

Effect of commercial probiotics on the survival and growth performance of goldfish *Carassius auratus*

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Abstract. The main aim of this work was to investigate the effects of commercial probiotic on the survival and growth performance of goldfish *Carassius auratus*. In this experiment, commercial probiotic was introduced in diet at three different levels (T1: 1%, T2: 3%, T3: 5%) and their dietary effects were compared to diet that contained no probiotic (control). Each treatment was triplicated with 10 fish each and the feeding trial was conducted for 28 days. Results showed that, supplementation of the basal diets at any level with commercial probiotic significantly yielded higher survival rate of the *C. auratus*. Similarly, growth performance of *C. auratus* including weight gain (WG) and specific growth rate (SGR) were significantly higher in all treatments than the control diet. Food conversion ratio in fishes that fed with diets containing probiotic was significantly lower than control groups. Meanwhile, total length was not significantly affected by the administered probiotic. The highest and optimum growth performances was recorded in fish fed on the control diet. The supplementation of dietary probiotics could serve as functional ingredients to enhance their survival and growth performances.

Key Words: ornamental fish, beneficial bacteria, survival rate, growth increase.

Introduction. Goldfish *Carassius auratus* and its varieties (Ahilan et al 2009) are among the most popular ornamental fish throughout the world as companion fish. In recent years, ornamental fishes are receiving increased attention due to high local and global demand and yield significant growth to import-export market and trade. In 2000, it was estimated that for about US\$ 3 billion worth the value for both freshwater and marine live ornamental animals (Andras 2012). Countries traditionally specializing in breeding and propagation of freshwater ornamental fishes are Japan, China, Singapore, Thailand, Indonesia and Malaysia. More recently ornamental fish are now cultivated in countries such as, Spain, Belgium, Czech Republic, Israel and Holland. Southeast Asia is the hub of ornamental fish trade, supplying up to 85% of the aquarium trade (Andras 2012).

The Japanese goldfish, *C. auratus*, is one of the most popular ornamental species in the world due to its varieties with attractive body shape and skin color (Zhou et al 2001). In addition to body shape and fins size, skin pigmentation (derived from the deposition of carotenoids in their tissues) of the *C. auratus* is one of the important quality criteria to set the market value of ornamental fish (Yanar et al 2008). The successful culture of *C. auratus* would depend extensively on prepared feed and cost effective diets that provide all the essential nutrients for the fish growth.

Some of the most utilized growth-promoting additives include hormones, ionophores, some salts and antibiotics (Fuller 1992; El-Haroun et al 2006). Antibiotics were commonly used in the early 1950s (Ahilan et al 2004) as traditional strategy for fish diseases management but also for the improvement of growth and efficiency of feed conversion. Cultured fish that faced continuous mortality due to disease are prompt to treat by using antibiotics but in some countries, this method is banned (De Paola et al 1995; FAO 2005; Watson et al 2008). Resistance bacteria evolved from the misuse and overuse of antibiotic (Watson et al 2008).

Hence, in connection with the ban of antibiotic growth promoters, new strategies in feeding and health management in fish aquaculture practice have received much attention (Balcázar et al 2006). This situation paved the way for the search for an alternative to the antibiotics (Kumar et al 2006; Wache et al 2006). Probiotics has proven to be the alternatives in promoting health and growth (Denev 2008).

Probiotics are now used in aquaculture as simple and safe additive to improve the health of the host and is increasingly viewed as alternative to replace antibiotic treatments. Probiotics have many advantages in aquaculture, such as modulating microbial colonization, providing nutrients, improving immune responses, increasing digestive enzyme activities, improving feed utilization and digestibility, controlling diseases, improving water quality and enhancing growth (Gatesoupe 1999; Verschuere et al 2000; Iriant & Austin 2002; Pérez-Sánchez et al 2014).

Cultivation of ornamental fish species, in addition to the aquaculture of food fish species, has gained ground nowadays, and research projects in this field have become of great interest. Since the economic importance of aquarium fish is not less than that of the food fish, it is therefore, important to investigate various aspects of their cultivation including survival and growth as well as ways of reduced cost of feeds by using probiotic. The commercial probiotic used in the present study contains Bacillus licheniformis and Bacillus subtilis. The advantage of these spore-forming bacteria is that they are able to survive the palletisation process. After transit through the stomach, they germinate in the intestine and use a large number of sugars (carbohydrates) for their growth and produce a range of relevant digestive enzymes, amylase, protease and lipase. The beneficial effects of probiotics include higher growth and feed efficiency, prevention of intestinal disorders and pre-digestion of anti-nutritional factors present in the ingredients. Moreover, the use of probiotics for enhancing bio-growth parameters and in improving disease resistance ability has been well documented in fish (Watson et al 2008; Wang et al 2006). Thus in the present study, the new strategy of promoting survival and growth of C. auratus juvenile by using probiotics will be studied.

