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Effects of probiotic (*Pediococcus acidilactici*) on growth and survival of kutum (*Rutilus kutum)* fingerlings

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Abstract

In the present study effects of different levels of probiotic *Pediococcus acidilactici*, on growth performance and survival rate of *Rutilus kutum* fingerlings were investigated. Kutum fingerlings with a mean weight of 1±0.235 g were reared for 8 weeks in 500-l fiberglass tanks (15 fish per tank) with 4 diet treatments (3 replicates for each). Treatments included 1×10^9, $2{:}1\times10^9$, 3×10^9 CFU kg^{-1} dry food and a control without probiotic. At the end of the study, specific growth rate (SGR) and survival rate (SR) were significantly higher in treatments supplemented with probiotic compared to the control ($p<0.05$), while the fish fed with probiotics showed lowest food conversion ratio (FCR) ($p<0.05$). The results indicated that the use of 3×10^9 CFU kg^{-1} dry food probiotics improved growth parameters and survival rate in *R. kutum* fingerlings.

Keywords: *Rutilus kutum*, Probiotic, *Pediococcus acidilactici*, Growth, Survival

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Introduction

Kutum fish (*Rutilus kutum,* Kamenski, 1901) is one of the valuable and unique bony fish species of the Caspian Sea and the world which is highly popular particularly in Iranian fish market because of its special flesh quality. The fish is omnivorous with a life span of 9-11 years in the sea. There are two Kutum forms; spring and autumn forms (Valipour and Khanipour, 2009). It also accounts for nearly 60 percent of the total bony fish landings in the Iranian side of the Caspian Sea with 10000 fishermen engaged in beach seine fishing operations (Valipour and Khanipour, 2009). Considering the plans to introduce Kutum fish in the national aquaculture development program, it is necessary to find a suitable specific diet for this fish. In this respect, the use of probiotics is of prime importance to upgrade feeding efficiency. Upon definition; Parker (1974), probiotics include those organisms that create a microbial balance within the gut. Also, probiotics should be capable of setting in the right areas within the digestive tract (Verschuere *et al.,* 2000a). Probiotics can help deter increment of bacterial pathogens in the digestive tract of the host animal by competing over food absorption and junction nodes, alter bacterial metabolism and stimulate the immunity system of the host (Gatesoupe, 1994).

In addition, most probiotic bacteria tend to have positive bearings on the growth of living organisms by generating vitamins and increasing mineral intake capacities for particularly trace elements along with making crucial digestive enzymes (Holzapfel *et al.,* 1998). Meanwhile, generating detoxifying agents, decomposing unconsumed feed and acting as an appetite stimulant in the host animals can be cited as important functions of probiotics. (Irianto and Austin, 2002).

The most widely used probiotic consumed in aquaculture operations are lactic acid bacteria. These are a group of tubular or round gram positive bacteria incapable of producing spores, negative catalase and negative oxidative agents and use carbohydrates as their main energy reserves and yield in lactic acids. Different lactic acid bacteria have adopted to survive in a variety of situations which are capable of propagation in the wild and can reside in areas as desperate as the alimentary canal of warm blooded animals such as mice, pigs, fowls and humans as well (Tannock, 1988). They are also found in dairy products (Sharp, 1981) and certain plants (Keddie, 1959). Jafarian *et al*. (2006) showed that an increase of *Bacillus* bacteria resulted in the promotion of life indices such as extended durability, blood factors and digestive enzymes among beluga sturgeons. Lara Flores *et al.* (2003) added two types of probiotic bacteria namely *Streptoccus faecium* and *Lacto bacillus acidophilus* as well as *Saccharomyces cervisiae* – a kind of ferment to be included in the diet of

