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Original Article

Effects of *Pediococcus pentosaceus* as a probiotic on intestinal microbiota and body composition of Siberian sturgeon, *Acipenser baerii* Brandt, 1869

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Abstract: An eight-week experiment was carried out to determine the effects of dietary *Pediococcus pentosaceus* as probiotic on the body composition and gut microbiota of Siberian sturgeon, *Acipenser baerii*. A total of 180 fish with mean body weight of 143±0.01g were randomly distributed into 12 200L fiberglass tanks as four treatments with three replicates, including groups fed with diet containing 2×10^7, 2×10^8 and 2×10^9 CFU g^{-1} of *P. pentosaceus* and probiotic-free as control group. Body composition of treatment groups was not influenced by *P. pentosaceus* except for fat and moisture. The bacteria had a significant colonization in the intestine of fish fed with supplemented diet with *P. pentosaceus*. High level of acid lactic bacterial load was found in the treatment fed with highest amount of the probiotic i.e. 2×10^9 CFU g^{-1}. The results showed that application of *P. pentosaceus* has positive effect on the body composition and intestinal microflora of the *A. baerii*.

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Introduction

Probiotics are commonly defined as 'Live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance' (Fuller, 1989) and play a beneficial role on the health of the host if used in adequate amount (FAO/WHO, 2002). The application of the probiotics in aquaculture is based on the concept that the balance of intestinal microorganisms in healthy animals increases resistance to diseases, and efficient digestion and maximum absorption of nutrients (Fuller, 1992; Verschuere, 2000). Probiotics can also improve the immune response system, reduce mortality, increase growth parameters, improve water quality, enhance stress resistance, and increase reproduction efficiency (Bairagi et al., 2002; Wang and Xu, 2004; Gomez-Gil et al., 2000; Martınez Cruz et al., 2012).

The selection of suitable strain of a microorganism is a primary requirement for the use of probiotics. Different types of microalgae, yeasts, gram-positive and gram-negative bacteria are considered as probiotics. Several bacterial strains which are common member of the non-pathogenic microflora are capable of inhabiting fish pathogenic bacteria in in-vitro assay (Joborn et al., 1997; Austin and Zhang, 2006; Gibson et al., 1998). This has been demonstrated for lactic acid bacteria (Joborn et al., 1997), *Vibrio* sp. (Austin and Zhang, 2006), and *Bacillus* sp. (Gibson et al., 1998) etc.

Pediococcus pentosaceus is a gram-positive, facultative anaerobic, non-motile, and non-spore forming bacterium. It is a member of the industrially important lactic acid bacteria that grows on lactobacilli MRS broth at 37°C. Ferguson et al. (2010) stated that dietary supplementation of *Pediococcus acidilactici* increases the intestinal microbial load of red tilapia, *Oreochromis niloticus*. Merrifield et al. (2011) also reported that this bacterium has a significant effect on intestinal microflora of rainbow trout, *Oncorhynchus mykiss*. They reported that this probiotic has a proper colonization in the gut of treated fish species.

Acipenseridae are the most commercially

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important fish species in the Caspian Sea basin. They are endangered due to loss of habitat, overfishing and deterioration of water quality (Bahmani et al., 2001). Among sturgeon fishes, the Siberian sturgeon, *Acipenser baerii*, is a proper candidate for aquaculture because it can easily be adapted to hand feeding in captive condition. Hence, in the present study, the effect of *P. pentosaceus* was examined as a probiotic on the body composition and intestinal microflora of the Siberian sturgeon, *A. baerii*.

Materials and Methods

Probiotic: *Pediococcus pentosaceus* was isolated from the intestine of Persian sturgeon, *A. Persicus* identifying by 16S rRNA gene based on Merrifield et al. (2009). The probiotic powder was prepared in Guilan Science and Technology Park (Rasht, Guilan Province, Iran) with 10^{12} bacterial count.

Experimental diet: A commercial diet composed of 36% crude protein, 14% lipid, 1% phosphorus, 11% moisture, 10% ash and 4% fiber was used as a control diet (Chineh commercial feed), and fish were acclimated to this diet for two weeks before beginning the experiment. The experimental diet was supplemented with *P. pentosaceus*. It was prepared by slowly spraying the mixture of 500 ml saline serum with 0.2, 2 and 20 g probiotic powder on 10 kg diet with 2×10^7, 2×10^8 and 2×10^9 CFU g^{-1} bacterial count, respectively, according to Merrifield et al. (2009).