Material and Method

Experimental fish. *C. auratus* specimens were obtained from local supplier in Terengganu, Malaysia. The fish (initial weight 8.88 ± 0.17 g) were maintained in a holding rectangular aquarium of $15 \times 13 \times 15$ cm in size with a constant flow of fresh water. The temperature and photoperiod of the culture were maintained between 29-32°C and day:light 12:12 hr, respectively. Experimental fish were acclimated to these conditions for at least 7 days prior to the feeding trial.

Diet preparation. Commercial *C. auratus* starter food was taken as a basal diet for the supplementation of probiotic. The diet content fish meal, soybean meal, rice bran, broken rice, vitamin and mineral (Table 1). The commercial probiotic levels of 1%, 3% and 5% (of the fish body weight), respectively were sprayed onto the feed slowly. Then, the feed was air dried under open air for 12 hours and was kept in the fridge. The commercial feed sprayed in the same way with sterile diluent alone served as the control diet (Aly et al 2008).

Table 1

Chemical composition of the diet used in this experiment

Item	Composition (%)
Crude protein	32
Crude fat	3
Moisture	12

Experimental design. The experimental tanks benefited by continuous aeration in order to maintain dissolved oxygen near saturation levels (>4mg/L). Four experimental groups were conducted to evaluate the effect of probiotics administered to the *C. auratus*

juvenile, each treatments were designated with different concentration of probiotic as (T1: 1.0%, T2: 3.0%, T3: 5.0%, C: 0%). Group C was fed with a commercial pelleted diet without any probiotics supplementation and was considered as control group. This study was performed in triplicate and stocked with 120 fish (10 fish per aquarium; 3 aquarium per treatment). *C. auratus* juvenile of different treatments were fed at the rate of 2% of the body weight initially with respective diet and the feeding rate was adjusted accordingly with respect to the bio-mass gain over a period of every 14 days. The fish were fed daily at 9.00 AM and 3.00 PM. Each aquarium was cleaned prior to morning feeding by siphoning fish faeces and other organic wastes and 50% of total water volume was change with dechlorinated fresh water. Mortality, external signs of infections and behavioral abnormalities were recorded daily and the survival percentage (SP) was calculated (Jindal et al 2010).

Every 2 weeks of sampling, the fish were taken for wet weight and total length measurement. Indicators of growth includes: body weight increase (BWI), specific growth rates (SGR), feed conversion ratio (FCR) and survival rate (SR) are expressed as following:

BWI=W2 -W1;

SGR = 100*In(W2) - In(W1)/T;

FCR=Dry weight of feed consumed by fish/Wet weight of fish (g);

Where, W1 and W2 are the initial and final weight, respectively, and T is the number of days in the feeding period.

Survival rate (SR %) = $\frac{\text{Final number of alive fish}}{\text{Initial number of fish}} \times 100$

Isolation of probiotic bacteria, Bacillus sp. The isolation of bacteria can be done from the gut of the fish pre- and post- oral administration of probiotics (Himabindu et al 2004). One fish was collected randomly from each tank of the feed trial and the fishes were brought to the laboratory alive and they were sacrificed (Ghosh et al 2007). The ventral surfaces were sterilized using 70% ethanol to remove any external microbial contamination (Chantharasophon et al 2011) and aseptically dissected to remove the intestines (Ghosh et al 2007). The intestines were opened by a longitudinal incision and thoroughly flushed with sterilized chilled normal distilled solution (NDS) to remove feed materials, dirt, and other impurities (Ghosh et al 2007). Gut sample was initially diluted 1:1 (wt:vol) in buffered peptone-water (Oxoid) and resuspended by vigorous vortexing until an evenly distributed suspension was obtained. Aerobic spore-forming was isolated by using heat. For heat treatment, the suspension was further diluted 1:10 in buffered peptone-water and incubated at 65°C for 20 min (Barbosa et al 2005). Subsequent plating of 0.1 mL aliguots of appropriate 10-fold serial dilutions in buffered peptonewater (up to 10-5) was aerobically on Luria-Bertani (LB) plates, which support germination (Barbosa et al 2005). Although no quantification was attempted, a measurable number of colonies (more than 10) was routinely obtained on 10⁻² to 10⁻³ dilution plates after 24 to 48 hours of incubation at 37°C (Barbosa et al 2005). Bacillus isolates was routinely grown aerobically at 37°C in LB or DSM (Barbosa et al 2005). The sample was stored at -81°C if the sample does not proceed at that day.

