Evaluation of Aqualase as Probiotic on Wild Carp (Cyprinius Carpio) Growth, Immunity Characteristics and Resistance to Streptococcosis

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Abstract: Aqualase is a yeast probiotic that include Saccharomyces cerevisiae and Saccharomyces elipsoedas. Fish were fed with carp commercial food contained 26% protein, 10% lipid and estimated digestible energy level of 3250 cal. Aqualase was supplemented in feed in three dosages of 0.1%, 0.15% and 0.2%. The study was carried out on wild common carp (Cyprinud carpio L.) fingerlings (Average 12.4 g), reared in 2×2×0.5 m³ polyethylene tank for 60 days. Each treatment tested in triplicate. The result showed feed enriched 0.15% and 0.2% Aqualase has been increased most growth factors including weight gained, condition factor, growth rate, special growth rate, however the differences with control was not significant P>0.05. Diet supplementation increased stimulated serum lysozyme levels as well as the value C3 and C4 complements, but maximum of immunoglobulin IgM level between two stages was exhibited in diet 0.15% Alkaline phosphatase was significantly difference after 60 day, using supplemented diet with 0.15% Aqualase. At the end of 60 days treatments, the fish were challenged with Streptococcus iniae for 15 days. The results were showed that mortality percent in the control groups was 13% but the treatment groups were not observed any mortality (p<0.05). The present study indicated that Aqualase positively enhanced growth performance and immune response and resistance to Streptococcus iniae infection. Optimum level of dietary Aqualase suggested is 1.5 g per kg diet (0.15%).

Key words: Aqualase, complement, immunoglobulin, lysozyme, probiotic, wild carp, Streptococcus iniae

INTRODUCTION

Wild common carp of Caspian Sea is one of the important fish in north of Iran. It has low growth rate comparing to farm adapted once. To increase the grow performance a series of experiment have been done before introducing it to farmers. In the other hand to reduce mortality, prevention and control of disease antibiotics used as a traditional strategy for aquatic disease management, but they exhibited suppress the immune system, potentially making aquacultured organisms more susceptible to viral or parasitic infections. Thus in recent years there has been heightened research in developing dietary supplementation strategies in which various health promoting compounds have been evaluated4,6).

Probiotics have been defined as viable microbial food supplements that beneficially influence the health of the host3]. The beneficial effects of probiotics may be mediated by competition for specific pathogen receptor sites on the mucosal surface, production of inhibitory compounds, competition for nutritional sub states, or by an enhancement of the host s innate and adaptive immune responses2,31). The Aqualase is a commercial probiotic that is produced by Italy country.

This material includes two species yeast of Saccharomyces cerevisiae and Saccharomyces elipsoedas31].

Present study was conducted to determine the effect of Aqualase on growth performance, innate and adaptive immunity and resistance of Caspian Sea wild carp to Streptococcosis.

MATERIAL AND METHODS

Fish Rearing: The study was carried out on wild Carp (Cyprinius carpio) fingering of Caspian Sea in two series of time of 2009 and 2010, with average weight 12.4 g, obtained from Restocking Center of S.R.Sari, Iran. The fish were acclimated in river water for two week before the start of the experiments. A hundred fish from each treatment were reared in 2×2×0.5 m³ polyethylene tank for 60 days. At the beginning of study the fish from each tank were randomly checked for any disease. Water quality was checked periodically (dissolved oxygen: 6.6±0.43 mg/L; pH: 8.27±0.15; nitrat: 0.31±0.28 mg/L) throughout the experiment. The water temperature during the experiment ranged about 28 ± 0.58°C.
**Experimental Diet:** In order to determine the effect of Aqualase on fish performance, fish were fed with carp commercial food 26% protein, 10% lipid, 6.5% fiber and 3250 cal digestibility energy (Abzian Co, Sari, Iran), contained three levels of Aqualase commercial probiotic (Tepax, Italia) as treatments (0.1%, 0.15% and 0.2%), also a group of fish was fed with commercial carp food as control.

**Sampling:** Fish were sampled at the days of 30 and 60 to determine the immunity parameters. 10 fish from each treatments were anaesthetized (clove oil: 20 mg/L) and blood taken from the caudal vein (Abdel-Tawwab et al., 2008) using a medical syringe (2ml). Serum was separated by centrifugation at 3000 g for 15 min at 4°C and it was stored at -80°C until analysis.

**Growth Performance:** Body weight (BW) (g) and total length (TL) (cm) were recorded for growth evaluation at each 10 days. The condition factor (CF) was calculated as \[ W / L^3 \times 100 \]. The growth rate (GR) was calculated as the increase of weight (g) in respect to a given time interval (days). The specific growth rate (SGR) was calculated as Ln gain growth in the time interval between two successive samplings.

