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Phototrophic Purple Bacteria as Feed Supplement on the Growth, Feed Utilization and Body Compositions of Malaysian Mahseer, *Tor tambroides* Juveniles

(Bakteria Fototrofik Ungu sebagai Makanan Tambahan kepada Pertumbuhan, Penggunaan Makanan dan Komposisi Badan Kelah Malaysia, Juvenil *Tor tambroides*)

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ABSTRACT

*The application of microbial techniques in aquaculture has been playing a vital role to increase the production yield by improving the nutritional values of the targeted fish. Phototrophic purple bacteria as single cell protein (*SCP*) appears to be a promising substitution for protein rich supplement for aquaculture feeds making them a promising growth enhancer in aquaculture industry. Two species of phototrophic purple bacteria,* Marichromatium *sp. and* Rhodopseudomonas *sp. were used in the commercial diet to compare the growth, survival rate and feed utilizationon for* Tor tambroides *juvenile. Purple bacteria were isolated from mangrove sediment and fish tank and mass cultured using 112 synthetic media under anaerobic light condition. Bacterial cells were included in the diets by mixing the fresh biomass with the crushed commercial pellet, re-pelleted and air-dried. The experimental diets were fed to the fingerlings twice per day for 10 weeks to satiation level. The results showed that there were trends of increased growth, better survival rate and improved feed conversion ratio when fed with diet 1 (*Marichromatium *sp.) compared with other diets. There was significant difference (*p*<0.05) between the sampling days. The specific growth rate and weight gain of the fish fed with diet 1 were 0.49 % and 4.92 g, respectively, compared to 0.42% and 4.11 g from the control. This study suggested that purple bacteria could be used in feed formulation as a supplement to promote growth and survival of freshwater fishes in Malaysia.*

Keywords: Feed supplement; Malaysian Mahseer; phototrophic purple bacteria; Tor tambroides*, 112 synthetic media*

ABSTRAK

*Penerapan teknik mikrob dalam akuakultur telah memainkan peranan yang penting untuk meningkatkan hasil pengeluaran dengan meningkatkan nilai-nilai pemakanan ikan yang disasarkan. Protein sel tunggal (*SCP*) daripada bakteria ungu fototrofik digunakan sebagai pengganti untuk makanan tambahan yang kaya dengan protein sebagai makanan akuakultur yang menjanjikan penambah yang baik untuk pembiakan di dalam industri akuakultur. Dua spesies bakteria ungu fototrofik* Marichromatium *sp. dan* Rhodopseudomonas *sp. Digunakan dalam diet komersial untuk membandingkan tumbesaran, kelangsungan hidup dan penggunaan makanan untuk juvenile* Tor tambroides*. Bakteria ungu telah diasingkan daripada sedimen bakau dan akuarium ikan dan dibiakkan menggunakan 112 media sintetik di bawah keadaan cahaya anaerobik. Sel bakteria dimasukkan ke dalam diet dengan mencampurkan biojisim segar dengan pelet komersial dihancurkan, dipeletkan dan dikeringkan. Diet uji kaji telah diberi kepada ikan dua kali sehari selama 10 minggu ke tahap kekenyangan. Hasil kajian menunjukkan bahawa terdapat kecenderungan pertumbuhan meningkat, kadar kelangsungan hidup yang lebih baik dan nisbah penukaran makanan yang lebih baik ketika diberi makan dengan diet 1 (*Marichromatium *sp.) berbanding dengan diet yang lain. Terdapat perbezaan bererti (*p*<0.05) antara hari-hari persampelan. Kandungan kadar pertumbuhan dan pertambahan berat badan ikan yang diberi makan dengan diet 1 ialah masing-masing 0.49% dan 4.92 g berbanding dengan 0.42% dan 4.11 g daripada kawalan. Kajian ini mencadangkan bahawa bakteria ungu boleh digunakan dalam rumusan makanan sebagai tambahan untuk menggalakkan pertumbuhan dan kelangsungan hidup ikan air tawar di Malaysia.*

Kata kunci: Bakteria ungu fototrofik; Mahseer Malaysia; makanan tambahan; media sintetik 112; Tor tambroides