Experimental design: The specimens of the Siberian sturgeons were obtained from Beheshti Sturgeon Reproduction and Restoration Center (Rasht, Guilan Province, Iran). A total of 180 fish having average body weight of 143±0.01 g were randomly distributed into 12 fiberglass tanks (with 2000 L capacity) each containing15 fish. Four treatments each with 3 replicates, including TA, TB, TC and C containing 2×10^7, 2×10^8, 2×10^9 CFU g^{-1} probiotic and probiotic free diet, respectively, were designed for this study. The treatments were fed on supplemented diet for 8 weeks at the rate of 2-2.5% of body weight 3 times a day (08:00, 14:00 and 20:00). The unconsumed feed was siphoned out six hrs after feeding. About 75% of water from each tank was changed daily with minimum disturbances to the fish.

Intestinal Microbiology: At the end of experiment, after 24 hrs starvation, three fish from each tank were anesthetized by 200 ppm clove powder solution. The intestine of fish was removed in a sterile condition then washed thoroughly three times with saline serum (Merrifield et al., 2009). Samples were diluted with saline serum and then the specimens were added to TSA (Tryptic Soy Agar) culture medium to count total number of intestinal bacteria (Rawling et al., 2009) and MRS (Man Rogosa Sharpe) culture medium to count slightly lactic acid bacteria (Merrifield et al., 2009).

Chemical analysis: At the end of experiment, three fish per tank were taken to analyze carcass composition according to protocols of AOAC (1990) for measuring moisture, ash, lipid and protein. Amount of moisture was determined by drying the fish muscle in oven at 103-105°C for 18-24 hrs. Thereafter, the dried samples were used to determine ash content by combusting them in a furnace. Amount of lipid was estimated by ether extraction in Soxhlet system. Micro-Kjeldahl system was used to determine the nitrogen content followed by multiplying the amount of nitrogen by 6.25.

Statistical Analysis: Data analysis were performed using SPSS program as installed by the University of Guilan. Differences between treatments were performed using One Way ANOVA and Duncan tests. The results were considered significant at the 95% ($P<0.05$) level.

Results

The results showed that *P. pentosaceus* has affected on fat and moisture of fish fed with probiotic-supplemented diets. The highest level of moisture was found in the control group and a significant differences were found between probiotic-fed and control fish ($P<0.05$). The maximum level of fat was found in TA (fish fed with 2×10^7 CFU g^{-1} probiotic in the diet) and a significant difference was found between TA and the other groups in term of fat level.

Table 1. Siberian sturgeon's body composition at the end of the experiment.

Parameter / Treatment	Moisture (%)	Ash (%)	Fat (%)	Protein (%)
C	76.383±0.12[a]	1.063±0.006[a]	6.53±0.33[b]	18.03±0.05[a]
TA	74.52±0.28[b]	1.133±0.006[a]	8.59±0.26[a]	16.14±0.05[a]
TB	75.073±0.12[b]	1.163±0.09[a]	7.28±0.21[b]	17.6±0.03[a]
TC	74.99±0.25[b]	1.136±0.008[a]	7.01±0.31[b]	17.82±0.05[a]

Different superscript letters denote significant (P<0.05) difference.

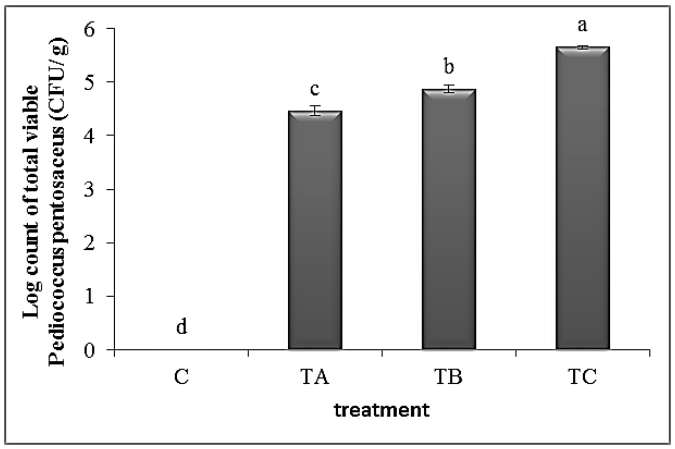

Figure 1. Effect of *P. pentosaceus* on Siberian sturgeon intestinal total viable count of bacteria (log).

