · *Daphnia* · Ammonia · Challenge · Bioencapsulated

INTRODUCTION

Acipenser persicus is an important native species in Iran. Live food (as *Daphnia magna*) have been used as vectors for delivering compounds of diverse nutritional and/or therapeutic value to larval stages of aquatic animals [1], in a process known as bioencapsulation. The *Daphnia magna* is common live food organisms used for the rearing of marine fish larvae. These have been considered as possible vectors for the delivery of different substances, such as nutrients and probiotics. Intensive rearing of marine fish larvae suffers from heavy mortalities, which may be attributed to bacteria introduced in the rearing system with live food [2]. In the last decade, the scientific community carefully examined roles and effects of probiotics in aquaculture as an alternative to antimicrobial drugs, demonstrating positive effects on fish survival [3], growth [4], stress resistance [5], immunosystem enhancement [6] and finally general welfare [7].

The use of natural prophylactic supplements in place of chemotherapeutics in aquaculture has received a great deal of attention in the past decade; such preventive products include probiotics. These biotics can be applied through external bathing or dietary supplementation and have been demonstrated to improve growth performance, feed utilization, digestibility of dietary ingredients, disease resistance and stimulate the immune response of aquatic animals [8-10]. The use of natural immunostimulants is promising in aquaculture because they are safe for the environment and human health, biocompatible and biodegradable [11]. Many studies have looked into the modulation of the immune system in fish as a means to prevent disease outbreaks [12]. Finding a definition for stress is a difficult task. Levin [13] is often quoted as being uncertain whether anyone attempting to define stress "either has an enormous ego, is immeasurably stupid, or is totally mad." The general definition by Selye [14] stating that, stress is the response of an organism to any demand placed on it such that it causes an extension

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of a physical state beyond the normal resting state, may be the most useful when considering fish in culture systems. Challenge tests were proposed as meaningful tools for assessing fish quality in the aquaculture industry, environmental resources management and researches [15]. The concept is based on the presumptions that stress loading above the acclimation capacity of an organism will weaken it and reduce performance in growth, survival and reproduction and that the reduction in performance can be quantified by assessing tolerance to reference stressors [16]. Performance tests which were described included; thermal, hypoxia, chemical and salinity tolerance tests, swimming performance, resistance to disease, crowding and handling tests. Literature on the use of stress challenge tests for determining finfish quality is scarce. Salinity challenges have been used to determine the hypoosmoregulatory capability (osmoltification) of juvenile anadromous salmonids [17]. McGeer, Baranyi and Iwama [18] proposed that a battery of challenge environmental degradation and verifying the effectiveness of tests could be useful in selecting for genotypes which are stress-tolerant, improving stock, monitoring management practices. The challenge tests were performed at VESO Vikan, which is licensed to perform challenge trials with fish pathogens. A standardized bath challenge [19] was applied.

Changes in the nervous and endocrine systems of fish after stress episodes have consequences on their immune system and thereby affect the ability to maintain immunocompetence. Fish mainly depend upon innate immune responses, which include a rich and powerful array of mechanisms that appear to be more potent than in higher vertebrates. Thus, fish provide a unique model to understand the evolution of immune defense system. When the organism is challenged by an antigen or by stressors, a number of responses of reactive nature are engaged in an attempt to counteract the threat and recover homeostasis. However, if the challenge is maintained, changes in the immune system become chronic and suppression can be observed in several key immune mechanisms, leading to maladaptation. Therefore, the time factor is of key importance in immune assessment. The range-finding tests were necessary because of the lack of literature on such short-term lethal tests. Taking into account this dynamic pattern of infection and stress, specific indicators should be identified in order to detect functional changes in the immune system. challenge test to be a useful tool for estimates of fish quality, particularly for farm gate testing where there may be rapid turnover of large numbers of fry, it was necessary to develop a test procedure that involved a shorter exposure period, that could be used by farmers .Another consideration in developing the test was that it should be easy to perform.

