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GROWTH AND LENGTH-WEIGHT RELATIONSHIPS OF *Trichopodus trichopterus* (Pallas, 1770) FED A SUPPLEMENTED DIET WITH DIFFERENT CONCENTRATIONS OF PROBIOTIC

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ABSTRACT

The aim of the present study was to evaluate the effects of diet with different microbial concentrations on the growth and length-weight relationship of *Trichopodus trichopterus*. For this aim a study was designed with four experimental treatments and the control to evaluate different microbial concentrations on growth and length-weight relationship. The counts of bacteria that were used for dietary supplementation were 1×10^4, 2×10^4, 3×10^4 and 4×10^4 CFU g^{-1} in T1, T2, T3 and T4 treatments, respectively. The results indicated that the use of 3×10^4 CFU g^{-1} concentration increased the length, and a positive allometric growth was observed. The higher length frequency was estimated in T3. The results indicated that the use of microbial additives with proper concentration causes different allometric growth.

INTRODUCTION

The genus *Trichopodus* is one of the oldest genus-group names within the Southeast Asian fish family *Osphronemidae* (Kottelat, 1998). Gourami fish is a member of the *Trichopodus* genus and is closely related to *Trichogaster* (formally *Colisa*). Species of both genera have long and threadlike pelvic fins known as "feelers" in the aquarium trade that are used to sense the environment. The species has become established in other non-Asian countries possibly due to the improvement of the aquarium trade (Allen, 1991). Nevertheless, in Iran, where Gourami had been introduced, it is regarded as a valuable and suitable species for breeding. This is a fresh water and benthopelagic specimen that tolerates a pH range between 6-8 and 22-28ºC (Riede, 2004; Riehl et al., 1991). Under native conditions, it lives in lowland wetlands (Vidthayanon, 2002), marshes, swamps and canals (Kottelat, 2001). It feeds on zooplankton, crustaceans and insect larvae (Westenberg, 1981). However, it feeds on formulating diets in captive breeding, and nutrient supplements, hormones and therapeutic agents as probiotic have special relevance in the development of rearing technologies for fish larvae and juveniles (Yufera et al., 2003). Growth process in fish reflects numerous effects (Brett, 1979). One of the important agents of growth of fish is feed supply (Elliott, 1976; Jones, 2002). Also reproduction factors such as egg size and fecundity were highly dependent on female traits such as body size and nutrition (Shimada et al., 2007). The most obvious method seems to be the incorporation of such compounds in the feed and administering them with the regular feed, as proven in the research on juveniles. In general, this stems from different feeding behavior and diet preferences but also from the fact that during the first weeks of life the digestive tract is still developing and its function is changing. This results in insufficient digestion and assimilation of nutrients provided in the dry supplemented diet and attention has recently been focused on the use of probiotics. FAO and WHO defined probiotics as live micro-organisms that when administrated in adequate amount confer a health benefit on the host (FAO/WHO, 2001). Probiotic operation has been well defined (Fuller, 1978, 1992; Fuller and Turvey, 1971; Parker, 1974; Roach and Tannock, 1980; Smoragiewicz et al., 1993). The aim of this study was to investigate the length-weight relationships and growth characteristics of three-spot Gourami fish in an in vitro study under different concentrations of *Bacillus circulance* and *Bacillus subtilis*.

MATERIAL AND METHODS

Bacterial strain and experimental diet

The basal commercial diet (BioMar-France) contained about

54% of protein, 18% of lipid and 9.7% of ash. The basal diet was used as the control diet. Out of the control diet, four experimental diets containing four graded levels of *B. circulance* and *B. subtilis* were prepared. The probiotic strains, *B. subtilis* and *B. circulance* used in this study were obtained from Protexin commercial products (London, England) and cultured on Tryptic Soy Agar (TSA) plates. The counts of bacteria in diets were approximately (T1) 1×10^4, (T2) 2×10^4, (T3) 3×10^4 and (T4) 4×10^4CFU g^{-1}.

