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EFFECT OF IN-FEED PROBIOTIC BLEND ON GROWTH PERFORMANCE AND INFECTION RESISTANCE OF THE GUPPY (*POECILIA RETICULATA*)

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Summary

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The present study was carried out to evaluate the dietary effects of *Saccharomyces cerevisiae* and *Bacillus* spore (*B. licheniformis* and *B. latrospore*) probiotic blend in guppy (*Poecilia reticulata*) larvae 30 days after the first feeding at ratios of 0.5%, 1% and 1.5%. *Bacillus* spores were used with the same density of 1×10^9 cfu/100 g in all treatments. There were no significant differences in larvae growth performance ($P>0.05$). A significant effect was observed when fish were challenged with *Ichthyophtirius multifilus* parasites ($P<0.05$). The highest survival percentage was observed in the group which received 1.5% yeast supplement and 1×10^9 cfu/100 g *Bacillus* spores. Also the highest mortality was related to group D1 which was fed a diet containing 1% of *Saccharomyces cerevisiae* and *Bacillus* spore blend. This information indicates that the blend of *Saccharomyces cerevisiae* and *Bacillus* spore can act as immune stimulator to treat fish ectoparasites, *Ichthyophtirius multifilus* instead of chemical drugs.

Key words: antiparasitic effect, growth performance, *Poecilia reticulata*, probiotics

INTRODUCTION

Guppies (*Poecilia reticulata*) are a fish species with attractive colours, bred and exported to other countries by ornamental fish companies. The species was discovered in the middle of the 19th century. The two main aspects that determine the trade and prosperity of the ornamental fish industry are health and nutrition of fishes. Success of larval rearing depends mainly on the availability of suitable food that is readily consumed, efficiently digested and that provides the required nutrients to support good growth and health (Tovar-Ramirez *et al.,* 2002; Waché *et al.*, 2006). Therefore, the use of probiotics in aquaculture has generated much interest. Lilley & Stillwell (1965) described them as substances secreted by one microorganism, which stimulated the growth of another. The range of probiotics examined for use in aquaculture has encompassed both Gram-negative and Gram-positive bacteria, bacteriophages, yeasts and unicellular algae (Irianto & Austin, 2002).

The ciliate protozoan *Ichthyophthirius multifilus* Fouquet, 1876, causing "Ich" or white spot disease, is recognised to be one of the most pathogenic diseases of orna-

mental fish that may result in 100% mortality especially in closed systems. *Ichthyophtirius* is a major problem to aquarists and commercial ornamental fish producers worldwide. While many protozoa reproduce by simple division, a single Ich organism can multiply into hundreds of new parasites (Gholipour-Kanani *et al.,* 2012).

Saccharomyces cerevisae yeast is a natural feed additive, which positively influences the non-specific immune responses of many aquaculture species (Thanardkit *et al.,* 2002). *S. cerevisae* contains various immunostimulating compounds such as β glucans, which are most commonly found in the cell wall of yeasts are generally considered as the main factor for the immunological mechanism (Tukmechi & Bandboni, 2013). The nucleic acids as well as mannon oligosaccharides are capable to enhance the immune responses (Abdel-Tawwab *et al.,* 2006).

In view of above mentioned factors, we incorporated the probiotic strain *S. cerevisae* and *Bacillus* spore blend in the diet of guppy fish (*Poecilia reticulata*) larvae to evaluate its effect on growth performance and fish resistance against *Ichthyophthirius multifilus.*

MATERIALS AND METHODS

Larvae rearing

The experiments were performed at a private ornamental fish laboratory in Tehran (Iran). A total number of 80 parasites-free guppy fish weighing 28.42 ± 13.84 mg were obtained from a private ornamental fish farm (Tehran, Iran). They were divided in 3 experimental treatments and a control with two replicates. Each 15 L tank was stocked with 10 guppies. They were kept under usual conditions at 27 ºC with proper aeration. Rearing water quality was controlled every two days exchanging 30% of the water to remove fish waste.

Experimental diets and feeding

Fish were hand-fed to apparent satiation, three times a day (7:00 AM; 3:00 PM; 11:00 PM), with an isonitrogenous (41% protein) diet, supplemented with dried yeast, *Saccharomyces cerevisiae* at levels of 0%, 0.5%, 1%, 1.5% as detailed in Table 1. Yeasts were mixed with 50 mL distilled water and then sprayed into the food and mixed part by part. In addition to yeast supplementation, *Bacillus* spores containing *Bacillus licheniformis* and *Bacillus latrospore* at a density of 1×10^9 CFU/100 g was mixed into the food. Control group was fed without *Saccharomyces cerevisae*.