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juvenile Nile tilapia. The results indicated a rise in the specific growth rate (SGR) and survival of juvenile tilapia and a decrease in food conversion ratio (FCR). Bairagi et al. (2004) investigated the effects of *Bacillus sircolance* addition in the diet of *Labea rohita*. The findings revealed diets added with this type of bacteria entailed growth rate improvement, decreased FCR and increased protein efficiency. The result of studies by Ghosh *et al.* (2008) revealed a significantly meaningful difference with the non-experimental group in terms of the impacts of probiotic bacteria- *Bacillus subtilis* extracted from the gut of *Cirrhinus mrigala* on the growth factor, durability of certain digestive enzymes and the overall health status of platy fish. The present study however, is aimed at investigating the effects of probiotic- *Pdiococcus acidlacticus*- inclusion in the juvenile Kutum fish diet and examining the possible results on the growth and survival of *Rutilus kutum* fingerlings produced and released as part of the stock rehabilitation program of the Caspian Sea.

Material and methods

Preparing fry and breeding environment

The kutum fry enjoying an approximate weight of 1.7±0.12 g were obtained from the 'Sijaval Bony Fish Hatchery Centre located in Bandar Turkman - Golestan Province. The juvenile fish were adapted under laboratory conditions for two weeks prior to the start of the study during which they received basic feed (without probiotics). This was followed by random selection of 15 pieces of kutum fingerlings in each of the 500 l fiberglass tanks provided with ongoing aeration.

Preparation of experimental diet

The experimental feed composition is illustrated in Table 1. The diets were isonitrogenus and isocaloric. In order to prepare the experimental diets, the primary dry materials were ground, mixed and later a certain amount of probiotic was added to it. Next it was supplemented with oil and water. The resultant paste was passed through a meat mincer turning it into pasta in string form and dried in an oven at 50^{o}C for 7-10 hours. After drying, it was turned into pellet form. The experimental feed in this study involved 4 treatments with three replications for each. The experimental treatments included probiotics at the rate of 1×10^{9} (T1), 2×10^{9} (T2), 3×10^{9} (T3) cfu/kg *P. acililactici* and the control treatment without the probiotic. The biochemical composition of the feed was determined through standardized analysis including identification of crude protein, (Lowery et al. 1951) crude fat (Folch *et al*., 1957), carbohydrate (Roe, 1955), ash and moisture (APHA, 2005) (Table 1).

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Table 1: Ingredients and proximate analysis of experimental diets of kutum.

Food ingredients	%
Gelatin	16.4
Starch	12.2
Wheat meal	6
Corn meal	5.2
Fish meal	30
Soybean meal	5
Cellulose	4.9
Fish oil	10.3
Vitamin premix[1]	2
Mineral premix[2]	2
Vitamin C	1
Colin chloride	1
Methionine	2
Lysine	2
Treat 1: *P. acidilatici (*cfu kg^{-1})	$\times 10^9$
Treat 2: *P. acidilatici (*cfu kg^{-1})	2×10^9
Treat 3: *P. acidilatici (*cfu kg^{-1})	3×10^9
Proximate analysis	
Crud protein (%)	40
Gross Energy (kcal/100g)	385
Crud fat (%)	13.5
Wet (%)	4.5
Ash (%)	4.73
Carbohydrate (%)	24.5
Fiber (%)	1.06
NFE (%)	11.71

[1] Vitamin Mix: Vit A, 1600000 IU; Vit D3, 400000; Vit E, 40g; Vit k3, 2g; Vit B_1 (Thiamin), 6g; B_2 (Riboflavin) 8g; B_3 (Pantothenic acid) 12g; B_5 (Niacin) 40g; B_6 (Pyridoxine) 4g; B_9 (Folic acid) 12g; Vit B_{12} 8g; Vit H_2, 0.24g; Vit C 60g; Inositol 20g; BHT 20g; Choline chloride (added to Mineral Mix) 12g (There is 0.5% from up amounts in per kg of Vitamin Mixture).

[2] Mineral Mix: Fe 26g; Zn 12.5g; Se 2g; Co 480mg; Cu 4.2g; Mn 15.8g; I 1g (There is 0.5% from up amounts in per kg of Mineral Mixture).

