The Effects of Dietary Probiotic *Pediococcus acidilactici* on the Growth Performance and Survival rate of Oriental Bream Fry (*Abramis brama orientalis)*

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Abstract

The present study tried to investigate the effects of *probiotic*, *Pediococcus acidilactici* in concentrations of 1×10^9, 2×10^9 and 3×10^9cfu kg^{-1} diet on growth and survival rate of oriental bream fry (*Abramis brama orientalis*, Berg 1949) in comparison to control diet (devoid of probiotic) for 60 days. In so doing, 180 specimens of oriental bream with initial weight of 2.69± 0.22 g were divided randomly into12 fiberglass tanks (capacity 110 l).They were fed at 3-5 percent body weight daily. A the end of the trial, growth and survival rates were measured. The best growth performance was viewed in treatments rather than the control because of the improvement of gut microflora balance, which performed by probiotic *P. acidilactici*, following by the enzyme secretion for increasing nutrient digestibility and fish appetite. The best bodyweight gain, specific and daily growth rates, and the lowest feed conversion ratio was observed in treatment, feeding with diet containing 2×10^9cfu kg^{-1} diet ($P<0.05$). The results of the current study revealed that it is the role of probiotic, *P. acidilactici* to the diet of oriental breamfry and *A. brama orientalis*, which resulted in growth performance increase and feed efficiency ratio improvement.

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Introduction

In recent decades, to keep pace with the global trends of demanding protein resources and increased number of hungry people, it is inevitable to establish sector's development such as agriculture and aquaculture (Nakagawa, Sato & Delber 2007). From ecological, biological and economical perspectives, the Oriental bream *A. brama orientalis* (Berg 1949) is one of the most important species. This can be find in Anzali wetland and some rivers (Sefidrod, Atrak and Aras) in southern coastline of the Caspian Sea that dischargeinto the Caspian Sea (Ghasemi, Keyvanshokooh, Shahriari-Moghadam, Khara & Sourinejad 2007). Recently, the oriental bream put on the endangered species list and many reasons caused this (Ghasemi *et al*., 2007). Therefore, the Stock Improvement programs of *A. brama orientalis* have implemented by artificial propagation. Thus, the Fisheries Institute of Iran rears oriental bream larva for nine months, and then fish fries (weight gains around 3 grams) released into the Caspian Sea. The culture of oriental bream needs serious attention and many attempts have been done to prevent species extinction. One of the most challenging difficulties encountered in culture of *A. brama orientalis* is larvae mortalities in the face of changing environmental conditions.

Over the two past decades, as an additive for use in diet of fish, the application of probiotic has been demonstrated a wide range of benefits. Many studies have been done on the effects of probiotics on growth performance and health of various species in aquaculture. For example, Effect of *Lactobacillus* spp on *Macrobrachium rosenbergii* (Venkat, Sahu & Jain 2004), *Bacillus subtitilis* on rohu, *Labeo rohita*, (Kumar, Mukherjee, Pani Prasad & Pal 2006), *Bacillus* spp *on* rainbow trout, *Onchorhynchus mykiss* (Bagheri, Hedayati, Yavari, Alizadeh & Farzanfar 2008), *Micrococcus* on Nile tilapia, *Oreochromis niloticus* (Osman, B. Ibrahim, Soliman & Aboud 2010) *Lactobacillus acidophilus* on rainbow trout, *O. mykiss, (*Faramarzi, Kiaalvandi, Lashkarbolooki, & Iranshahi 2011*),* *Lactobacillus sporogenes* on freshwater prawn, *Macrobrachium rosenbergii* (Seenivasan, Saravanabhavan, Radhkrishnan & Shanthi 2012) and *Pseudomonas aeruginosa* on rohu, *Labeorohita* (Sankar Giri, Sankar Sen, & Sukumaran 2012). Dry LAB bacteria are dietary supplements containing live microorganisms, and when managed in enough quantities, may improve host health and could stimulate growth of animals (Ringø & Gatesoupe 1998; Balcazar, Deblas, Ruiz-Zarzuela, Cunningham, Vendrell & Muzquiz 2006). Of the most widely researched topics in recent years on LAB have been the *lactobacilli* and *bifidobacteria* (Ross &Toth 1974; Corcoran, Ross, Fitzgerald & Stanton 2004; Senok, Ismaeel & Botta 2005). Meanwhile, some studies have been recently investigated on the effect of *P. acidilactici* on Gold fish *Carassius auratus* (Perera 2007); shrimp *Litopenaeus stylirostris* (Castex, Chim, Pham, Lamaire, Wabete, Nicolas Schmidely &