Table 1. *Saccharomyces cerevisiae* and *Bacillus* spore densities in the diet of *Poecilia reticulata* groups during this experiment

Groups	Probiotic	
	Bacillus spores (CFU/100 g)	*S. cerevisiae* (%)
Group D1	1×10^9	0.5
Group D2	1×10^9	1.0
Group D3	1×10^9	1.5
Control	1×10^9	0

All diets were kept frozen (−20 °C) until distribution. During the study period all dead fish were removed from tanks and recorded. After 30 days, study fish were anaesthetised with 0.1% extract of *Eugenia caryophyllata* and their weights and lengths were measured.

Guppy growth parameters

The following guppy growth parameters were evaluated: condition factor (CF), specific growth rate (SGR), food conver-

sion ratio (FCR), relative food intake (RFI) and growth coefficient efficiency (GCE) at the end of experiment based on standard formulae as followed:

$CF = W/L^3$ (Lagler *et al.*, 1962)

$SGR = [(LnFBW–LnIBW) / (t_1–t_0)]\times100$ (Helland *et al.*, 1996)

$RFI = \{[feed\ intake] / [0.5\times (FBW–IBW) \times(t_1 – t_0)]\times100\}$

$FCR = F/(FBW–IBW)$
(Helland *et al.,* 1996).

$GCE = (SGR / RFI) \times 100$
(De Silva & Anderson, 1995)

where: W=fish weight (wet weight, g); L=fish length (cm); FBW=final body weight (g); IBW=initial body weight (g); $t_1–t_0$=experiment's duration; F=feed fed (g)

Collection of Ichthyophthirius multifiliis tomonts

Fish with natural heavy parasitic infection (5 days post-infection) were anaesthetised with extract of *Eugenia caryophyllata*, washed with water and the skin was scraped to dislodge the tomonts. The isolated tomonts were concentrated with 70 µm mesh. The collected tomonts with 70 µm mesh were transferred into 1 L glass containing 1000 mL water as a modification of the method of Noe & Dickerson (1995).

Exposure to Ichthyophthirius multifiliis

After the last biometery, 5 remaining fish in each experimental tank were challenged with *Ichthyophthirius multifiliis* to measure level of resistance to infection in each group. Fish were infected with *Ichthyophthirius multifilus* via exposure to a high dose of collected tomonts using the immersion methods as described by McCallum (1986). Following exposure for 5 hours in the dark, fish were transferred to a glass aquarium with 15 L capacity supplied with aerated dechlorinared tap water. After fish exposure to *Ichthyophthirius miltifiliis* they were fed on the control diet and similar water temperature and quality parameters were maintained as observed in growth trial for 10 days. Upon termination of experiment, each fish was individually examined for the number of *Ichthyophthirius multifiliis* through direct counting of parasites on a microscope. Total number of *Ichthyophthirius multifiliis* present on each fish were counted and recorded to estimate the intensity of infection.

Statistical analysis

The differences in growth rates and parameters among the different experimental treatments were calculated using one-way ANOVA followed by Duncan's multiple range test to examine statistically significant differences (SPSS v. 19 software).

RESULTS

The growth and nutrient utilisation data obtained from this study are presented in Table 2. At the end of experiment, it was found that the weight gain (%) had no significant difference ($P>0.05$) among all yeast-supplemented groups.

Likewise, no significant difference ($P>0.05$) was observed among yeast-supplemented groups for food conversion ratio (FCR), specific growth rate (SGR) and condition factor ($P>0.05$). The same result was observed in relative food intake (RFI) and growth coefficient efficiency (GCE). The mean weight of groups is presented on Fig. 1.

There was no significant differences among mean lengths of fish after treatments (Fig. 2).

Table 2. Growth parameters of *Poecilia reticulata* reared with diets containing different concentration of *Saccharomyces cerevisiaee* in presence of *Bacillus* spore.

Parameters	Groups			
	Group D1	Group D2	Group D3	Control
Weight, mg	10.73±7.63	11.05±8.08	10.78±6.95	9.31±4.53
CF, %	1.97±1.12	2.23±1.35	2.90±2.80	2.22±1.47
FCR	1.17±0.15	1.14±0.11	1.15±0.13	1.14±0.10
SGR, %	6.39±2.18	6.58±1.86	6.53±1.90	6.38±1.41
RFI, %	34.91±30.60	28.87±22.34	31.01±26.20	28.82±20.26
GCE, %	40.99±36.45	42.37±41.63	40.65±33.85	32.26±20.75

CF=condition factor, FCR=food conversion ratio, SGR=specific growth rate, RFI=relative food intake, GCE=growth coefficient efficiency.