Feeding, biometrics and maturation during the experimental period

The juvenile fish were fed four times at 8:00, 12:00, 16:00 and 20:00 hours at 5 percent of their body weight. The animal biometrics involved measuring of body length (cm) and weight (g) at the beginning, once in every other two weeks and at the end of experiment. Measurements were carried out 12 hours prior to and after feeding. The duration of the study was 8 weeks and all of the experimental tanks were supplied with continuous aeration. 30-40% of water from each tank was renewed daily so as to drain out fish fecal matter and waste feed. The lighting system was set to provide 12 hours of darkness followed by 12 hours light. Dissolved oxygen content and water temperature were measured daily and their average was 6.1±1.24 mg L^{-1} and 25±1.8^{o}C, respectively. The water pH remained relatively stable at 7.5±0.7.

Growth and Survival measuring

At the end of experiment, growth parameters and survival rate were measured by the following formulae:

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Weight Gain (WG) Percent (Tacon, 1990):

$$WG = \frac{\text{Final weight average (g)} - \text{Initial weight average (g)}}{\text{Initial weight average (g)}} \times 100$$

Specific Growth Rate (SGR) Percent (Tacon, 1990):

$$SGR = \frac{\text{Ln Final weight average (g)} - \text{Ln Initial weight average (g)}}{\text{Days of experiment}} \times 100$$

Food Conversion Ratio (FCR) (Lin *et al*, 1997):

$$FCR = \frac{\text{Food consumption (g)}}{\text{Weight gain (g)}}$$

Survival Rate (SR) (Hile, 1936):

$$SP = \frac{\text{Number of fish in end of experiment}}{\text{Number of fish in start of experiment}} \times 100$$

Statistical methods

This study was implemented in a completely random design with four experimental treatments with three replications for each. Normality of data was determined by Shapiro-Wilks test. Given the data was normal, effects of diets on growth and survival were examined by one-way ANOVA. Levels of significance were determined using Duncan's Multiple Range Test, with critical limits being set at $p<0.05$. In addition EXCEL 2010 software was utilized to illustrate the data and the statistical analysis of data based on SPSS17.

Results

Considering figures 1 and 2, the increased level of probiotics in fish diets resulted in an increase in growth factors such as body weight increase percentage and specific growth rate of the fish in the experimental treatment which were not significantly different from those in the control group ($p>0.05$). Treatment 3 fed diet containing *P. acidilatici* (3×10^9 cfu kg^{-1}) led to the highest weight gain and specific growth rate among the experimental juveniles showing a significant difference with those in treatment 1 and the control group ($p<0.05$).

FCR in the diet containing probiotics at 3×10^9cfu kg^{-1} turned out to be the lowest, representing a significant difference with treatment 1 and the control ($p<0.05$). The control group showed the highest FCR making it significantly different from the other treatments ($p<0.05$) (Fig. 3).

As seen in figure 4, with an increase in the use of probiotics in the diet, the survival rate among experimental fish tended to rise significantly. Of course a similar increase was also observable in treatment 1 and 2, but their difference was not significant ($p>0.05$). In addition, treatment 3 showed the highest survival rate that was significantly different from the control ($p<0.05$) (Fig. 4).

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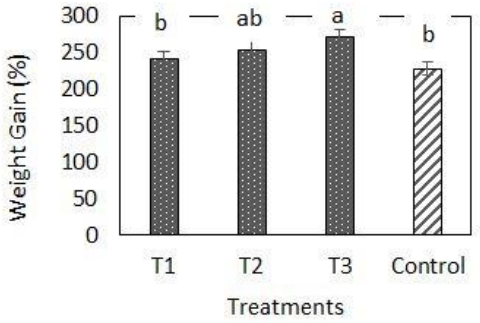


Figure 1: Weight gain percent in kutum fed with experimental diets.