The aim of this study was to determine the effects of yeast product against stressor factor such as pH, salinity, temperature and ammonia in *Acipenser persicus* larvae after 28 days feeding by bioencapsulated *Daphnia.*

MATERIALS AND METHODS

Ten-day old healthy larvae of Persian sturgeon (*Acipenser persicus*) with initial weight of 80±7 mg and total length of 22±5 mm were obtained from Hatchery of Marjanii sturgeon center, Golestan, Iran. The Baker's yeast (*Saccharomyces cerevisiae*) product under the commercial title of Amax (contain 1×10^{10} cells mg^{-1}) was prepared from Doxal Co. (Italy). The *Daphnia magna* was cultured in earthy ponds in Marjanii sturgeon center. Three concentrations of yeast suspension (50, 100 and 150 mg/l Amax in suspension of broth) were provided. Twelve fiberglass tanks (capacity of 50 liters) with three replicates for experimental treatment and control were used. The density of fish larvae was 70 fish/tank. After 10 h the bioencapsulated *Daphnia magna* was collected on a 120 mm-pore-size sieve, washed with fresh water and used as a live food and vector to carry Amax to digestive system of *Acipenser persicus* larvae. In experimental treatments of T1, T2 and T3 the Persian sturgeon larvae were fed by bioencapsulated *D. magna* by 50, 100 and 150 mg/l Amax. In control the fish larvae were fed on unbioencapsulated *D. magna*, each treatment was in triplicate. Sturgeon larvae were fed based on the 30% of their body weight for six times a day at 2.00, 7.00, 12.00, 17.00 and 22.00 with bioencapsulated *Daphnia magna* in experimental treatments and unbioencapsulated *Daphnia magna* in control treatment respectively.

Each rearing tank was supplied with running fresh water which had been filtered through the special cotton filter (flow rate was one L min^{-1}). Water quality parameters from every tank were monitored each week throughout the experiment. The water temperature was 19.8±0.6°C, pH was 7.6-8.3 and water oxygen level was maintained above 7.5 mg-1 $^{-1}$ during the experiment by setting electrical air pump. In the termination of experiment (after 28 days), 5 larvae from each replicate collected randomly and transferred to twelve 10-liter uncircular fiberglass test tanks (5 larvae per each test tank) without use any electrical air pump in these tanks.

Range-Finding Tests: To determine the range of concentrations to be used for 10 minute LC50 testing, larvae which had been conditioned for packing were exposed to salinities of 0 (control), 30, 60, 90, 120 and 150 ppm for 10 minute. For temperature range, larvae were exposed to 20, 25, 30, 35, 40°C. To determine the range of pH, five concentrations of 11, 11.5, 12, 12.5 and 13 (for alkalinity challenge) and concentrations of 1, 1.5, 2, 2.5 and 3 (for acidity challenge) were used. For assign range of ammonia, *Acipenser persicus* larvae were exposed to concentrations of 1, 1.5, 2, 2.5 and 3 mg l^{-1}, three replicates of each concentration were used. Mortality at 10 minute was noted for calculation of LC50.

pH Challenge: For pH challenge after feeding trial 60 fish (5 fish than each replicate) were exposed to natural acid (average pH 2) and non-acid source water (average pH 13). Sulfuric acid (H2SO4) added into water to increase toxicity and limestone or sodium-silicate to reduce toxicity. At the period of challenge, pH was checked by pH meter. After exposure, fish mortality was counted.

Salinity Challenge: A 120 ppm saltwater (SW) static aquarium was produced by adding salt to freshwater from the fish's environment. Test fish are quickly captured and placed in the SW aquarium. Three groups of experimental feeding fish and one group of control fish were placed into separate tank. At the end, dead time of fish was recorded in seconds.

Thermal Challenge: To determine effect of feeding with Amax in comparison control treatment thermal challenge was conducted. Five *Acipenser persicus* larvae were collected from each tank. The larvae were exposed to heat shock (35°C). Tank water temperature was monitored minutely from beginning to end of the experiment. After heat stress, larval resistance and mortality against stressor was recorded. Death was defined as the point at which fish lost balance.

Ammonia Challenge: To examine the effect of high ammonia exposure, five *Acipenser persicus* larvae from each tank were challenged with elevated water ammonia. *Acipenser persicus* larvae were exposed to 2.5mg/l for challenge (pH 7.5) during exposure. At the end of the exposure period, time of resistance against stressor was recorded.

Data Analyze: Results analyzed by one-way ANOVA and significant differences determined by Duncan test. All statistical analyses were performed using the software SPSS 15.0 for Windows.