Experimental animal and design

Healthy larvae of three-spot Gourami (*Trichopodus trichopterus*) were obtained from an ornamental fish farm (Golestan, Iran). Prior to the start of the experiment, the fish were fed a control diet for a week in the same tank with 100 L volume to enable the larvae to adapt to the experimental diet and environment. The larvae of uniform size (50 ± 0.8 mg, 4 ± 0.6 mm) were randomly distributed in 15 fiber glass tanks with a capacity of 15 liters with each of three replicates following a complete randomized design. Each fiber-glass tank was stocked with a density of 3 larvae/L. The fish were hand-fed to apparent satiation four times daily at 6:00, 12:00 a.m. and 18:00, 24:00 p.m. for 30 days. During the experimental period, water temperature ranged from 27.5 to 28°C, and pH value was 7.8. At the end of the study, the total number of the fish in each tank was counted and mean bodyweight and length of the fish in each tank was measured to investigate the length-weight relationship and growth.

Fish capture and sampling

Sampling was performed from each treatment after the experimental period. Three-spot Gourami were collected with handheld fishing nets with 0.1 mm mesh from each tank. In the sampling location, fish specimens were immediately anesthetized with the extraction of *Eugina caryophillata*. The fish specimens were weighed (W, ± 0.01) on a digital scale (Kern model, Germany) and total length was measured with calipers (TL, ± 0.1 mm). Length-weight relationships were calculated for the entire population. The exponential equation: $W = aTL^b$, where *W* is the weight (g), L the length (cm) and *a* and *b* are regression parameters.

Statistical analyses

The significance of difference in growth rate and parameters among the different experimental treatments was calculated by one-way ANOVA followed by Duncan's multiple range test (DMRT) to examine which of them varied significantly. The slope of length-weight regressions was compared for differences using analysis of variance (ANOVA) (SPSS, Version 19).

RESULTS

Growth and population structure

Three-spot Gourami (*T. trichopterus*) ranged from 1.9-4 cm in total length and 0.07– 0.87 g in total weight. Length frequency distribution (Fig. 1) indicated that the most frequent size classes in the samples were 1.5- 2 cm for control, T1 and T2 groups, 3-3.5 for T4 and 3.5-4 for T3 group, respectively.

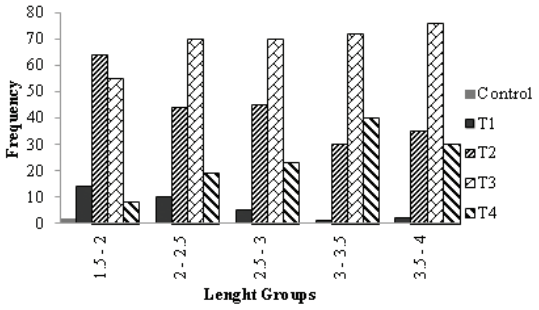

Fig 1. The total length (cm) frequency of different treatments of *T. trichopterus.* Control showed the frequent size classes of 1.5-2 and other size classes were not observed in the control groups. The effect of different microbial concentrations on growth performance of three-spot Gourami are presented in Table 1.

The sex of the remaining individuals could not be identified and therefore were not included in the study. Length estimates revealed that the used probiotics during study caused maximum size of 4 cm in T1, T2 and T3 groups (Fig. 1). Length-weight

Table 1. The growth response of Gourami (*T. trichopterus*) fed diets with graded level of *B. circulance* and *B. subtilis*. (mean±SD, n=142)

	Treatments				
Parameters	Control	T1	T2	T3	T4
Final weight (mg)	334.75±120.95^a	349.51±122.62^a	340.76±117.74^a	330.83±108.03^a	332.58±105.28^a
Finial length (cm)	2.85±0.37^d	3.03±0.35^c	3.10±0.36^{bc}	3.21±3.11^a	3.14±0.33^{ab}
Condition Factor	1.59±0.23^c	1.77±0.28^a	1.70±0.25^b	0.69±0.10^d	1.74±0.40^{ab}
Average daily growth of length (%)	19.06±2.47^d	20.20±2.36^c	20.67±0.46^{bc}	21.43±2.07^a	20.97±2.26^{ab}

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

Table 2. Parameters of the regression ($W = aTL^b$) between total length (TL, cm) and total weight (W, g) for three-spot Gourami (each treatment).

