Phototrophic Bacteria as Fish Feed Supplement

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ABSTRACT : Single cell of an indigenous phototrophic bacterium, *Rhodovulum sulfidophilum*, was incorporated in commercial fish feed for *Oreochromis niloticus*. The bacterial cell was analyzed for nutritional value and tested for toxicity and acceptability as an aquaculture feed supplement. The results showed higher survival rate and significantly higher growth rate ($p<0.001$) in *O. niloticus* fed with the bacteria incorporated fish feed. It is suggested that *R. sulfidophilum* can be utilized as an aquaculture feed supplement. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 7 : 991-994*)

Key Words : Single Cell Protein, Fish Nutrition, Phototrophic Bacteria, *Oreochromis niloticus*, Microbial Biotechnology

INTRODUCTION

Microbial feeds produced through biotechnological processes have been actively investigated as alternative or unconventional feed supplement as well as probiotic resources for aquaculture and aquaculture systems.

Microorganisms of interest include zooplankton, algae, fungi and phototrophic bacteria. Phototrophic bacteria are widely distributed in nature. These bacteria are found in freshwater, seawater, aquaculture and wastewater ponds. They are dominant in polluted areas because they are very efficient in converting wastes into useful products. (Kobayashi, 1982; Sasikala et al., 1993). Phototrophic bacterial cells are rich in proteins, vitamins and amino acid. The biomass of phototrophic bacteria, especially Rhodospirillaceae, is reported to be highly nutritive by workers such as Kobayashi and Kurata (1978), Shipman et al. (1975), Sasaki et al. (1981) and Kobayashi and Kobayashi (1995). The single cell protein values were found to be comparable to those of single cell protein from algae and yeast (Kobayashi and Kurata, 1978). When grown in cheap organic wastes, they offer a good option to substitute the expensive fish and prawn feeds (Getha et al., 1998). Also, phototrophic bacterial cells are said to contain physiologically active substances/chemicals that act as probionts. Growing public dissatisfaction over the use of antibiotics in feed additives has encouraged commercial interest in alternative therapy.

Very little is known about the feasibility of using phototrophic bacteria as a source of growth supplement in feed formulations for tilapia. The present experiment was conducted to ascertain whether phototrophic bacteria could be incorporated as a supplement in fish diet.

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MATERIALS AND METHODS

Test animals

Fries of *Oreochromis nilotica*, of the same batch, having mean total length and body weight of 1.75 cm and 0.078 g respectively, were obtained from the Research Farm of University of Malaya. Groups of 25 fishes were kept in fiberglass tanks containing 100 L of aerated fresh water at ambient temperature. Aeration was done by air stone diffusers on the bottom of the tanks. The fishes were acclimatized to the system for one week prior to the start of the experiment. All experimental treatments were replicated twice and each group was fed a commercial diet incorporated without or with phototrophic bacteria. The experiment was conducted under conditions of natural photoperiod for four months. Ten fish fries were sampled randomly every 21 days for four months. The total length was determined with a measuring board whereas the weight was determined with an electronic balance.

Microorganism

Rhodovulum sulfidophilum, a purple non-sulfur phototrophic bacteria, was selected from the culture collection of Institute of Postgraduate Studies and Research, University of Malaya, for the study. It was isolated from the seawater of Port Dickson, Malaysia and was grown in 10 L glutamate malate medium (GMM) and centrifuged to get the bacterial biomass. This bacterial biomass was incorporated into the commercial fish feed as a supplement.

Diet formulation

The experimental feed was prepared by incorporating 1:2 (w/w) of phototrophic bacterial cells grown in GMM into the commercial fish feed, the control diet of the experiment. The commercial fish feed and fresh biomass was mixed and pelletized with an extruder. The pellets were stored at a room temperature and used throughout the experiment. Analysis of the experimental and commercial fish feed

was done at the Veterinary Diagnostics Laboratory, Petaling Jaya, Malaysia.

Feeding regime/protocol

The experiment consisted of two groups, one given the phototrophic bacteria incorporated commercial feed and the other only commercial feed, with two replicates for each feed. Feeding of the experimental diet commenced 7 days after the fish were stocked in the tanks. The experimental and control fishes were fed twice a day *ad libitum*, every day.