Statistical analysis. One way analysis of variance (ANOVA; SPSS, 10.0) was used to determine whether significant variation between the treatments existed. Difference between means were determined and compared by Tukey's HSD test. All tests used a significance level of P < 0.05. Data are reported as means \pm standard errors.

Results and Discussion

Growth performance and survival rate. Growth performance including weight gain, feed conversion ratio, specific growth rate had higher significant differences (p<0.05) between fish groups offered diet contained probiotic compared to the control group offered basal diet. The results showed that the highest weight gain was achieved with fish fed diet T2 followed by diet T1, T3 and control. The weight gain of fish fed with control diet was significantly lower (P>0.05) from all diet treatments. Meanwhile, total length was not significantly affected by the administered probiotic at any level. Results of Table 2 shows that, incorporation of probiotics, significantly (P<0.05) improved FCR level. All treatments showed significantly lower of FCR compared to control. Meanwhile, the SGR in all treatments were significantly higher compared to control. Fish fed diet T2 showed the highest SGR which significant difference (P>0.05) among all treatments.

Table 2

Growth index	T1	T2	Т3	C (control)
Survival (%)	96.67 ± 3.33^{a}	100.00 ± 0.00^{a}	86.67 ± 6.67^{ab}	76.67±3.33 ^b
Weight gain (g)	1.89 ± 0.13^{a}	2.61 ± 0.21^{a}	1.99 ± 0.14^{a}	0.74 ± 0.03^{b}
Length (cm)	6.11 ± 0.06^{a}	6.06 ± 0.05^{a}	5.78 ± 0.01^{a}	5.61 ± 0.20^{a}
Food conversion ratio	2.92 ± 0.07^{a}	2.20 ± 0.12^{a}	2.67 ± 0.30^{a}	6.45 ± 0.42^{b}
Specific growth rate (%/day)	0.67 ± 0.03^{a}	0.92 ± 0.05^{b}	0.71 ± 0.04^{ab}	$0.29 {\pm} 0.03^{c}$

Growth performance and survival of *Carassius auratus* juvenile fed basal diet and diet supplemented with probiotics for 28 days

Values are mean of triplicate groups and presented as mean \pm SE. Values with different superscripts in the same row are significantly different (p<0.05). C (control): fish fed with basal diet. T1, T2 and T3: fish fed with basal diet supplemented with 1%, 3%, and 5%, respectively.

According to the Tuan et al (2013), commercial probiotic was only tested on *Oreochromis niloticus* and never used for other species yet. Mehrim (2009) reported that, addition of 0.3% commercial probiotic to the diet increased the survival rate of tilapia compared with the control diet (without commercial probiotic). Abdelhamid et al (2002) showed that survival rate of Nile tilapia *Oreochromis niloticus* was increased as commercial probiotic level increased from 0 to 0.4%. Similar results were recorded in this present study where, administering probiotic showed significantly improves higher survival in all treatments, except for T3. Survival rate for fish fed on the control diet T2 was found to be 100%. The positive effect of the commercial probiotic used in this study may be due to its effects which serve as antitoxic, antibacterial and antifungal agents, which may lead to improve the survival rate. *B. subtilis* may play a vital role to control the pathogen. Ghosh et al (2007) indicated that incorporation of *Bacillus* species in fish diets significantly increased survival and decreased mortality.

Application of *B. subtilis* and *B. licheniformis* in this present study resulted in significant improvement in weight gain. In examples of growth improvement in ornamental fishes, in guppies, *Poecilia sphenops*, *P. reticulata*, and southern platyfish, *Xiphophorus maculatus*, and green swordtail *X. hellerii*, the incorporation of intestinal isolate of *B. subtilis*, isolated from *Cirrhinus mrigala* into their diet for 50 and 90 days has been evaluated. The growth of these tested fish was increased as length and weight of the ornamental fishes were improved. The elevated specific activities of proteases and amylases in the digestive tract were reflected as significant increases in survival and growth of *Xiphophorus* and *Poecilia* (Ghosh et al 2008).

Numerous studies have shown that, the application of probiotics can improve weight gain, feed conversion ratio, specific growth rates of salmonids (Merrifield et al 2010). Supplementation of *Bacillus* spp. resulted in significant improvement of rainbow trout fry feed conversion ratio (FCR), specific growth rate (SGR), weight gain and protein

efficiency ratio (PER) after 2 months feeding trial (Bagheri et al 2008). While, in the present study, supplementation of the basal diet with probioic on the *C. auratus* juvenile throughout 28 days also resulted in similar higher weight gain, FCR and SGR compared to the control diet as described in Table 2.

Results of Table 2 stated that, the best FCR values observed with probiotic contained diets significantly (P<0.01) improved FCR compared to control diet. In practical terms, this means that supplementation of fish diets with probiotics improved feed utilization or optimized protein use for the growth which can decrease the amount of feed necessary for fish growth, which could result in production cost reductions (Ringo & Gatesoupeb 1998).