INTRODUCTION

Freshwater fish culture practices have shown a demand for efficient, balanced and economical fish feed in last decades. Fish is today considered as the most promising inexpensive alternative source of animal protein. Thus the assessment of feed ingredients is an essential need to nutritional research and feed development for aquaculture species. Many of these ingredients are more complex which required thorough evaluation in order to determine their nutritional value towards the use of prospective diets (Glencross et al. 2007). There are two main goals to be achieved in aquaculture, which are maximizing growth rate and minimizing production costs. Thus some strategies must be utilized to achieve these goals including reducing the feed processing and production cost, maximizing food conversion, controlling disease outbreaks and maintaining a good nutrition for the fish (Huntington & Hasan 2009). The dependency on expensive commercial fish feed should be reduced for the success in aquaculture. Due to cost and the need to reduce the environmental footprint of aquaculture, the amount of fish meal is reduced as much as possible as the fish grow and replaced with cheaper alternative protein sources, such as phototrophic bacteria (Bender & Phillips 2004).

Collectively, the use of phototrophic purple bacteria is prominent and mainly applied in sewage treatment processes because they can consume toxic substances and organic compounds, which are abundant in the wastewater (Imhoff 2006). Diseases may cause major economic losses in hatcheries and farmed based aquaculture practices. The port of entry of these pathogens could be the gastrointestinal tract which has been implicated as a site of colonization. Currently, either treatment with chemotherapeutic agents or vaccination is used to protect fish against different bacterial diseases in hatchery conditions. The former method may alter the profile of a healthy gut microflora, while the latter is stressful for fish. The use of chemotherapeutic agents might lead to the occurrence of antibiotic resistant bacteria and thus their use should be restricted. Preventing diseases in juvenile fish is of significant economic importance, since small fish have high mortality rates and are too small for vaccination (Ashraf et al. 2011; Swarnendu et al. 2010). The microbial feed such as phototropic bacteria may provide an alternative way to reduce the use of antibiotics in aquaculture and simultaneously avoid the development of antibiotic-resistant bacteria. However, in recent years phototrophic purple bacteria are seen to be an increased interest in aquaculture industry as it able to satisfy the increasing demand for affordable, safe and high quality of feed ingredient. The beneficial effects of these bacteria are known in different areas especially as feed supplement because they are rich in protein, lipid, minerals, carotenoids and other biological cofactors (Banerjee et al. 2000). Due to these benefits, they have been used in plankton production, in the shrimp culture and as a source of food for fish and chicken.

Malaysian Mahseer or Kelah (*Tor tambroides)* is the most expensive fresh water fishes in Southeast Asian countries. In fact feeding with commercial pellet alone is inadequate to nurture the good growth and survival of infant to juvenile of this fish (Syahnon & Abol-Munafi 2012). Mahseer is being considered as an endangered fish species in Malaysia due to the declines population of this fish in their natural habitat. Extensive research has been conducted to find the right nutrition requirements needed for Mahseer (Islam 2005; Jalal 2000; Jalal et al. 2005, 2001). Based on above evidences, this study was aimed to improve the growth, survival and feed utilization of fingerlings of *Tor tambroides b*y using phototropic purple bacteria.

Materials and Methods

Bacterial Biomass Production

Marichromatium sp. was isolated from mangrove sediment at Kuantan, Pahang while *Rhodopseudomonas* sp. was isolated from fish rearing tank at Institute Oceanography and Maritime Studies (INOCEM), Pahang. Synthetic 112 medium was used to culture both isolates as well as in preparation of mass culture for the production of bacterial biomass. About 5% of inoculum were inoculated into 2 L of 112 broth medium and incubated under 2500 lux light intensity at 30±2°C under anaerobic conditions for 48-72 h. The cultures were centrifuged to obtain the fresh biomass to be used in the diet preparation (Jalal 2005; Swarnendu et al. 2010).

Experimental Feed Preparation

The commercial Kelah feed (Sanyu Sdn Bhd) was used in this experiment. The pellet was grounded into powder by a grinder and mixed with fresh biomass of the bacteria in 1:2 ratio. The experimental feeds were pelletized and air-dried in the laminar flow before storing at 4°C for short-term storage (Ashraf et al. 2012; Jalal et al. 2005; Shapawi et al. 2012).