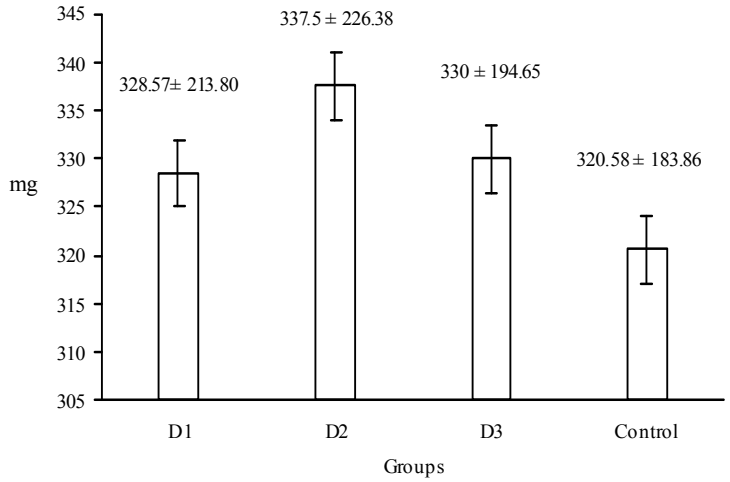

Fig. 1. Mean weights (mg) of *Poecilia reticulata* reared on diets containing different concentrations of *Saccharomyces cerevisiae* (Group D1: 0.5%; group D2: 1.0%; Group D3: 1.5%) in presence of *Bacillus* spores.

The effect of diets on fish survival rate and mortality are presented on Fig. 3. The group D3, which was fed 1.5% yeast showed no mortality, while a high mortality rate (30%) was observed in group D1, which received the 0.5% yeast-supplemented diet.

Significant difference (P=0.05) among groups were observed in the mean abundance of *Ichthyophthirius multifiliis*, as presented in Table 3. The lowest infestation after challenge infection was observed in group D3, which was supplemented with 1.5% yeast and 1×10^9 cfu/100 g.. There was a significant difference between D3 and all other groups ($P<0.05$). No significant difference was observed between D1 and D2 or between D1, D2 and the control.

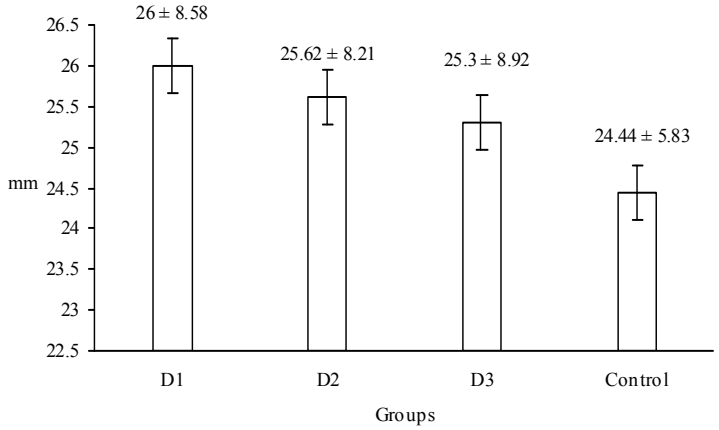

Fig 2. Mean lengths (mm) of *Poecilia reticulata* reared on diets containing different concentrations of *Saccharomyces cerevisiae* (Group D1: 0.5%; group D2: 1.0%; Group D3: 1.5%) in presence of *Bacillus* spores.

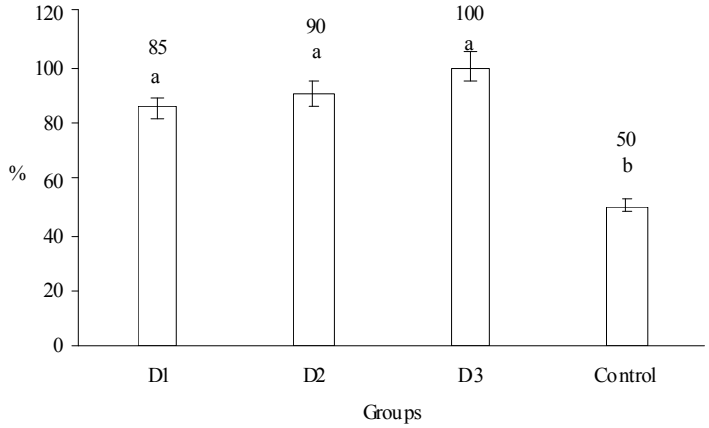

Fig. 3. Survival rate (%)of *Poecilia reticulata* reared on diets containing different concentrations of *Saccharomyces cerevisiae* (Group D1: 0.5%; group D2: 1.0%; Group D3: 1.5%) in presence of *Bacillus* spores. Values with different superscripts are significantly different ($P<0.05$).

