

EFFECT OF BIOBUDS AS A COMMERCIAL PROBIOTIC PRODUCT IN CULTURED TILAPIA

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Abstract

The study was conducted over a two-month period, in order to evaluate the potential benefit of super biobuds as a probiotic agent in Nile Tilapia (*Oreochromis niloticus*) feeds. There were three treatments, including a control (C), each consisting of four replicates of 25 fish/replicate (mean individual initial weight = 20 ± 3 g), each reared in aquarium (60X 70 X 50 cm). Fish were fed a balanced diet of 25% protein along the period of experiment. Two doses of super biobuds (1.0 and 2.0 g kg⁻¹ diet fed) were incorporated in the feed for two months. Various parameters were recorded and challenge was done immediately after the period of application. The challenge infection was done to the experimented fish by inoculation of both *Aeromonas hydrophila* and *Pseudomonas fluorescens* (0.5 ml of culture suspension of either pathogen containing 10⁸ bacteria ml⁻¹). Body weight gain was significantly (P < 0.001) high in all treatments than in the control group. The hematocrit values and nitroblue tetrazolium activities (NBT) were increased in both treated groups than the control. The survival rate was significantly high in all treatments in comparison to the control group. The percents level of protection among the two treated groups after challenge infection using *A. hydrophila* and *P. fluorescens* were higher than control. It could be concluded that, super biobuds can improve body gain, survival and enhance resistance to challenge infection. However, further studies including full commercial cost benefit analysis, is necessary before recommending their application in aquaculture.

KEY WORDS: Biobuds , *probiotic*, survival, growth, resistance to challenge, *Oreochromis niloticus*.

INTRODUCTION

In recent years, there have been growing concerns about the adverse effects of the bacterial diseases in the aquaculture of many economically important marine and freshwater fish species including Nile tilapia (Bowser *et al.*, 1998). These bacterial diseases may cause heavy losses from mortality, reduced growth and unmarketable appearance in various fish species. Some reported occurrences of the human disease were associated with puncture wounds or abrasions and handling of infected fish or contaminated water (Greenlees *et al.*, 1998). Therefore, an effective preventive strategy is not only needed to limit economical loss in aquaculture, but also to protect the health of aquaculturists and fish processing workers.

In aquaculture, traditional methods for treating infective pathogens include a limited number of Government-approved antibiotics and chemotherapeutics. However, the disadvantages such as marginal effectiveness and high cost are obvious (Sealey and Gatlin, 2001). These treatments also may cause the accumulation of chemicals in the environment and/or fish, thus posing potential threats to consumers and the environment. An alternative strategy, besides vaccine development, is nutritional modulation of immune responses and disease resistance of aquaculture species. The doses and time of administration have been recognized to have important effects on immunostimulant function (Sakai, 1999). Yeast by-products from the brewing industry are natural diet additives that have been shown to positively influence non-specific immune responses (Siwicki *et al.*, 1994 and Anderson *et al.*, 1995) as well as growth (Rumsey *et al.*, 1991; Oliva-Teles and Goncalves, 2001) of some fish species.

The present study was conducted to determine the effects of graded levels of super biobuds (*Saccharomyces cerevisiae*) on the growth performance and immune responses as well as resistance of cultured Nile tilapia to infection by *Aeromonas hydrophila* and *Pseudomonas fluorescens*.

MATERIALS AND METHODS

Fish

A total of 300 *Oreochromis niloticus* fingerlings (20 ±3 g) were equally distributed and reared in 12 glass aquaria of 50 x 60 x 70 cm and fed on a balanced diet (25% protein).

Diets were prepared using locally available ingredients. The ingredients were mixed mechanically and oil was added gradually to ensure even distribution of the ingredients. Super biobuds (*Saccharomyces cerevisiae*) was procured from the local market, crushed and two doses, i.e. 1 and 2 g of Biobuds kg⁻¹ feed were mixed with the balanced diet in pellets. Pellets (0.5 cm) were prepared and allowed to air-dry at room temperature for 24 h before use. The required amount of the diet was prepared every two weeks, and stored in a refrigerator. Fish were fed at a rate of 3 % of body weight per day, with the ration being split into two meals.

Experimental design

Two different doses (1 and 2g/ kg) of Biobuds (B1 & B2, respectively) were tested. This compound was added to the diet prior to pelletizing. Three treatment groups (100 each equally divided into 4 replicates) were used i.e. two groups received fish diet with tested doses of Biobuds and the control group (C), fed the fish diet only. Four replicates were used per treatment and these were randomly assigned to glass aquaria that daily examined and fish that died naturally were removed.