Experimental Fish and Preliminary Trial Protocol

The feeding trial was carried out at the Aquaculture Lab, Kulliyyah of Science, International Islamic University Malaysia (IIUM). *Tor tambroides* fingerlings were obtained from the Aquaculture Development Centre, Department of Fisheries, Perlok, Pahang. 50 L capacity of plastic aquariums with working volume of 40 L of dechloronized tap water were used as culture units. Three different treatments were employed in triplicates during the study. The aeration in the aquariums was supplied through air stone diffusers on the bottom of the aquarium. The water recirculation system was applied in each aquarium with auto-filter to make the water free of contaminants. Groups of 10 fishes (8.19±0.05 cm in standard length and 12.27±0.25 g in body weight) were randomly distributed into 9 aquariums (Shapawi et al. 2012).

Feeds and Feeding

Three types of diet were prepared throughout the experiment. The diets were *Rhodopseudomonas* sp. mixed with commercial pellet (Diet 1), *Marichromatium* sp. mixed with commercial pellet (Diet 2) and commercial pellet without bacteria (Diet 3). The fingerlings were fed twice daily: at 9.00 am in the morning and at 5.00 pm in the evening to satiation levels. A record of supplied feed was kept to determine the feed conversion ratio (FCR). During experimental period, the water quality was maintained by changing 50% of the water every day. Uneaten foods and the dead fishes were removed from the aquarium. The physicochemical characteristics such as dissolved oxygen, pH, nitrite, nitrate, ammonia and temperature were monitored every day. The experiment was conducted for 10 weeks. All ten fish from each tank were taken every 14 days to measure the standard length, weight and numbers individually with measuring board and analytical balance. The growth parameters such as survival rate, weight gain and specific growth rate were determined at the end of the experiment (AOAC 2000; Banerjee et al. 2000);

Weight gain = Final weight (g) - Initial weight (g)
Survival rate = (Number of fish at the end /Number of fish that was stocked at the start of experiment) × 100
Specific growth rate = (ln final mean weight- ln initial mean weight)/ Experimental days × 100
Feed Conversion ratio = Feed fed (g) / Gain in weight of fish (g)

Proximate Analysis

Proximate compositions (%) of the body carcass and feeds including moisture and ash were determined. While, crude protein and crude lipid were determined by Kjeldahl method and Soxhlet extraction method, respectively (AOAC 2000).

Statistical Analysis

The quantitative data are presented as mean ± standard deviation (SD) of three replicated diets. One-way ANOVA was performed with Statistic 6.0 (windows package) to compare the growth, survival and body composition of the fish with different types of supplemented diets.

Results and Discussion

The survival rate of *Tor tambroides* fingerling was generally high (96-100%) with Diet 2 showed no mortality (100%) and also no significance different (p>0.05) detected between the treatments (Table 1). The highest mean weight gain (g) was obtained from Diet 2 with 4.92±0.88 g, followed by control (4.11±0.48 g) and Diet 1 (3.99±0.29 g) (Figure 1). There was a significance different observed (p<0.05) between sampling days for the weight gain. Meanwhile, for length gain, Diet 2 also showed the highest length increment, which was 1.22±0.70 cm as compared with Diet 1 and Diet 3 which were 0.81±0.06 and 0.80±0.11 cm, respectively, as can be seen in Figure 2. However, There was no significance different (p>0.05) detected for the growth in length for all diet treatments. The specific growth rate (SGR) was generally low and fish of Diet 2 showed the highest SGR (0.49%) but no significance different (p<0.05) detected among the diets. The best feed conversion ratio (FCR) was achieved by Diet 2 with the lowest FCR value which was 1.77.

Table 2 shows the whole body composition of *Tor tambroides* fed with different diets. Lipid (15.5-19.0%), protein (33.37-36.46%), moisture content (71.2-73.67%) and ash content (8.5-12.73%) were observed through proximate analysis of the carcass. However the inclusion of the phototrophic bacteria in the commercial pellet did not significantly (p>0.05) affect the whole body composition of *Tor tambroides* fingerlings. While Table 3 shows the proximate composition of experimental feeds, which were moisture, ash, crude lipid and protein content ranging from 8.35-10.0%, 8.77-10.43%, 4.77-8.13% and 39.55-42.70%, respectively. The water quality parameters in all the culture

TABLE 1. Growth performance and feed conversion ratio of experimental fish at the end of feeding trial