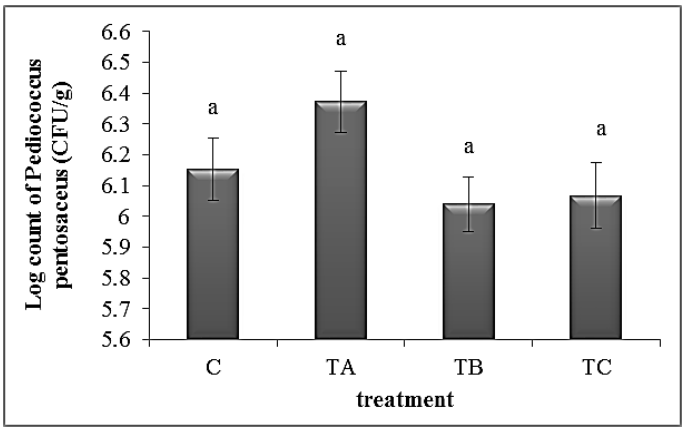

Figure 2. Effect of *P. pentosaceus* on the number of Siberian sturgeon intestinal *P. pentosaceus bacterium* (log).

The lowest level of fat was observed in control group. There were no significant differences between probiotic-fed fish and control group in terms of protein and ash (Table 1).

TSA (Tryptic Soy Agar) culture medium was used to calculate the Total Viable Count (TVC). The results showed that TVC ranged from log 6.37 CFU g^{-1} in TA (fed with 2×10^7 CFU g^{-1} of the probiotic) to log 6.15 CFU g^{-1} in C (control group). However, no significant differences were found between treatments (Fig. 1).

The results of MRS (Man Rogosa Sharpe) culture medium showed that the amounts of *P. pentosaceus* ranged from log 4.46 CFU g^{-1} in TA (fed with 2×10^7 CFU g^{-1} of the probiotic) to log 5.65 CFU g^{-1} in TC (fed with 2×109 CFU g^{-1}) and the differences between the probiotic-fed fish were significant (P<0.05) (Fig. 2).

Discussion

In the present study, the isolated *P. pentosaceus* from the intestine of Persian sturgeon was used as a probiotic in the diet of Siberian sturgeon. Based on the results, the supplementation of *P. pentosaceus* as probiotic affected the fat and moisture of body composition but it had no significant effect on protein and ash of treated fish. Merrifield et al. (2010) used *Chlorogloeopsis cyanobacter* as probiotic on the diet of *O. niloticus* and reported no significant effect on the body composition viz. crude protein, crude lipid and ash of the treated fish. Hoseinifar et al. (2011) and Merrifield et al. (2011) found similar results in *H. huso* and *O. mykiss*, by using *Saccharomyces cerevisiae* and *P. acidilactici*, respectively, as probiotics.

In contrast to our findings, Bagheri et al. (2008) showed that the diet containing commercial *Bacillus* probiotic has a significant effect on body composition of *O. mykiss*, but they did not observe significant difference between the groups consumed the lowest level of probiotic and control group. Bagheri et al. (2008) also observed significant

differences in carcass moisture and lipid content between the groups fed with the probiotic. El-Rhman et al. (2009) also showed that the body composition in *O. niloticus*, were significantly affected by the *Pseudomonas* and *Micrococcus lateus* as a probiotic, except for moisture. The difference between above mentioned findings and our results may refers to different bacteria and host.

The results of the present study showed no significant differences in TVC among all treatments, but the highest number of *P. pentosaceus* was observed in group fed with 2×10^9 CFU g^{-1} probiotic. This means that probiotic has been colonized in the intestine of treated fish. An important action of probiotic is competitive exclusion by competition for adhesion sites, nutrient, oxygen, and by-product inhibitory compounds (Irianto and Austin, 2002). Intestinal microbial flora change after application of probiotic and then pathogen bacteria can decrease in host animal. In addition, probiotic can reduce the pathogen by secretion of bacteriocins and then reduce intestinal pH (Irianto and Austin, 2002).