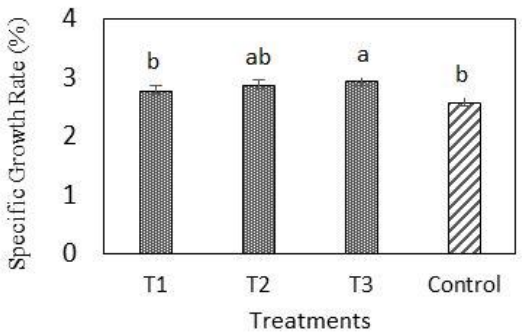


Figure 2: Specific growth rate percent in kutum fed with experimental diets.

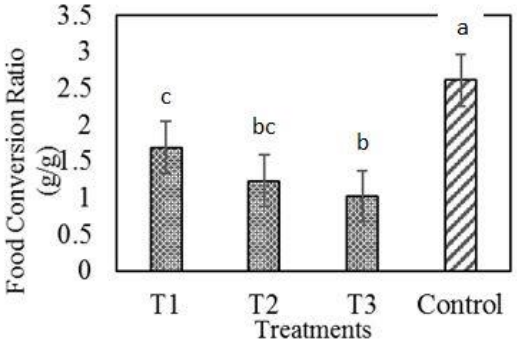


Figure 3: Food conversion ratio in kutum fed with experimental diets.

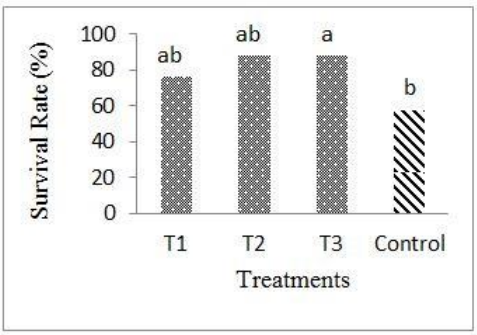


Figure 4: Survival rate percent in kutum fed with experimental diets.

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Discussion

There has been a good number of researches concerning the impacts of probiotics on farmed fish species suggesting improvement gained in fish survival rate (Moriarty, 1998), resistance against disease (Gatesoupe, 1994), adherence and formation of colonies within guts (Joborn *et al.,* 1997), the increased ability in diminishing bacterial load in kidneys and generation of polyamine and digestive enzyme functions (Tovar *et al*., 2002), development of non-specific immunity system by intracellular mechanism such as phagocytes and lysozyme activity (Guillan *et al.,* 2004; Irianto and Austin, 2004) and better growth function and nutritional efficiency (Ringo *et al.*, 2010).

Asadi *et al.* (2002) showed the inclusion of 2×10^9 cfu *Pediococcus acidiactici* per kilogram of fish feed for juvenile sea bream (*Abramis brama orietalis* Berg, 1949) resulted in weight gain, improved specific growth rate and daily growth rate, and a decline in FCR. The increased growth rate among Rohita (*Labeo rohita*) using *Bacillus circulans* extracted from the gut of the fingerlings may again prove the facilitative effects of probiotics on fish growth rates (Ghosh *et al.,* 2002). Ahilan *et al.* (2004) carried out a research on Gold fish (*Carassius auratus)* using three types of probiotics; S*porolac, Lactobacillus* and ferment. Also, it was found that probiotic added feeds resulted in greater weight gains within the experimental fish, and those treated with *Sporolac* accounted for the highest weight gains. Rengpipat *et al*. (1998) maintained that *Bacillus* S11 included in shrimp diet (*Penaeus monodon*) caused higher growth rates. Garriques and Arevalo (1995) applied *Vibrio alginolyticus* in the formulated diets of *Penaeus vannamei* in Ecuador. The result was a higher growth rate in treatments receiving shrimp feed containing probiotics than in other groups. The positive impacts of probiotics can also be traced to their role in boosting appetite which is achieved through production of vitamins and detoxification of feeds or because of decomposition of undigested compounds (Irianto and Austin, 2002). This might be attributed to the generation of certain enzymes such as peptide by probiotic bacteria that hydrolyze micro molecular compounds converting them to peptides and amino acids (Fuller, 1989; Kennedy *et al.,* 1998; Gatesoupe, 1999).