The feed conversion ratio (FCR) was calculated for each experimental group as used feed (g) to gain of body weight (g). The weight of feed dispersed to each tank was recorded daily and uneaten feed collected, weighted and not included for FCR calculation\(^{[1,20,23]}\).

**Immunological Parameters:** Serum lysozyme activity was measured using a modified turbidimetry method described by Ellis\(^{[1]}\). Briefly, a standard suspension of 0.375 g/ml *Micrococcus lysodeikticus* (Sigma) was prepared in 1 mL PBS (pH 5.8). Carp serum (25 mL) was added to 175 mL of bacterial suspension, and the decrease in absorbance was recorded at 15s and 180s at 600 nm in a Biophotometer (Eppendorf Co, Germany). One unit of lysozyme activity was defined as reduction in absorbance of 0.001/min. The units of lysozyme present in sera were obtained from a standard curve made with hen egg white lysozyme (Sigma).

Complement activity, Immunoglobulin M (IgM) and alkaline phosphatase were measured by Pars Azmon Kite (Pars Azmon, Iran) in Autoanalyser, Eurolyser (hoshmand-fanavar Co, Tehran, Iran).

**Challenge Experiment:** Fish fed with Aqualase and the non supplemented diet fed fish were challenged with *Streptococcus iniae*. on day 60. A challenge study was performed by intraperitoneal injection of 0.1 mL of 18h grown culture of *Streptococcus iniae* at a concentration of \(9 \times 10^7\) cfu/mL (final dilution \(9 \times 10^6\) cfu/mL). Mortality was recorded daily up to 15 days and relative percentage survival was calculated following methods of Rairakhwada et al., 2007. *Streptococcus* sp was confirmed after sampling from kidney, heart, liver of died fish. The fish were counted after 15 days from the start of the challenge to determine the survival rate. Survival rate was calculated with counting of the dead fish after challenge. Survival rate\(\% = \frac{[\text{Number fish in the start of the challenge} - \text{Number fish in the end of the challenge}]}{\text{Number fish in the start of the challenge}} \times 100^{[17]}\)

**Statistical Analysis:** The results are presented as means ± SD. Differences between treatments were analyzed by one-way analysis of variance (ANOVA), and significant means were subjected to a multiple comparison test (Duncan) at the \(P = 0.05\) level.

**Result:**

**Growth:** The results were showed after 60 days, treatment with Aqualase indicate high value than control for factors gain weight, length, FCR and SGR and GR (\(p>0.05\)). Treatments with Aqualase could not influence significantly on CF of wild carp (\(p>0.05\)), but it had influence on FCR that was significant. The results of fish growth parameters are shown in Table1.

**Lysozyme Activity:** Based on Sampling carried out at the end of treatment, Lysozyme activity increased after feeding of Aqualase supplemented feeds from 2.5 mg/dl in control to 4.5, 5.8 and 4.9 mg/dl after using 0.1, 0.15 and 20 mg/kg feed respectively. Result showed significant difference between fish fed Aqualase and control group (\(p<0.05\)). The highest activity was observed on 0.15% treatment with 5.8 mg/dl. Result of lysozyme activity was observed on the 60 days.

**Complement Activity of C3:** In the present work, not significant activity of C3 and C4 were observed in control and treatment group at initial stage of sampling (\(p>0.05\)), but one month feeding with aqualase made the fish to have more C3 and C4 comparing to control (\(p<0.05\)) (Table2).

**Immunoglobulin M (Igm):** Maximum activity of IgM was observed in 0.15% at both stages (\(p<0.05\)), but maximum value was observed in 0.15% treatment between two stages (\(p<0.05\)) (Table2).

**Alkaline Phosphatase (ALP):** Maximum activity of ALP was observed in 0.15% treatment at both stages of sampling (\(p<0.05\)).

**Challenge Study:** The survival rate of groups challenged with *Streptococcus iniae* was showed that fish fed with Aqualase didn’t show disease symptoms and mortality, however control group showed 13% mortality.
Discussion: The use of biological products namely the probiotic either alone or in combination with prebiotics is recently the goal of the disease biocontrol strategy in aquaculture as they improve the fish health and modify the fish associated microbial community[7,36]. The results revealed that any three groups received probiotic-supplemented diets showed higher growth rate than those kept on a basal diet suggesting that the addition of probiotics enhanced the growth performance and feed utilization and mitigated the effects of population density in the tank.

The statistical analysis of growth performance of wild carp in the end of experimental period (Table 1) indicated an increase in the body weight gain (W.G.) and growth rate (GR) in group receiver probiotic than control group. Also, the most specific growth rate (SGR) were observed in two groups receiving the supplemented with 0.15% and 0.2% probiotic, however the feed conversion ratio (FCR) of wild carp kept on a basal diet (control) was higher than other three groups receiving the diets supplemented with probiotic.