DISCUSSION

Ornamental fish and especially live-breeders are the most popular pet fish and breed easily. Guppies are one of these fishes and exhibit great variation. The species is the best known as a good choice for beginner aquarists, since they are hardy and reproduce rapidly. Our probiotic food fed fish exhibited no significant difference in growth performance when compared to the control fish. This result implies that ornamental guppies utilise dietary nutrients with the same efficiency, regardless of whether their food is supplemented with yeasts or not. In contrast,

Table 3. Response of guppy fish reared on diets with different concentration of *Saccharomyces cerevisiae* after exposure to *Ichthyophthirius multifiliis* infection

Parameters	Groups			
	Group D1	Group D2	Group D3	Control
No. of fish exposed to *I. multifiliis* infection	5	5	5	5
Average number of *I. multifiliis* per fish	1.5±1.29 ^{bc}	1.4±1.34 ^{bc}	0.0±0.0 ^{a}	0.44±0.20 ^{b}

Values in the same row with different superscripts are significantly different (P<0.05).

Tukmechi & Bandboni (2013) found a significant improvement in weight gain and survival in trout fed food containing *Saccharomyces cerevisae*. Baker's yeasts have a cellular membrane which reduce its digestion efficiency, and it may cause insignificant growth. The present study found no significant impact of yeast supplementation on FCR and SGR, in contrast to Gunasundari *et al.* (2013), who reported that the use of *Saccharomyces cerevisiae* in clownfish (*Amphiprion percula*) diet improved the specific growth rate and reduced food conversion ratio. The same result was observed by Dharamaraj & Kandasamy (2010) in *Xiphophorus helleri* fed *Streptomyces* as a probiotic.

In our study, the lowest FCR, RFI and GCE rates were observed in the D2 group. While our results were not statistically significant, this trend is in agreement with the earlier findings by different authors (Carnevali *et al.,* 2006; Ghosh *et al.,* 2007). We did however find a significant difference in survival rate. Group D3 (fed feed with 1.5% yeast) showed a 100% survival rate. The highest survival rate was related to group D3, followed by D2, the control group, and last came group D1. Similar results were reported by Gunasundari *et al.* (2013). The use of 2 and 3% of yeasts resulted in 99% survival in comparison with controls. The increased survival rate could be possibly due to the fact that the baker's yeasts are a source of nucleic acids and β-1,3 glucans that effectively enhance immune functions, as reported in the African catfish (Yoshida *et al.,* 1995). However the use of *Bacillus licheniformis* and *Bacillus latrospore* spores increased the survival rate of *Hypophthalmichthys molitrix* larvae reported by Sahandi *et al.* (2012), but was not responsible for significant survival rate alone in this study.

The effects of probiotics have been widely studied in cultured aquatic species, particularly the enhancement of the non-specific immune system (Irianto & Austin, 2002). Yeasts are able to provide β-glucans and nucleotides that stimulate the immune system of fish (Tuckmechi & Bandboni, 2013). Challenge infection with *Ichthyophthirius multifiliis* tomonts induced significant differences among groups after 10 days. Group D3 showed the highest response against *Ichthyophthirius multifiliis,* with no parasites were found on fish skin. There was no significant difference among other groups, but a significant difference was observed between D3 and the other treatments (P<0.05). Similarly, the use of *Saccharomyces cerevisiae* was found to increase disease resistance in Rosy Barb and Black Tetra fish challenged with *Aeromonas hydrophila* and *Pseudomonas fluorescens*

(Türnau *et al.*, 2000). However this enhancement of immune response and survival rate was not observed by Duncan & Klesius (1996) in catfish challenged with *Edwardsiella ictaluri.* Thus, *Saccharomyces cerevisiae* and *Bacillus* spores can improve fish response against parasites, and therefore, fish health and production.

CONCLUSION

The present study demonstrated the beneficial effect of *Saccharomyces cerevisiae* on *Poecilia reticulata* immune response against *Ichthyophthirius multifiliis,* despite the lack of significant effect on fish growth. Probiotics can thus be used as important supplements in fish diets to improve their responses against parasites, especially *Ichthyophthirius multifiliis*. Mitigation of fish disease would improve their production, which would be economically profitable to the ornamental fish industry and promote the production of safe fish.

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