Similar to our results, Ferguson et al. (2010) reported that the number of lactic acid bacteria (LAB) is significantly higher in the intestine of the probiotic-fed *O. niloticus*. However there was no significant difference between the population of heterotrophic anaerobic and aerobic bacteria (Ferguson et al., 2010). Merrifield et al. (2011) stated that *O. mykiss* had a successful colonization of *P. acidlactici* in its digestive tract and the TVC of mucosal bacteria was significantly higher in the probiotic-fed fish. In another study, the TVC of heterotrophic bacteria in *Huso huso* were not affected by *Saccharomyces cerevisia*, but the levels of autochthonous lactic acid bacteria significantly increased in the fish fed with 2% yeast (Hoseinifar et al., 2011).

The effects of *Lactobasillus curvatus* and *Leuconostoc mesenteroides* on the gut microflora of *H. huso* and *A. persicus* were examined by Askarian et al. (2011). They reported that the *A. persicus* fed with *L. mesenteoides* had higher levels of LAB than fish fed with *L. curvatus* and LAB mixture, but the highest level of LAB in *H. huso* was observed when the fish were fed with *L. curvatus*. They reported that the levels of LAB in *H. huso* and *A. persicus* fed with LAB mixture, were similar to control group.

As conclusion, the findings of this study showed that application of *P. pentosaceus* as a probiotic has positive effect on the body composition and intestinal microflora of the Siberian sturgeon.

References

Abd El-Rhman A.M., Khattab Y.A.E., Shalaby A.M.E. (2009). Micrococcus luteus and Pseudomonas species as probiotics for promoting the growth performance and health of Nile tilapia, *Oreochromis niloticus*. Fish and Shellfish Immunology, 27: 175-180.

AOAC (1990). Official Methods of Analysis of Association of Official Analytical Chemists (AOAC), 15th ed, Washington, USA.

Askarian F., Kousha A., Salma W., Ringo E. (2011). The effect of Lactic acid bacteria on growth, digestive enzyme activity and gut microbita in Persian sturgeon (*Acipenser persicus*) and beluga (*Huso huso*) fry. Aquaculture Nutrition, 17: 488- 495.

Austin B., Zhang X.H. (2006). *Vibrio harveyi*: a significant pathogen of marine vertebrates and invertebrates. Letters in Applied Microbiology, 43:119-124.

Bagheri T., Hedayati S.A.A., Yavari V., Alizade M., Farzanfar A. (2008). Growth, survival and gut microbial load of rainbow trout (*Onchorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. Turkish Journal of Fisheries and Aquatic Sciences, 8: 43-48.

Bahmani M., Kazemi R., Donskaya P. (2001). A comparative study of some hematological features in young reared sturgeons (*Acipenser persicus* and *Huso huso*). Fish Physiology and Biochemistry, 24: 135-140.

Bairagi A., Ghosh K.S., Sen S.K., Ray A.K. (2002). Enzyme producing bacterial flora isolated from fish digestive tracts. Aquaculture International, 10: 109-121.

FAO/WHO.2002. Guidelines for the evaluation of probiotics in food. London Ontario Canada. 11 p.

Ferguson R.M.W., Merrifield1 D.L., Harper G.M., Rawling M.D., Mustafa1 S., Picchietti S., Balcazar J. L., Davies S.J. (2010). The effect of *Pediococcus*

acidilactici on the gut microbiota and immune status of on-growing red tilapia (*Oreochromis niloticus*). Journal of Applied Microbiology 109(3): 51-62.

Fuller R. (1989). A review, probiotics in man and animals. Journal of Application in Bacteriology, 66: 365-378.

Fuller R. (1992). History and development of probiotics. In: R. Fuller (Ed.). Probiotics, the scientific basis. London, UK: Chapman and Hall. pp: 1-8.

Gibson L.F., Woodworth J., George A.M. (1998). Probiotic activity of *Aeromonas media* on the Pacific oyster, *Crassostrea gigus*, when challenged with *Vibrio tubiashii*. Aquaculture, 169: 111-120.

Gomez-Gil B., Roque A., Turnbull, J.F. (2000). The use and selection of probiotics bacteria for use in the culture of larval organism. Aquaculture, 191: 259-270.