Water quality

During the experimental period the water quality was maintained by changing water every day. Un-eaten food and feces that settled on the bottom of the tanks were removed by siphoning at the end of the day after the fish were fed. Proper aeration was ensured by using an air compressor. The physico-chemical characteristics of the water such as temperature, pH, dissolved oxygen (DO) and ammonia concentration were monitored both in experimental and control tanks once every week.

RESULTS

The effect of the bacteria supplemented diet and the commercial diet was compared by measuring the average individual weight gain and calculating the mean weight gain and specific growth rate. Percent survival of tilapia fry fed each diet was also compared.

Analysis of the experimental fish feed supplemented with phototrophic bacterial cells and the commercial fish feed showed that they have protein content of 47% and 42% respectively (table 1). The amount of amino acids in the experiment feed was comparable to that of the commercial feed.

There was no difference in the water quality in the test and control tanks. The summary of the water quality data is shown in table 2.

It was found that the survival rate in the experimental fishes fed with the bacteria incorporated feed was 16% higher than the control fishes fed with the commercial feed (table 3). Survival rate was only recorded at the end of the experiment. Periodic recording of survival rate will be followed in the next experiment.

Table 1. Proximate analysis of experimental and control feeds (%)

Composition	Experimental	Control
Crude protein	47	42
Crude fat	4.2	3.6
Crude fiber	3.4	2.9

Table 2. Summary of water quality data

Parameter	Experimental tank	Control tank
Water temperature (℃)	27.0 - 28.5	27.0 - 28.5
pH	7.5 - 7.8	7.6 - 7.9
Dissolved oxygen (mg/L)	7.1 - 7.3	7.0 - 7.3
NH_3 (mg/L)	<0.05	<0.05

Since there was no significant differences ($p<0.05$) in mean fish weights between replicates within diet groups, average was taken to calculate the different parameters pertaining to growth. Analysis of variance showed highly significant difference ($p<0.001$) in growth rate between days and between the two types of feed.

At the beginning of the experiment, the mean size of the tilapia fry was 0.078 g and their mean total length was 1.75 cm. (table 3). During the first 6 weeks of rearing, there was not much difference in the mean weight gain of tilapia fed with phototrophic bacteria incorporated feed and the commercial feed. The mean weight gain at the end of 77 days varied between 5.845 g and 6.883 g in control and experimental fishes respectively (figure 1, table 3). However, at the end of 119 days, when the experiment was terminated, it was observed that the final mean body weight was 23.18 g and 30.13 g in control and experimental fishes respectively (figure 1,

Table 3. Effect of phototrophic bacteria incorporated diet on growth of tilapia

Parameter	Commercial feed	Bacteria supplemented feed
Total number of fish	50	50
Initial mean body weight (g)	0.078 ± 0.05	0.078 ± 0.04
Initial mean total length (cm)	1.75 ± 0.03	1.75 ± 0.14
Final mean body weight (g)	23.18 ± 0.19	30.13 ± 0.05
Final mean total length (cm)	11.07 ± 0.15	12.80 ± 0.12
Net weight gain (g)	23.10	30.05
Mean weight gain (mg/fish/day)	194	252
Specific growth rate (%)	19.40	25.25
Survival rate (%)	76	92

table 3). Similarly the highest mean weight gain (252 mg/fish/day) and specific growth rate (25.25%) was obtained by tilapia fry raised on phototrophic bacteria incorporated commercial fish feed diet (table 3). The result of the present experiment demonstrated a successful incorporation of phototrophic bacteria strain *Rhodovulum sulfidophilum* in the commercial fish feed enhancing growth and survival rate.

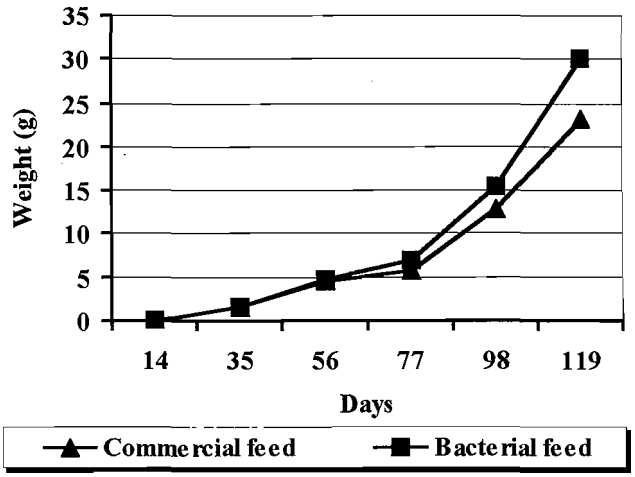

Figure 1. Effect of phototrophic bacteria on growth in tilapia.