The best FCR values was observed with probiotic-supplemented diets suggested that, the addition of probiotics improved feed utilization, in practical terms this means that probiotic used can decrease the amount of feed necessary for animal growth which could result in production cost reduction. Similar results have been reported by Lara-flors et al[14]. Condition factor (CF) didn’t show significant different between two groups receiving the diets supplemented with probiotic and control groups. Similar study was done by Taghavi[31], she studied effect of Aqualase on rainbow trout (Oncorhynchus mykiss). The result was showed in end of stage, FCR and body weight were increased. Li and Gatlin[14] reported that Striped bass receiving the diets supplements with S.cerevisiae improved increase body weight and FCR. Lim et al[16] showed that naplus enriched with S.cerevisiae in tilapia larva diets is increased improved growth and length. The use of Aqualase in rainbow trout (Oncorhynchus mykiss) diet is increased SGR in treatment group in compare control group[22].

It is probably due to high absorb of food. Lysozyme is humeral non-specific defence protein widely distributed in nature, including fish[13]. It is a cationic enzyme that attacks the β-1,4 glycosidic bond between N-acetyl muramic acid and N-acetylgalactosamine in the peptidoglycan of bacterial cell walls[5]. In the present study the lysozyme levels was increased in all treatment than to control group and maximum addition was observed in 0.15% treatment. Abdell-Tawwab et al[17] reported that the addition of S.cerevisiae in diet increased lysozyme levels of Nil tilapia (Oreochromis niloticus). Also, Sakai et al[16] showed that RNA of S.cerevisiae increased lysozyme level of common carp (Cyprinus carpio).

The complement system is composed of more than 35 soluble plasma proteins that play key roles in innate and adaptive immunity[3]. The complement proteins have multifunctional roles in the defence against microorganisms ranging from the opsonisation, lysis and killing of bacteria, to chemotaxis and anaphylaxis[8]. In the present study the value C3 complement in the serum was increased in all treatments than control especially more than 0.15%
treatment. The most increase of C₄ complement was in 0.20% treatment. This result is similar to observations by Panigrahi et al.[26] that elevated levels of complement activity resulted from feeding Lactobacillus rhamnosus to rainbow trout (Oncorhynchus mykiss).

However, this result wasn’t agreement with researches Selvaraj et al.[28] that showed enough change of complement in feeding common carp (Cyprinus carpio) by glucan of yeast.

Serum immunoglobulins are major component of the hemoral immune system and IgM is the main immunoglobulin present in fish[33]. Amount IgM in the serum was increased in all treatment than to control between two stages. Reyes-Beceril et al.[28] founded that the addition of yeast in diet improved IgM levels of Mycteropepera rosacea exposed to stress. Also, Nayak et al.[19] reported that glucan of yeast improved production antibody of carp (Cyprinus carpio) infected with Aeromonas hydrophila.

Alkaline phosphatase exists to three forms (intestinal, bone and liver). Change of the level of this enzyme is depend on water chemical, food absorb, temperature, age[12] and compound phosphorous[30]. In the present study the alkaline phosphatase (ALP) in serum was increased in all treatments than to control specially in 0.15% treatment (p<0.05). Result of Wache serum was increased in all treatments than to control the present study the alkaline phosphatase (ALP) in serum was increased in all treatment than to control收拾 between two stages. Reyes-Beceril et al.[28] founded that the addition of yeast in diet improved IgM levels of Mycteropepera rosacea exposed to stress. Also, Nayak et al.[19] reported that glucan of yeast improved production antibody of carp (Cyprinus carpio) infected with Aeromonas hydrophila.

In the present study the alkaline phosphatase (ALP) in serum was increased in all treatments than to control specially in 0.15% treatment (p<0.05). Result of Wache et al.[34] on effect of S.cerevisiae as a feed additive on increase AP levels of rainbow trout (Oncorhynchus mykiss) and reports Tovar-Ramirez et al. on effect of live yeast in diet on improve digestive enzyme levels of Sea bass (Dicentrachus labrax) is in accordance with the result of present study. Survival of wild carp of this study to Streptococcus iniae challenge was significantly higher in the all groups fed the Aqualase compared to the control group. This result showed that Aqualase has potential of increase resistance of fish against pathogen bacteria and decrease the risk of disease. There are many report confirm the beneficial effect of probiotics and prebiotics such as S.cerevisiae[14] glucan of yeast[20], Debaryomyces hansenii[36] Carnobacterum divergence[9], Bacillus spp[29] when challenging with pathogen bacteria. As conclusion, the present study indicated that Aqualase positively enhanced growth performance and immune response and resistance to Streptococcus iniae infection and optimum level of dietary Aqualase is about 1.5 g per kg diet (0.15%).

REFERENCE