Treatment	a	b	b- interval	n	R^2	F
Control	0.012	2.86	2.68-3.04	143	0.87	954.1
T1	0.015	2.66	2.41-2.85	143	0.74	625.3
T2	0.015	2.62	2.41-2.82	143	0.81	625.3
T3	0.006	3.28	3.03-3.54	143	0.82	649
T4	0.010	2.93	2.27-2.85	143	0.84	310.2

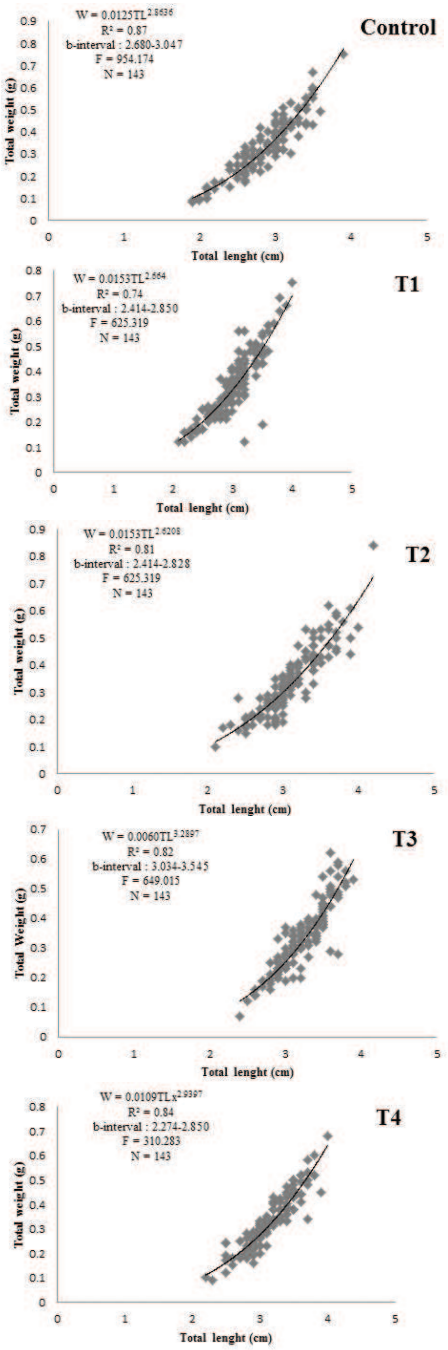

Fig 2. Relative growth curves (total length-total weight) for *T. trichopterus* fed on different probiotic concentrations.

regression was calculated separately for each treatment and presented in Table 2. Significant differences were observed at length-weight relationships (Fig. 2), while *b*-value implied that the body shape displays a negative allometric growth in all experimental groups except T3.

Positive allometric growth was observed in group T3 (*b* value 3.28) which was fed on lower probiotic concentration (3×10^4CFU g^{-1}) than T4 groups (4×10^4CFU g^{-1}). The highest growth mean was observed in T1 group but it did not show a positive allometric growth. However, T3 group with lower growth mean than T1 showed a positive allometric growth.

DISCUSSION

The aim of the present study was to describe data on the growth and length-weight relationship of *T. trichopterus* fed on different microbial concentrations. Three-spot Gourami is a very hardy fish; it has been produced commercially in various color forms: gold and opaline. It is prolific and easy to breed (Sandford, 2007). The ornamental fish sector is a global component of international trade, fisheries and aquaculture development, and is one of the most economic and profitable areas of fish farming (Ghosh et al., 2007). The two main aspects that determined the trade and prosperity of the ornamental fish industry were fecundity and growth. The fish fed probiotic food exhibited no significant difference in the growth of weight when compared to the fish fed control feed. This result implies that *T. trichopterus* utilize dietary nutrients with the same efficiency, regardless of whether or not the feed is supplemented with Bacillus. Similarly, Boyd et al. (1984) and Sahandi et al. (2013) reported that adding commercial probiotic did not have any significant effect on the weight of channel catfish and guppy fish, respectively, and the addition of probiotic to the rearing system of halibut larvae (*Hippoglossus hippoglossus* L) did not increase the growth of larvae (Makridis et al., 2001). According to DeVrese and Marteau (2007), mechanism and function of probiotic effect depend mainly on the interactions between probiotic species and microbiota of the host or immunocompetent cells of the intestinal mucous. However, the use of probiotics in feed increased the growth in length significantly ($P<0.05$). Significant difference of average daily growth of length, and also the condition factor, showed that T3 has the highest length in comparison with other treatments ($P<0.05$). Also the total length frequent size classes of 1.5-2 observed in the control and other size classes were not observed in the control group. The highest frequent