The trial extended for two months where the cumulative mortalities were recorded and the experimented fish were investigated for:

(i) Body weight gain, Specific growth rate (SGR) and condition factor (CF) (Laird and Needham 1988).

$SGR = [\ln \text{ final mean body weight (g)}] - \ln [\text{initial mean body weight (g)}] \div \text{time interval (days)} \times 100.$

$CF = \text{weight (g)} \div [\text{length (cm)}]^3.$

(ii) Nitroblue tetrazolium test (NBT) using 20 blood samples from each treatment (Siwicki *et al.*, 1985).

(iii) Response to challenge infection using a suspension of pathogenic *A. hydrophila* and *Pseudomonas fluorescens* (0.5 ml of 4.0×10^8 bacterial cells ml^{-1}) via I/P route to 20 fish/ treatment/ challenge (Salah *et al.*, 2008). The relative level of protection (RLP) among the challenged fish was determined (Ruangroupan, *et al.*, 1986) using the following equation:

$RLP \% = 100 - (\text{per cent of immunized mortality} \div \text{per cent of control mortality}) \times 100$

(iv) Statistical analysis was performed to all experimented fish and collected samples using analysis of variance (ANOVA) and Duncan's Multiple Range Test (Duncan 1955) (mean at significance level of $P < 0.05$). Analysis was carried out using the SAS (2005) package.

RESULTS

Body weight gain was significantly ($P < 0.001$) high in all treatments than in the control group. The body gain, condition factor and specific growth rate showed no significant difference between the two treated groups after the two months of administration; however they revealed significant increase in the condition factor than that of the control group. The hematocrit values and nitroblue tetrazolium activities (NBT) were none significantly increased in both treated groups than the control. The survival rate was significantly high in both treatments than that of the control group (Table 1).

The mortality rate after the challenge infection using *A. hydrophila* was 45, 40 and 75% for groups treated with 1 and 2 g of biobuds /kg feed as well as the control group respectively. On the other hand, the mortality rate after the challenge infection using *P. fluorescens* was 45, 35 and 80% for groups treated with 1 and 2 g of biobuds /kg feed as well as the control group respectively.

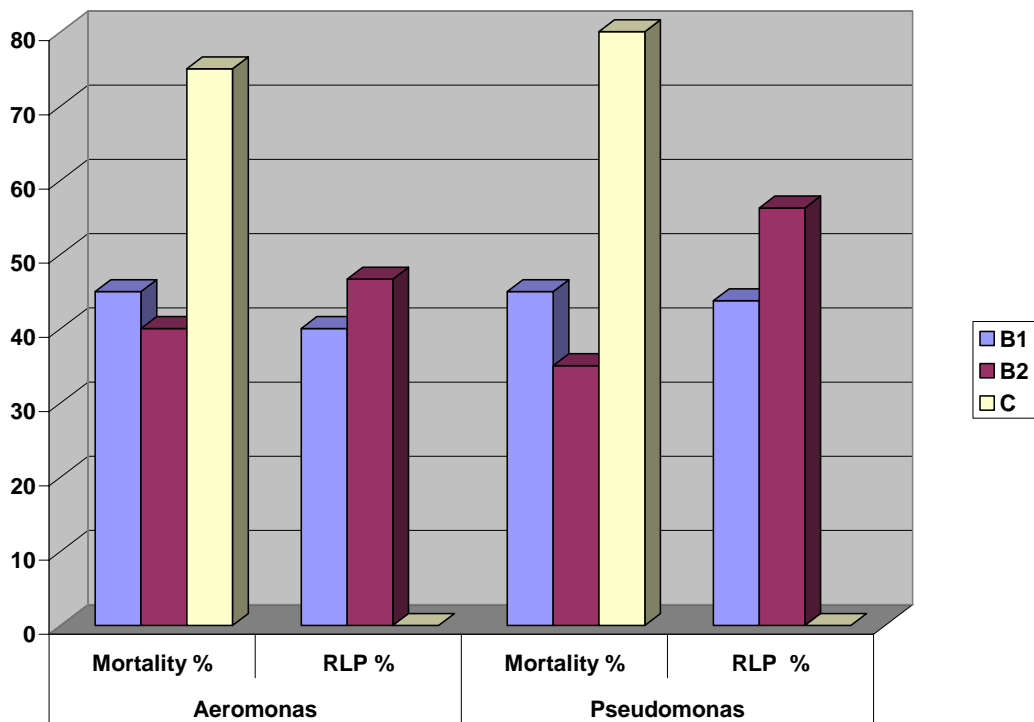
The percents level of protection among the two treated groups after challenge infection using *A. hydrophila* was 40 and 46.67 for the groups treated with 1 and 2

g of biobuds /kg feed, respectively. However it was 43.75 and 56.25 after challenge infection using *Pseudomonas fluorescens* for the same groups, respectively (Graph 1)

Table 1. Survival, growth performance, some immunological and hematological parameters in Nile Tilapia by the end of experiment (2 months).