DISCUSSION

The use of growth promoter in animal feed is not new (Abalos et al., 1990). It provides an economic advantage as it enhances growth and improves feed utilization (Abalos et al., 1990). It is evident from reports of Shipman et al. (1975), Kobayashi and Kurata (1978), Kobayashi and Kobayashi (1995), that phototrophic bacterial cells are enriched with high quality proteins and other physiologically active substances for inclusion in animal feeds. Phototrophic bacteria are reported to contain 40-69% (w/w) protein, 0.09-0.80 mg carotenoids per gram dry cell weight, 30-79 mg vitamin B12 per kg dry cell weight and an essential amino acid composition comparable with egg, algae and soybean (Kobayashi and Kurata, 1978; Vrati, 1984).

Proximate analysis of the experimental feed supplemented with phototrophic bacterial cells and the commercial feed showed that they have protein content of 47% and 42%, crude fat content of 4.2% and 3.6%, crude fiber content of 3.4% and 2.9% respectively. The amino acid profile was equally comparable to that of the commercial fish feed. In the initial experiment, attempts were not made to make the two diets isocaloric or of similar protein level. In future experiments, these considerations will be kept in mind during the planning process of the trial.

However, research data on the use of phototrophic bacterial cells as feed supplement have been rather few, although application of phototrophic bacterial cells to fish farms is quickly taking hold in Japan (Sasaki et al., 1991). These bacterial cells have been used on a practical scale as a nutritional ingredient in feed for yellowtail and sunbeam fish cultures in Japan.

Kobayashi and Kobayashi (1995) identified that fry of fishes such as loach, goldfish, carp, ark shell and sweet fish are direct predators of phototrophic bacteria soon after hatching. This results in an increase in weight and survival rate of more than two folds within 2-4 weeks after hatching. Live cells of phototrophic bacteria added to the feed of crucian carp soon after hatching also increased the rate of survival. Intriago and Jones (1993) successfully used these bacteria as an exclusive diet for *Artemia*.

According to Sasaki et al. (1991), phototrophic bacterial cells were readily accepted and utilized and no toxicity was found in the goldfish. Similar results were also obtained in red tilapia and carp. Growth was significantly superior in terms of growth rate (9.9-11.69 %/day) and weight gain (99.1-116.97 mg/day, 18.03%). Our initial experiments with *R. sulfidophilum* also indicated promising results. The overall results indicated a better survival rate (16%). One of the reasons for better survival rate may be the phototrophic bacterial cells serve to supply essential proteins, to increase the survival rate of young fish fry (Kobayashi and Tchan, 1973).

In the present experiment the bacterial cell supplemented feed was acceptable and utilized by *Oreochromis nilotica* which resulted in the increase of growth, specific growth rate being 19.40% and 25.25% for control and test fishes respectively. Early growth rates between 14 and 56 days were found to be much less than expected. This is perhaps due to the fact that the experimental fishes required some time to get adjusted to the bacterial feed. Similar results have been reported in other fishes also. The microorganisms are able to synthesize precursors of cell macromolecules and vitamins which may be the reason for better growth obtained in the experiment (Manju and Dhevendran, 1997).

Based on its characteristic merit where various effective by-products, such as feeds, are produced, utilization of phototrophic bacteria to treat agro-industrial wastes in this country is being studied. Besides having suitable culture conditions, Malaysia has a variety of wastes from the agriculture and the industry sector that can be used to produce bacterial biomass of phototrophic bacteria. The outcome would save the cost of the import substitute for aquaculture industry and also aid in the treatment of the effluent

of the sago and sardine processing industry-a predominant industry of the country.

The present study demonstrated that phototrophic bacteria strain *R. sulfidophilum* is a useful supplement in feeds of juvenile tilapias. It can be incorporated in the feed to a possible extent in formulated diets. The results are preliminary and wider screening is necessary to get better bacterial strains for this purpose and to determine the optimum level of incorporation in fish diet.

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