Mariojouls, 2008; Castex, Lemaire, Wabete & Chim, 2009; Castex, Lemaire, Wabete & Chim 2010); rainbow trout, *O. mykiss* (Aubin, Gatesoup, Labbe & Lebrun 2005 ;Merrifiel, Bradley, Harper, Baker, Munn & Davies 2009) and Nile tilapia, *Oreochromis niloticus* (Ferguson, Merrifield, Harper, Rawling, Mustafa, Picchietti, Balcazar & Davies 2010).

Inspired by the aforementioned literature, the researchers tried to evaluate the probable impact of probiotic *P. acidilactici* on *A.s brama oriental* is fries on growth performance and survival rate.

Table 1 Composition of the experimental, basal and treatment diets (%)

Ingredients	C	Treatment 1	Treatment 2	Treatment 3
Probiotic* (cfu kg^{-1})	0	1×10^9	2×10^9	3×10^9
Fish meal	38.38	38.38	38.38	38.38
Soybean meal	26.38	26.38	26.38	26.38
wheat meal	9.38	9.38	9.38	9.38
wheat bran	3.00	3.00	3.00	3.00
Corn meal	9.38	9.38	9.38	9.38
Corn oil	2.47	2.47	2.47	2.47
Antioxidant	0.10	0.10	0.10	0.10
Binder	2.00	2.00	2.00	2.00
Vitamin premix**	2.00	2.00	2.00	2.00
Mineral premix***	1.50	1.50	1.50	1.50
Mild inhibitor	0.40	0.40	0.40	0.40
DL-methionine	1.00	1.00	1.00	1.00
Lysine	1.00	1.00	1.00	1.00
Garlic	2.00	2.00	2.00	2.00
Cholin chloride	1.00	1.00	1.00	1.00
Approximate analysis			%	
Crude protein			41.00	
Crude lipid			13.50	
Carbohydrate			26.00	
Fiber			2.50	
Ash			8.00	
Gross energy (kcal kg^{-1})			3420.27	

*= *Pediococus acidilactici* (CNCM MA 18/5 M), bactocell_ (Lallemand Inc., France).

**= Vitamin A, vitamin D, vitamin E, vitamin K (menadione), vitamin B_{12}, niacin, pantothenic acid, riboflavin, carrier and oil.

***= Copper, iodine, iron, manganese, selenium, zinc, carrier, oil.

Dietary groups: C = control; Treatment 1= 1×10^9cfu kg^{-1} of diet; Treatment 2= 2×10^9cfu kg^{-1} of diet; Treatment 3= 3×10^9cfu kg^{-1} of diet.

Material and Methods

Fish and experimental conditions

The oriental bream (*A. brama orientalis*) used in the experiment obtained from main ponds in Sefidrod Fishery Research Station, which located in Guilan province, North of Iran. Their initial weight was 2.62± 0.22 gram and was reared at the same station in 12 fiberglass tanks (capacity of each tank: 110 L). The rearing water conditions were stable during the whole period of the experiment and regularly monitored. The temperature maintained at 24.00± 1.15 ºC (measured daily). The oxygen concentration and pH value were 9.34± 0.56 mg/L (determined once a week in the mornings) and 7.61±0.06, respectively. The photoperiod set at a 12 h light/12 h dark and water replacement filter of 30 percent of water volume did every two days.