In this study, the use of probiotics caused an increase in body weight and specific growth rate during the course of the study, to the extent that treatment 3 with 3×10^9 cfu kg^{-1} of *P. acidlactici* resulted in the highest body weight and growth rate compared to the control group. The improved body weight and specific growth rate observed in this study might be attributed to factors mentioned above. In addition, the use of the probiotic, *Micrococus luteus* had a boosting effect on specific growth coefficients in farmed tilapia (Khattab *et al.,* 2005).

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A similar effect was also confirmed in Japanese flounder (*Paralichthys olivaceus*), showing a remarkably greater body weight and body length in fish fed with probiotics (Taoka *et al.*, 2006). Furthermore, Gullian *et al.* (2004) examined the effects of *Bacillus* P64، *Vibrio* P62 and *V. alginolyticus* on *Penaede vannamei* as the result revealed growth improvement. Bagheri *et al.* (2008) concluded that rainbow trout fed diets containing *Bacillus* spp. enjoyed higher specific growth rates, better environmental factors and protein efficiency than trout receiving no probiotics. The increased growth rate among farm-raised fish consuming probiotic added feeds may be accounted for by the increased reactions of digestive enzymes, morphological transformation of the fish guts or the probiotic fermentation within the guts (Dimitroglou *et al.*, 2010, Maslowski and Mackay, 2010). Suzer *et al.* (2008) indicated that enzymatic functions within the gut and pancreas, the specific growth rate and survival percentage of Godhead sea bream larvae fed with *Lactobacillus* bacteria in diet increased in comparison to the control group.

Gullian *et al.* (2004) noted an augmented growth rate among shrimp fed with *Bacillus*. Meanwhile, El-Dakar and Goher (2004) identified the possibility of increasing shrimp growth rate by including bacteria such as *Bacillus subtilis* in their diet which gives rise to greater enrichment of digestive enzymes inside gut through the synthesis of vitamins and the related co- factors. This is done through generation of certain enzymes including protease and lipase. Most probiotic bacteria contain extra cellular enzymes such as amylase and lipase that stimulate the digestive system and enhance microbial interactions that naturally occur in intensive feeding (Gildberg *et al.*, 1997). Therefore the mentioned bacteria upgrade nutritional efficiency and ultimately speed up growth rate as a result of increased digestion and high nutrient intake that follows (Gatesoupe, 1999).

In the present study however, the lowest FCR was found to occur in treatments with diets containing probiotic, more specifically treatment 3 whereas the highest FCR level belonged to the control group, suggesting lower feed consumption due to the use of probiotics (Arslan, 2004). In a research done by Arslan (2004) that involved *Lactobacillus bulgaicus* on carp, a low FCR was observed among the probiotic treated fish. Khattab *et al.* (2005) obtained a yet lower FCR in feeding farmed tilapia (*Oreochromis niloticus*) using *Micrococcus luteus.* The use of probiotic-*Bacillus subtilis* in feeding ornamental fish (Ghosh *et al.*, 2008) and *Bacillus* spp in rainbow trout diets (Bagheri *et al.*, 2008) led to a noticeable boost in growth, survival percentage and a declined FCR. Meanwhile in a study by Jafari *et al.* (2011) and Bagheri *et al.* (2013) probiotic treated group of fish gave a lower FCR compared to non-probiotic treated fish. The results obtained in the present research

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however, correlate well with those of the above stated research.

Nevertheless, contrary to earlier studies, no significant differences could be established in terms of the two different types of bacterial *(Cho et al., 2006)*. Bagheri *et al*. (2008) investigated the effects of varying degrees of probiotics on growth performance of rainbow trout, showing no important differences in survival rates across treatments. However, in this study it was observed that varying probiotic levels could secure higher survival to the extent that treatment 3 yielded the highest survival rate with the lowest pertaining to the control. In general considering the higher weight gain percentage, specific growth, FCR, and survival rate in treatments containing the probiotic, it is safe to point out that *P. acidilactici* tended to act as a growth promoter and that it can reinforce immunity system if included in the diet of juvenile Kutum fish.

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