Diets	Diet 1	Diet 2	Diet 3 (control)
Initial weight (g)	12.55±0.12	12.08±0.32	12.17±0.73
Final weight (g)	16.54±0.41	17.0±0.87	16.28±0.53
Weight gain (g)	3.99±0.29	4.92±0.88	4.11±0.48
Specific growth rate (%)	0.39±0.02	0.49±0.09	0.42±0.06
Survival rate (%)	96.7±1.91	100.0±0	96.7±1.91
Feed conversion ratio	2.44±0.61	1.77±0.33	1.90±0.12

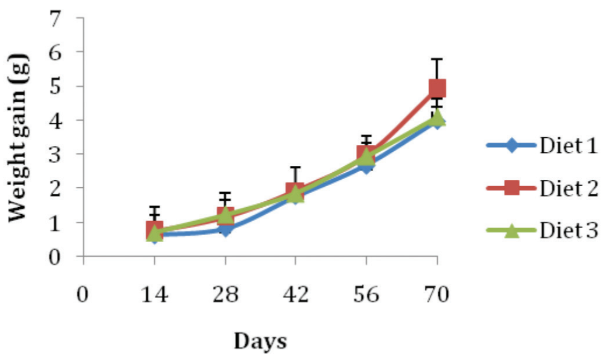

FIGURE 1. Weight gain of *Tor tambroides* fingerlings fed with Diet 1, 2 and 3

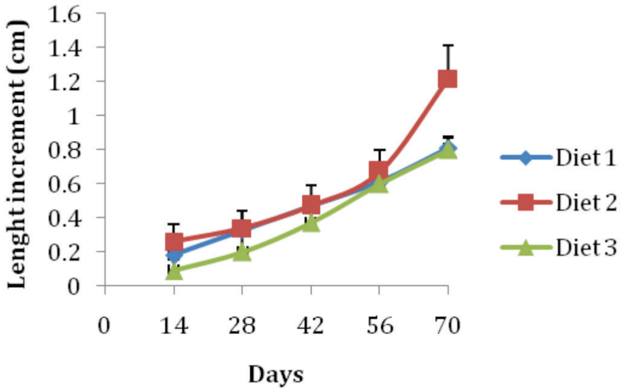

FIGURE 2. Length gain of *Tor tambroides* fingerlings fed with Diet 1, 2 and 3

TABLE 2. Analyzed proximate composition of experimental carcass on % dry matter basis

Diets	Initial	Diet 1	Diet 2	Diet 3
Lipid content (%)	25.61±1.68	19.0±5.20	22.50±8.67	15.50±8.85
Protein content (%)	24.50±3.64	33.76±3.76	36.46±4.94	33.37±1.31
Ash content (%)	7.01±4.37	12.73±0.95	10.86±0.55	8.50±2.09
Moisture content (%)	63.67±1.84	71.20±0.76	73.67±4.19	72.83±2.08

TABLE 3. Analyzed proximate composition of experimental feeds on % dry matter basis

Diets	Diet 1	Diet 2	Diet 3
Lipid content (%)	5.47±2.08	8.13±8.20	4.77±3.11
Protein content (%)	39.90±0.99	42.70±2.97	39.55±1.48
Ash content (%)	9.10±0.20	10.43±0.55	8.77±0.31
Moisture content (%)	8.35±1.96	8.85±7.11	10.0±0

units were almost the same and within the range of 25-27°C, 6.5-8.1 mg/L and 7.0-7.4 for temperature, dissolved oxygen and pH, respectively. While nitrite, nitrate and ammonia showed 0.17-0.34, 0.35-0.70 and 1.71-1.75 mg/L, respectively.

The findings of this experiment indicated that *Tor tamboides* fingerlings responded positively to the feed supplemented with phototrophic purple bacteria. The diet with the inclusion of *Marichromatium* sp. consistently showed higher growth and survival than other diets. On the other hand, it was observed that the magnitude of weight gain in the control diet was consistently less than diet of *Marichromatium* sp. The addition of bacterial cell in the fish feed of various cultured fish species have been investigated by a number of researchers (Banerjee et al. 2000; Shapawi et al. 2012). However, the effect of different bacterial species for fish varies from species to species due to their feeding behavior, habitat and size (Huntington & Hasan 2009).