Diet Preparation Cycle

One hundred and eighty *A. brama orientalis* fries fed with an isocaloric and iso-nitrogenous basal diet in the laboratory for two weeks (adaptation period) formulating by Winfeed software, version, 6 (Table 1). At first, for making basal diet, dry ingredients carefully mixed, and then water and oil added until the ultimate mash formed. The ultimate mesh grinded in a meat grinder, and then placed in drying laboratory oven at a temperature of 60 ^{o}C for 8 hours. Having broken up into pellets of 0.03 mm diameter, the researchers let them to be cool under a sterile hood for one hour, and then stored them at 4 ^{o}C until used.

Experimental Design

At theend of the adaptation cycle, the tanks holding 15 fish and each divided in four groups of three replicates. Every day before fish feeding, *Pediococcus acidilactici* in concentrations of 1×10^{9} CFU kg^{-1}(T_1), 2×10^{9} CFU kg^{-1}(T_2) and 3×10^{9} CFU kg^{-1} (T_3) of diet, separately coated on basal diet for making of three experimental diets. The control group (C) was also fed with basal diet (without probiotic-supplementation).The commercial probiotic used in our experiments was Bactocell_ PA 10 (Lallemand Animal Nutrition S.A., Blagnac, France) which formulated with live *P. acidilactici* MA18/5M (Institute Pasteur, Paris, France).

The specimens fed three times per day (9 AM, 13 PM, and 19 PM). The daily portion was 3–5 percent of biomass day^{-1} for 60 days.

Statistical Analysis

Fish weighed individually at the beginning and at the end of the experiments. Estimating the growth performance done by calculating the weight gain (WG), length gain (LG), specific growth rate for weight and length (SGR_wandSGR_l), feed conversion ratio (FCR), percent of body weight increase (BWI),condition factor (K), and survival rate (SR) by the formula.

WG (g $fish^{-1}$) = [(W_f-W_i) / W_i] x 100(Yanbo & Zirong 2006)

LG (mm $fish^{-1}$) [(L_f-L_i) / L_i] x 100(Yanbo & Zirong 2006)

FCR = $\frac{\text{Feed intake}}{\text{average daily gain}}$ (Yanbo & Zirong 2006)

SGR (weight) = ($\ln W_f$ - $\ln W_i$x100) / t **(**Hevroy, Waagbo, Sandness, Rund & Hermerr 2005)

SGR (Length) = ($\ln L_f$–$\ln L_i$x100) /t **(**Hevroy et al. 2005)

BWI= 100[(Wf-Wj)/Wi]/t. (Tacon 1990)

$K = 100W/L^3$ (Raibeiro, Crain & Moyle 2004)

SR = 100 (live fry –dead fry) / Live fry (Tacon 1990)

At the end of the experiment, the effects of *pediococcusacidilactici* as probiotic on survival rate and growth parameters examined. Analytical procedure performed in SPSS (version, 16) for Windows 7(SPSS Inc., Chicago, IL, USA). To check the normality and homogeneity of data, Kolmogorov-Smirnov test has utilized. Also, data analyzed by One-Way Analysis of Variance (ANOVA). When the difference observed, Bonforoni's test as post hocused for mean comparison of treatments at a level of 95% ($P<0.05$). About descriptive statistics, mean and standard deviation (SD) have reported.

Results

Table 2 depicts the growth indicators and survival rate of *A.brama orientalis* after 60 days of trial. According to the results, compared with the control group, fish, which fed with diet containing probiotic (*P. acidilactici*) gave the best growth performance rates.

The highest weight gain (WG) and daily growth rate (DGR) were obtained in fish fed on treatment 2 (2×10^9 cfu kg^{-1} of diet) ($P<0.05$). The least and the highest feed conservation ratio (FCR) obtained in fish fed on the treatment 2 and control group, respectively. There were no statistically significant differences ($P>0.05$) between treatments and control group in the condition factor and survival rate.