After 28 days of experiment, *C. auratus* juvenile fed with probiotic showed significantly higher (p<0.05) survival rate compared control except for T3. From Table 2 fish group fed with diet T2 exhibited the highest percent of survival (100%) followed by T1 (96.67%) and T3 (86.67%). Meanwhile, control fish groups exhibited the lowest percent of survival (76.67%).

Water quality parameters. During the whole experimental period about 4 weeks, water temperature ranged from 27.45 to 29.33° C, dissolved oxygen from 4.16 to 6.67 mg L⁻¹, pH from 7.60 to 7.90 and total ammonia from 0.11 to 0.14 mg L⁻¹. The results indicated that, the water parameters are in acceptable level and the experimental diets had no adverse effects on the surrounding water quality of experimental fish.

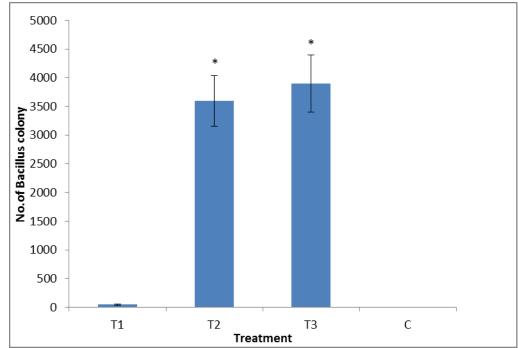
Isolation of Bacillus sp. Figure 1 showed that, the total bacterial counts of *Bacillus* spp. in the intestinal flora of *C. auratus* juvenile fed diets containing probiotics, increased with greater level of probiotic supplement in treatments. All treatment shows significantly higher in number of *Bacillus* species except for T1 which non-significant different (P>0.05) compared to the control group. The relative proportion of *Bacillus* spp. in the intestine of *C. auratus* juvenile fed diets containing probiotics, increased with greater density of probiotic supplement in treatments.

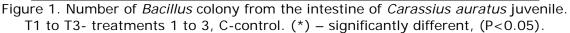
According to Ringo et al (1995), high proportion is probably related to an increase in suitable attachment sites as a result of histological and functional development of fry and improved internal environmental conditions for bacterial growth (Vine et al 2006). Other than that, high proportion of *Bacillus* sp. indicated that the given probiotic is suitable to settle and grow.

However, Son et al (2009) mentioned that high concentration of probiotic does not indicate good growth performance as the effectiveness of the probiotic is also depend on fish species, temperature, enzyme level, genetic resistance an also water quality (Cruz et al 2012). This statement goes paralleled with the results obtained from the experiment. The specific growth rate throughout the experiment was improved in T2 (3% of probiotic) not in T3 (5% of probiotic), it can be certainly suggested that the more probiotic cells in diets and host intestine necessarily does not result in the more improved survival and growth. Better growth, as observed in T2 which 100% of survival, may establish better health conditions in *C. auratus* juvenile and therefore, decrease mortality.

The *Bacillus* species secret a wide range of exoenzymes (Moriarty 1998), which might have supplied digestive enzymes and certain essential nutrients to promote better growth. *B. subtilis* and *B. licheniformis* can break down proteins and carbohydrates (Farzanfar 2006). So it can be suggested that administration of *Bacillus* bacteria to *C. auratus* juvenile results in enhanced digestion of food and improved growth, including high weight gain, low FCR, and high SGR.

From the results in the present study showed that, supplementation of *C. auratus* juvenile diet with the proper density of commercial *Bacillus* probiotic, could be beneficial for survival and growth of *C. auratus*, especially in fast growing conditions, where it would be essential to stimulate the precocious maturation of digestive system (Wache et al 2006). No clear effect of probiotic on diversity of *C. auratus* juvenile intestine flora was detected, but high rate of probiotic bacteria colonization was observed.





Conclusions. Based on the obtained results, it is recommended to supplement *C. auratus* diets with probiotics, as natural feed additives in their nutritional needs. Thus, could be replacing the use of antibiotics in the animal industry. It can be concluded that the *Bacillus* species were play an important role in enhanced the survival, weight gain, FCR and SGR of reared *C. auratus* juvenile with optimum concentration of 3%. Future work must focus on applications of probiotics to detect the mode of action of probiotics on digestibility, immune response and stress resistance especially on ornamental fishes. Also, it is important to define the probiotic levels administered to fish to avoid overdosing and under-dosing with resultant lower efficacy and unnecessary costs. While, in order to achieve the maximum efficiency of natural alternative growth promoters to be used in functional feedstuffs, probiotic treatments must be used in concert with effective farm management and husbandry.

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