Hoseinifar S.H., Mirvaghefi A., Merrifield D.L. (2011). The effects of dietary inactive brewer's yeast *Saccharomyces cerevisiae* var. *ellipsoideus* on the growth, physiological responses and gut microbiota of juvenile beluga (*Huso huso*). Aquaculture, 318: 90-94.

Irianto A., Austin B. (2002). Probiotics in aquaculture. Journal of Fish Diseases, 25: 633-642.

Joborn A., Olsson C., Westerdahl A., Conway P.L., Kjellberg S. (1997). Colonization in the fish intestinal tract and production of inhibitory substances in intestinal mucus and faecal extract by *Carnobacterium* sp. strain K. Journal of Fish Diseases, 20: 383-392.

Martínez Cruz P., Ibáñez A.L., Monroy Hermosillo O.A., Ramírez Saad H.C. (2012). Use of Probiotics in Aquaculture. ISRN Microbiology, ID 916845: 1-13.

Merrifield D.L., Dimitroglou A., Bradley G., Baker R.T.M., Davies S.J. (2009). Probiotic applications for rainbow trout (*Oncorhynchus mykiss* walbaum) I. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria. Aquaculture Nutrition, 10: 1365-2095.

Merrifield D.L., Güroy D., Güroy B., Emery M.J., Llewellyn C.A., Skill S., Davies C.J. (2010). Assessment of *Chlorogloeopsis* as a novel microbial dietary supplement for red tilapia (*Oreochromis niloticus*). Aquaculture, 299: 128-133.

Merrifield D.L., Bradley G., Harper G.M., Baker R.T.M., Munn C.B., Davies S.J. (2011). Assessment of the effects of vegetative and lyophilized *Pediococcuc acidlactici* on growth, feed utilization, intestinal colonization and health parameters of rainbow trout (*Oncorhynchus mykiss* walbaum). Aquaculture Nutrition, 17: 73-79

Verschuere L., Rombaut G., Verstraete W. (2000). Probiotic bacteria as biological agents in aquaculture. Microbiology and Molecular Biology, 64(4): 655-671.

Wang Y.B., Xu Z.R. (2004). Probiotic treatment as method of biocontrol in aquaculture. Feed Research, 12: 42-45.

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چکیده فارسی

اثرات *Pediococcus pentosaceus* به‌عنوان پروبیوتیک بر فلور میکروبی روده و ترکیب بدن تاسماهی سیبری، *Acipenser baerii* Brandt, 1869

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چکیده:

یک آزمایش هشت هفته‌ای به منظور تعیین اثرات تغذیه‌ای *Pediococcus pentosaceus* به‌عنوان پروبیوتیک بر ترکیب بدن و فلور میکروبی روده تاسماهی سیبری اجرا گردید. تعداد ۱۸۰ قطعه ماهی با وزن متوسط ۰/۰۱±۱۴۳ گرم به‌صورت تصادفی بین ۱۲ تانک فایبرگلاس ۲۰۰ لیتری به‌عنوان چهار تیمار با سه تکرار شامل گروه‌های تغذیه شده با خوراک‌های حاوی $2x10^7$، $2x10^8$ و $2x10^9$ CFU g^{-1} باکتری *P. pentosaceus* و خوراک بدون پروبیوتیک به‌عنوان تیمار شاهد تقسیم شدند. براساس نتایج ترکیب بدن گروه‌های تیمار به جز چربی و رطوبت به‌وسیله باکتری *P. pentosaceus* تحت تاثیر قرار نگرفت. باکتری سبب کلنی‌سازی معنی‌داری در روده ماهیان تغذیه شده با خوراک حاوی پروبیوتیک شد. سطح بالای لود باکتری‌های اسیدلاکتیک در تیمار تغذیه شده با بیشترین مقدار پروبیوتیک به‌عبارت دیگر تیمار $2x10^9$ CFU g^{-1} یافت شد. نتایج نشان داد که کاربرد باکتری *P. pentosaceus* اثرات مثبتی بر ترکیب بدن و میکروفلور روده تاسماهی سیبری دارد.

کلمات کلیدی: ماهی‌خاویاری، پروبیوتیک، میکروفلور روده، ترکیب بدن.