Commercial feed without any additives seemed to have very limited acceptability, which agrees with the previous findings during the study on feed additives for *Tor tambroides* fry (Jalal et al. 2005). The addition of additive is important as it supplied the essential nutrients needed by the fry and fingerlings at their initial stage of life cycle. Phototrophic purple bacteria were known to contain enzymes that were found to be an important factor for increasing weight for fish. Extracellular enzymes from phototrophic purple bacteria help their early digestion and enhanced metabolism of the fingerling. Other than its cheap, halal and easy cultivation, it was evident that the nutritional values of these bacteria are crucial such as vitamins B12, proteins and sulfur containing amino acid makes them as a potential group of bacteria for the valuable source of fish feed (Banerjee et al. 2000).

Tor tambroides is a slow growing species, which takes a longer time to grow to a bigger size compared to other species. Thus it is reasonable to achieve a low specific growth rate (0.39-0.49%) which similar result was also reported also by other authors (Ismail et al. 2012; Kamarudin et al. 2012). During the first 4 weeks of experiment, the growth of the fingerlings was less than expected. However, same outcome also reported in other research as the fingerlings took some time to adjust with supplementation of bacterial cells (Vikineswary 1994). In the second month of feeding, the fingerlings started to show the attraction and likeness towards the bacterial cells when the fingerlings finished the feeds in 5 min. Both genus *Rhodopseudomonas* sp. and *Marichromatium* sp. used in this experiment were safe to the *Tor tambroides* fingerlings as there were no diseases observed among the fingerlings throughout the study period. Thus, phototrophic purple bacteria are safe to be incorporated in the fish diet (Shapawi et al. 2012; Vikineswary 1994).

Tor tambroides is known as a very sensitive freshwater fish. It can only tolerate high-oxygenated cold water. The experiment has been carried out in running water, aerated and the temperature was maintained to 23-25°C. The fish showed better growth performance within the water temperature range 20 to 24°C (Kamarudin et al. 2012). Although no published reports are available on the optimum temperature for their growth but it can be postulated that a temperature above 30°C may not be suitable for its growth based on the condition of their natural habitat (Muhamad & Mohamad 2012).

FCR values obtained in the present study showed similar trends with the FCR values reported for *Tor putitora* which ranged from 1.12 to 2.21 (Hossain et al. 2002). A lower FCR value showed that the fish needs less feed to grow at optimum weight. This is important as it can reduce the consumption of excess feed that will produce excess fecal material, uneaten foods and urinary nitrogen. It is not only wasteful in terms of money but also increase waste management problem. The FCR value in the present study showed that diet 2 with *Marichromatium* sp. as the supplement which can maximize the fish growth and minimize the waste at the same time.

The proximate analysis of the fish flesh showed that the addition of *Marichromatium* sp. has improved the nutritive values of the fingerling flesh in term of protein content, ash content, lipid and moisture content. However, lipid content for Diet 1 and 3 decreased after the feeding trial. This might be due to low lipid content in the diets. A complete diet should contain 10-25% of lipid (Ahmed et al. 2014; Webster & Lim 2002). It was reported that rainbow trout fed with probiotic resulted in higher protein content and decreased lipid content in the carcass (Ahmed et al. 2015; Bagheri et al. 2008). Proximate analysis of the feeds showed that the inclusion of phototrophic cells has significant effects on the nutritive values of the feeds. Lipid content of experimental feeds (Diet 1 and 2) showed a better percentage compared to commercial pellet (Diet 3). Feed supplemented with *Marichromatium* sp. (Diet 2) has a greater lipid and protein content compared to *Rhodopseudomonas* sp. Protein content reported in this study was comparable (42.7%) which was achieved by the feed supplemented with *Marichromatium* sp.

CONCLUSION

The present study demonstrated that the incorporation of *Marichromatium* sp. in fish feed consistently giving better values in growth performance, survival rate, proximate composition and lowest FCR, which were most desired requirements in modern aquaculture practices. In fact, it was evident that the phototrophic bacteria was able to control the bacterial disease and maintain the water quality of the fish cultured tank. Hence, the proposed experimental diet *Marichromatium* sp. could be a useful as feed supplement during initial stages of fresh water fishes. Nevertheless, it can be concluded that this microbial feed might be an alternative approach to replace antibiotics in fish feed to boost the rapidly growing aquaculture industry in Malaysia and South-east Asian countries.

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