RESULTS

The stress parameter levels are indicated in table 1. Our results revealed that, *Saccharomyces cerevisiae* product (Amax) has been shown to enhance the non specific cellular immunity were significantly increased at a dose 150 mg/l Amax at 4th week post treatment in compared with the control group ($P<0.05$). It was observed that the mortalities among the challenged fish are dose related. Generally, mortality rates post challenge tests were significantly lesser in experimental treatments in comparison to control ($P<0.05$). In the first experiment (pH challenge), the mortalities of larvae fed with Amax were significantly lower than the mortality than control group for both of alkalinity and acidity challenge ($P<0.05$). Moreover, in this experiment, *Acipencer percicus* larvae showed higher resistance for exposure the acidity condition compares alkalinity environment. In the second experiment (Thermal challenge), there was no significant difference in resistance between experimental treatment and the control in thermal challenge ($P<0.05$). In the third experiment (Salinity challenge), the mortality of larvae were fed with yeast probiotic was also significantly lower (258, 296 and 318 second, respectively) than the control group (178 second). In the fourth experiment (Ammonia challenge), fishes used for ammonia exposure showed significant differences in mortality and resistance ($P<0.05$).

Table 1: Evaluation of resistance in larvae against challenge test based on second

	Treatment			
Stressor	C	T1	T2	T3
Ph(13)	117+11.78b	158+6.08a	178+3.78a	175+10.50a
Ph(2)	277+10.22b	349+8.51a	364+9.47a	364+12.22a
Salinity (120 ppm)	178+14.01c	258+12.65b	296+13.58ab	318+11.13a
Temperature (35°C)	53+16.09a	83+15.03a	87+15.18a	89+18.17a
Ammonia (2.5 mg/l)	136+9.01c	214+12.27b	244+15.24a	255+13.22a

The larvae witch were fed with enriched *daphnia* had higher ability to resist stressor comparison larvae were fed not enriched Daphnia (control).

DISCUSSION

The effects of probiotics have been widely studied in cultured aquatic species, particularly the enhancement of the non-specific immune system [20] which is favorable to pathogen control [21]. Yeasts are particularly interesting probiotic, because they provide β- glucans and nucleotides that stimulate the immune system of fish [22, 23]. There is evidence that the administration of glycans extracted from the cell wall of *Saccharomyces cerevisi*ae induces increased resistance to infection by *Vibrio anguillarum*, *V. salmonicida* and *Yersinia ruckeri* in Atlantic salmon (*Salmo salar*) [24, 25], also showed an increased of the survival and digestive enzyme activity in *Dicentrarchus labrax* larvae fed diet containing *Debaryomyces hansenii* [26]. This investigation demonstrated that beta-glucans and nucleotides might be useful to increase disease resistance of fish. When these immunostimulants were added to the feed, one possible explanation for the results of the first experiment is that the mechanism of action of these compounds may be through the stimulation of the nonspecific immune system, which has been observed in other investigations [27, 28]. The efficacy of beta-glucans in increasing disease resistance has also been documented in other investigations. Whereas, lower mortality was observed in swordtails, rosy barbs and black tetras challenged with *Aeromonas hydrophila* or *Pseudomonas fluorescens* [29] fed beta-glucans. However, in some cases, an enhancement of the nonspecific immune response but no increased survival was measured in fish fed glucans, as in catfish challenged with *E. ictaluri* [30] and in turbot challenged with *Vibrio anguillarum* [31]. *Saccharomyces cerevisiae* product (Amax) was able in enhancing the non specific cellular immunity at a dose 150 mg/l for 4th week feeding procedure. Similarly, rainbow trout fed a commercial diet supplemented with 100 mg/kg diet for 1 week enhanced the cellular immune response, leukocytes peroxidase content, respiratory burst and phagocytic index in gilthead seabream fish [32]. Mortality rates among the challenged *Acipencer percicus* larvae with pH, thermal, salinity and ammonia were significantly lower in the experimental groups in comparison with control. Several studies have also observed increased disease resistance in fish fed diets supplemented with *Saccharomyces cerevisiae*. Similar studies dealing with the capacity of yeast or structural polysaccharides to improve disease resistance in fish showed their capacity to reduce mortalities associated with infection by pathogens such as *Aeromonas* [33].

In conclusion, this study supports the hypothesis that beta-glucans and nucleotides can help improve disease resistance in fish. However, their effectiveness may vary among facilities and production units because of the influence of many confounding factors (genetics, nutrition quality, stress, water temperature and handling). It is important to develop educational and demonstrative programs for commercial fish farmers to promote and demonstrate the efficacy of preventive medicine that include also the use of vaccine and immunostimulants, instead of the mass application of drugs and antibiotics.

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