Table 2 Growth performance and survival rate of *Abramis brama orientalis, which* were fed with *P. acidilactici* diets for 60 days

Parameters	C	Treatment 1	Treatment 2	Treatment 3
WG[1]	0.43±0.04[d]	0.95±0.07[b]	1.25±0.01[a]	0.68±0.02[c]
LG[2]	2.23±0.14[b]	3.97±1.11[ab]	4.45±0.57[a]	3.02±0.56[ab]
SGR_W[3]	0.25±3.79[c]	0.53±2.31[a]	0.63±6.03[a]	0.36±2.52[b]
SGR_L[4]	0.06±1.53[b]	0.08±6.35[a]	0.10±2.52[a]	0.06±0.00[b]
BWI[5]	15.36±1.95[c]	38.46±1.98[a]	45.13±5.03[a]	24.91±2.55[b]
DGR[6]	0.007±0.0006[d]	0.01±0.0012[b]	0.02±0006[a]	0.01±0006[c]
CF[7]	0.84±0.03	0.85±0.01	0.88±0.02	0.85±0.02
FCR[8]	6.39±0.22[c]	2.68±0.19[a]	2.34±0.12[a]	3.98±0.39[b]
S[9]	100± 0.00	100± 0.00	100± 0.00	100± 0.00

Dietary groups: C = control; Treatment 1= 1×10^9cfu kg^{-1} of diet; Treatment 2= 2×10^9cfu kg^{-1} of diet; Treatment 3= 3×10^9cfu kg^{-1} of diet

$^{a, b, c, d}$ values in the same row with different superscript are significantly different ($P< 0.05$)

1-Weight gain, 2-Lenght gain, 3- Specific growth rate for weight, 4- Specific growth rate for length, 5- Body weight increase , 6- Daily growth rate, 7- Condition factor, 8- Feed conservation ratio, 9-Survival rate

Discussion

Growth performance rate, which has increased in treatments fed with Probiotic-supplemented diet over 60 days of trial. As the results revealed, the highest growth rates obtained in fish fed on treatment 2 (artificial diet coated by 2×10^9 cfu kg^{-1}*P. acidilactici*) compared with the others. Probiotics are supplements that help to increase metabolism and digestion in fish by improving gut microflora balance. Accordingly, the decreasing growth performance rate in treatment1(artificial diet coated by1×10^9 cfu kg^{-1}*P. acidilactici*) can be attributable to low bacteria density of *P. acidilactici* in diet and less effect of probiotic on natural intestinal flora than treatment 2. Moreover, due to the excessive use of probiotic, which may interfere with normal metabolism in the body, the growth rate in treatment 3(artificial diet coated by 3×10^9 cfu kg^{-1} *P. acidilactici*) decreased. Generally speaking, LAB display antagonism by decreasing pH, the consumption of available nutrient, and the production of specific inhibitory compounds (Kosin & Rukshit 2006)

By reducing nutrient requirement, the use of probiotics as a supplemental constituent of diet can increase the feed efficiency ratio and reduce culture costs (Yanbo & Zirong 2006). Also, knowing the active modulation of the gastrointestinal tract (GIT) could confer antagonism against pathogens which is in consistent with probiotics activity, then, it can help development of the immune system, provide nutritional benefits and aid the intestinal mucosal barrier (Vaughan, deVries, Zoetendal, Ben-Amor, Akkermans & de Vos 2002). In recent years, many studies have shown the use of probiotics can improve growth performance and survival rate of aquatic animals. These studies evaluated the effect of *Enterococceu sfaecium* on european eels *Anguilla anguilla* L. (Chang & Liu2002), *Bacillus cereus* on dentex *Dentex dentex* L. (Hidalgo, Skalli, Abellán, Arizcun & Cardenete 2006), *Bacillus* spp *on* rainbow trout *Onchorhynchusmykiss* (Bagheriet al. 2008), *Microccus* sp. on Nile tilapia *O. niloticus* (Osman et al. 2010), *Lactobacillus* on clown fish *Amphiprion sebae* (Pushparaj, Ramesh & Ambika 2012), and *L. sporogenes* on freshwater prawn *M. rosenbergii* (Seenivasan et al. 2012). The effect of dietary probiotic on growth performance and survival rate depends on the target species, level and frequency of application and environmental conditions (Gomez- Gil, Roque & Turnbull 2000). In the same vein, several results have shown that *P. acidilactici* improved growth performance in