Parameters	Control	Biobuds (1g/kg feed)	Biobuds (2g/kg feed)
NBT (mg/ml)	00.18 ^A ± 0.03	00.22 ^A ± 0.04	00.24 ^A ± 0.02
HCV (%)	28.67 ^A ± 1.42	31.10 ^A ± 1.88	30.78 ^A ± 1.50
Body gain (g)	14.09 ^B ± 1.08	20.41 ^A ± 2.78	20.68 ^A ± 0.39
Condition factor	01.70 ^B ± 0.03	01.81 ^A ± 0.03	01.85 ^A ± 0.03
Specific growth rate	00.94 ^A ± 0.05	01.19 ^A ± 0.13	01.18 ^A ± 0.02
Survival (%)	76.33 ^B ± 3.17	87.33 ^A ± 3.81	92.00 ^A ± 4.00

Super script variable litters are significantly different at $p < 0.001$



Graph 1. Mortality and relative Level of protection (RLP) among experimented Nile tilapia after challenges with *Aeromonas hydrophila* and *Pseudomonas fluorescens*.

DISCUSSION

The growth performance, in the present study, showed significant (Body weight gain and condition factor) to non significant (specific growth rate) increase in all treatment groups when compared with the control. It may be possible to use more than 50% yeast in the diets of salmonid fishes as growth promoters (Rumsey *et al.*, 2007). Mortazavi-Tabrizi *et al.* (2008) evaluated the effects of different levels of yeast *Saccharomyces cerevisiae* (0.05%, 0.1%, 0.15%, and 0.2 %) as growth promoters of rainbow trout. They found that, specific growth rate and feed conversion rate were significantly ($P < 0.05$) different among treatment groups, also SGR and SR were higher compared to the control. On the other hand, Bagni *et al.*, (2005) found that the long-term period treatment with dietary yeast β -glucan had no significant differences in the growth performances between the treated and control seabass fish.

The survival rate, of the experimented fish in the current study, was significantly high in the two treated groups than that of the control group. Nearly similar findings were reported by Mortazavi-Tabrizi *et al.* (2008). On the other hand, Bagni *et al.*, (2005) found that the survival after long-term period treatment with dietary yeast β -glucan had no significant differences in the treated and control seabass.

Bagni *et al.*, (2005) found that the long-term period treatment with dietary yeast β -glucan had no significant differences in innate and specific immune parameters in treated and control sea bass fish. Moreover, El-Boushy and El-Ashram (2006) showed that the β -glucans was able to enhance the non-specific immunity of the African catfish (*Clarias gariepinus*) efficiently more than *Saccharomyces cerevisiae*. The findings of our study were similar to those previously mentioned where the hematocrit values and nitroblue tetrazolium activities (NBT) were non significantly increased in both treated groups than the control.

The mortality rate after the challenge infection using *A. hydrophila* and *P. fluorescens* was higher in control group than that of groups treated with 1 and 2 g of biobuds /kg feed. The percents level of protection among the two treated groups after challenge infection using same two pathogens was 40 & 46.67 for *A. hydrophila* and 43.75 & 56.25 for *P. fluorescens* in the groups treated with 1 and 2 g of biobuds /kg feed, respectively. Li and Gatlin (2004) observed that a significantly enhanced survival rate after bath exposure to *Streptococcus iniae* due to the feeding on yeast compared to fish fed the basal diet. Yeast β -glucans seem to modulate the specific immune response by increasing the serum antibodies secreted by plasma cells against *Edwardsiella ictaluri* in catfish (Chen and Ainsworth 1992) and against *Yersinia ruckeri* in rainbow trout, when given in combination with vitamin C (Verlhac *et al.*, 1996).

It can be concluded that the two doses of super biobuds may enhance the growth rate, improve the survival and increase resistance of Nile tilapia to the challenge

infection. It seems beneficial to use biobuds at lower doses for two months. However, more extensive field trials and economic studies are recommended.

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