size classes of 3.5-4 were observed in T3 which showed the highest length growth (P<0.05). The growth in length might be caused by probiotic interaction that increased mineral absorption (Tewe et al., 1999). Sahandi et al., 2012 also reported the same results that demonstrate the effect of *Bacillus spp* on the length of Silver carp and increased length growth from 2.85±0.37 mm in the control to 3.21±3.11 cm in T4. The exponents of the length-weight relationship (Table 2) of three-spot Gourami were estimated between experimental treatments and the control and showed a negative allometric growth (T1: b = 2.41; T2: b = 2.41; T4: b = 2.93 and the control: b = 2.86). Also, a positive allometric growth was observed in T3: b = 3.28 fed on diet containing 3×10^4 CFU g^{-1}. The length-weight relationship provides information on growth patterns. Peters (1983) reported that fecundity in fishes is positively correlated with the length and the amount of energy available for egg production, and the egg accommodation capacity of the body cavity increases with fish size. Also other researchers report that fecundity may increase with increased body size in fish (Lawson, 2011). The improvement of fish fecundity in aquaculture will give rise to sustainable expanse in this industry. The use of probiotic caused length growth in the present study, however, the probiotic concentration was positively correlated with the growth of length (r=0.822, P<0.05). The use of probiotic is suggested for better growth performance and increase of feed digestibility (Jafaryan et al., 2008; Sahandi et al., 2012; Adineh et al., 2013; Ljubojević et al., 2013).The b-values observed in this study conform to the suggestion of Carlander (1969) that b value normally falls between 2.5 and 3.5. The length-weight relationship exponent indicated that growth is positively allometric in T3 treatment, with a b-value > 3 indicating that fish becomes longer as weight increases with probiotic concentration. However, the length-weight relationship demonstrates that growth is negative allometric in other treatments and the control (b-value <3). A weight-length relationship has not previously been studied in nutritional experiments of fishes. Ismen (2005) reported that the functional regression of b value is directly related to weight and is affected by environmental parameters such as food supply. There are some studies that have been done on the length-weight relation and feeding habits in fish (Hosseini et al., 2009; Lawson, 2011). In the present study, feed supply with different probiotic concentrations was studied and a positive allometric growth was observed in the treatment which was fed on 3×10^4 CFU g^{-1} level of *B.subtilis* and *B. circulance*. Based on condition factor as a biological parameter, Jafaryan et al. (2008) reported the same result that demonstrates the effect of Bacillus on the positive allometric growth of *Acipenser nudiventris* experimental treatments in comparison with the control. Growth coefficient values showed that three-spot Gourami fed on a diet containing 3×10^4CFU g^{-1} of *B. circulance* and *B. subtilis* approached the asymptotic length faster than other treatments and the control. The fish length is the best indicator of production efficiency (Ghorbani et al., 2012). Increase in the length of three-spot Gourami under the Bacillus consumption is not completely understood but, according to Lilly and Stillwell (1965), probiotic improved the gastrointestinal microbial populations and thus increased enzyme secretion (Jafaryan, 2006) and increased utilization of nutrients and protein anabolism, as length and weight gain occurs in fish. The growth (length in cm) was significantly changed in experimental trials fed on a diet supplemented with probiotic (P<0.05) when compared with the control (Figure 3). The result of this study showed that the use of different concentrations of *B. subtilis* and *B. circulance* improved the length of three-spot Gourami. The growth in length might be increased on fecundity of *T. trichopterus*.

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Sažetak

RAST I DUŽINSKO MASENI ODNOSI VRSTE *Trichopodus trichopterus (Pallas,* 1770) HRANJENE NADOMJESTKOM RAZLIČITIH KONCENTRACIJA PROBIOTIKA

Cilj ovog istraživanja bio je ispitati učinke hranidbe nadomjestkom različitih koncentracija mikroorganizama na rast i dužinsko masene odnose *Trichopodus trichopterus*. U svrhu tog cilja, studija je dizajnirana s četiri eksperimentalna tretmana i kontrolom za procjenu različitih koncentracija mikroorganizama na rast i odnos duljine i mase. Broj bakterija koje su se koristile za hranidbeni dodatak su 1×10^4, 2×10^4, 3×10^4 i 4×10^4 CFU g^{-1} u T1, T2, T3 i T4 tretmanu. Rezultati su ukazali da se upotrebom 3×10^4 CFU g^{-1} koncentracije povećava duljina, a uočen je pozitivan alometrijski rast kod istraživane vrste. Veća frekvencija duljina procijenjena je u T3. Dobiveni rezultati ukazuju da primjena različitih koncentracija probiotičkih organizama u odgovarajućem slučaju uzrokuje različit alometrijski rast riba.

Ključne riječi: dužina, Trichopodus trichopterus, rast, probiotici, alometrija

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