aquatic animals. Cases in point, Ferguson *et al.* (2010) have shown improving growth performance by *P. acidilactici* in rainbow trout *oncorhynchusmykiss* and tilapia *Oreochromisniloticus*, respectively. The reason for this could be because of improving gut microflora balance by *Pediococcusacidilactici* and the enzyme secretions for increasing nutrients digestibility and fish appetite. Proteases, amylase, liposomal enzymes and other enzymes that support natural digestion secreted by LAB can improve digestibility and safety of the host (Gill1998; Izvekova 2005; Ziaei-Nejad, Rezaeib, Takamic, Lovettd, Mirvaghefia & Shakourie 2006). Nonetheless, some previous studies have reported conflicting results from the use of *P. acidilactici* on aquatic animals. No statistically significant increasing in growth performance and feed use gained on fish (rainbow trout *O. mykiss*), which fed with probiotic (vegetative and lyophilized *P. acidilactici*) (Merrifield et al. 2009).

The results of the present study showed that there was a statistically significant decrease in treatments with calculation of the feed conservation ratio (FCR). In other words, this probiotic could improve the feed efficiency ratio as well. The previous studies support the results of the current study indicating the decrease in FCR by *P. acidilactici* on shrimp *L. stylirostris* (Castexet al. 2009) and red tilapia *O. niloticus* (Ferguson et al., 2010). Practically speaking, this means that probiotic use can decrease the amount of feed necessary for animal growth culture, which could result in production cost reductions.

At the end of the experiment, survival rate in all groups gained 100 percent. This means that no mortality observed during the trial. Merrifield *et al*. (2009) has assessed the effect of *P. acidilactici* on rainbow trout *O. mykiss* and seabass, *Dicentrachus labrax*, respectively. They did not observe statistically significant difference in the survival rate between treatments and control group. However, the main reason for this could be traced to the good condition of experiment, good water quality for all specimens and no symptoms during the trial. However, because of the high sensitivity of *A. brama orientalis* to changing environmental condition, we conclude that *P. acidilactici* cannot be ineffectual on survival rate and mortality and could be effective on health and continuance of existence in target fish.

All in all, this study has revealed the use of probiotic-supplemented *P. acidilactici* diet improved growth performance of *A. brama orientalis* fries and this probiotic could be a good dietary supplement for Oriental bream. Meanwhile, at the end of the trial the results showed that add probiotics *P. acidilactici* with concentration of 2×10^{9}cfu Kg^{-1} of diet improved growth performance of Oriental bream fries better than other treatments and has chosen as suitable and sufficient dose for culture of *A. brama orientalis.*

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تأثیر سطوح مختلف پروبیوتیک *Pediococcus acidilactici* برعملکرد رشد و بازماندگی بچه ماهی سیم

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چکیده

در این تحقیق اثر پروبیوتیک پدویوکوکوس اسیدی لاکتیسی در مقادیر10^9×1، 10^9× 2 و 10^9 ×cfu3 در هر کیلوگرم جیره بر شاخص های رشدو نرخ بقا بچه ماهی سیم در مقایسه با غذای شاهد (بدون پروبیوتیک) در مدت 60 روز بررسی شد. تعداد 180 عدد ماهی با وزن اولیه 2/69±0/22 گرم در12عدد وان فایبرگلاس 110 لیتری به طور تصادفی توزیع شدند. غذادهی روزانه بین 3-5 درصد وزن توده زنده، در طول مدت پرورش، انجام می شد. در انتهای دوره آزمایش، شاخص های رشد و نرخ بقا اندازه گیری شد. بالاترین میزان افزایش وزن بدن، نرخ رشد ویژه وزن، نرخ رشد روزانه و پایین ترین ضریب تبدیل غذایی، در تیمار تغذیه شده با 10^9× cfu2 پروبیوتیک در هر کیلوگرم جیره مشاهده شد (P<0/05). نتایج نشان داد که افزودن پروبیوتیک به جیره غذایی بچه ماهیان سیم،باعث افزایش عملکرد رشد، کارایی تغذیه در ماهیان می شود. بنابراین می تواند مکمل مناسبی برای جیره غذایی بچه ماهی سیم باشد.

کلمات کلیدی : پروبیوتیک، رشد، تغذیه، نرخ بقا، شاخص‌های خون شناسی، ماهی سیم

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