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Effects of dietary Lactic acid bacteria on some indices of growth, survival and intestinal microbiota of cutured Persian Sturgeon (*Acipenser persicus*)

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ABSTRACT

Current study has been done for assessing the effect of additive probiotic of Lactic Acid bacteria (LAB) including equal ration of bacteria *Lactococcus lactis* (JF831150) , *Pediococcus pentosaceus* (JF831149) and *Weissella cibaria* (JF831160) (dose 10×10^7 per gram) in diet of Persian Sturgeon. 312 Persian Sturgeon with average weight of 27.80±0.20 gr were fed up for 8 weeks by 3 different concentrations of LAB including 0/2 (B 0.2), 0/3 (B 0.3), 0/4 (B 0.4) gr bacteria per kilogram food and a control group (without adding bacteria). Treatments were distributed randomly into 12 fiber glass tanks with density of 28 ones in each tank in the form of 4 treatments with 3 repeats. At the end of period the result showed that final length did not have significant difference in treatments and control ($P>0.05$) but the maximum final length has been observed in (B 0.2) treatment. Food conversion ratio showed significant difference between B(0.2) and B(0.4) treatments ($P\leq0.05$) and minimum FCR has been observed in (B 0.2). Final weight, body weight increase, Average daily growth, specific growth rate, percent of body weight increase and condition factor showed higher significant difference in (B 0.2) treatment than other treatments ($P\leq0.05$). Survival of fishes in all treatments and control was 100% and didn't have meaningful difference ($P\leq0.05$). Also there hasn't been observed significant difference in the total viable bacteria in intestine between treatments and control ($P>0.05$) but its count in control treatment was higher than other bacteria treatments. The number of LAB of intestine in treatments (B 0.2), (B 0.4) and control has had significant difference ($P\leq0.05$) and it was the highest in (B 0.4) treatment and the least in control treatment. Therefore by the increase of concentration of LAB in food the number of intestine Lactic Acid bacteria increased.

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INTRODUCTION

Development of aquaculture in many regions in the world leads to increase of request of applying and using new chemical materials. As in recent years many chemicals and industrial compounds were studies accurately to be used aquaculture economically and healthy. Using proper quality of food ingredients and supplements stimulating immunity system, to the increase of growth of fishes, has been paid attention by nutritionist. In this direction extensive research has been done in the field of improvement of formulated diets used at culturing Sturgeon fishes. It seems that ecosystem of intestine of fishes with food habits, directly has adapted with the materials that is available for bacteria [10].

In recent years, fallowing identification of Lactic Acid bacteria (LAB) in intestinal microbiota of fish and distinguishing their function in health and growth of hostess, researches have tended toward introducing supplements in this field [11,10,16]. Therefore they generally were candidate for using as probiotics and are known as improver of immunity response and growth performance in fishes. Soltani *et al*., [20] by studying microbial society of intestine of Persian Sturgeon (*Acipenser persicus*) reported that the majority of the society of LAB of intestine of this fish is related to the bacteria of potential pathogen (*Lactococcus garvieae*) to the ratio of 42.55% and probiotic bacteria (*Lactococcus lactis*) with the ratio of

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36.17% and at the less ratio bacteria with (*Pediococcus pentosaceus*) with ratio of 14.90%, (*Weissella cibaria*) with ratio of 4.25% and (Enterococcus faecalis) with ratio of 2.13%. Sturgeons are such regional, national, international living valuable resources that are important ecologically, biologically and economically for Iran. Persian Sturgeon was absolutely valuable Sturgeon fishes that its meat and caviar has high food value [19]. Therefore the goal of this research is evaluating potential of mixture of three bacteria *Lactococcus lactis* (JF831150), *Pediococcus pentosaceus* (JF831149) and *Weissella cibaria* (JF831160) to the equal ratio on some indices of growth, survival and intestinal microbiota of cultured Persian Sturgeon (*Acipenser percicus*).

MATERIALS AND METHODS

This study carried out at the center of Propagation and Culturing Sturgeons of Shadid Marjani in Gorgan located in 45 kilometers northeast of Gorgan, Iran, in Agust to December 2013. 312 young Persian Sturgeons (*Acipenser persicus*) were chosen after biometry (measuring weight and length) with average weight of 27.80±0.20 gr and after two weeks of adaptability with basic diet, they were introduced randomly to the 12 tanks with density of 26 ones. Biomass in all tanks was equal and average weight didn't have significant difference (P>0.05). Experimental groups by 3 levels of LAB treatments including equal ratio of bacteria *Lactococcus lactis* (JF831150) ،*Pediococcus pentosaceus* (JF831149) and *Weissella cibaria (*JF831160) with the dose of 10×10^7 count per gram and the concentration of 0.2 (B0.2), 0.3 (B0.3) and 0.4 (B0.4) gr bacteria per kilogram food and a control group with three repeat was done. Feeding of young fishes was done based on 2%- 2.5% of biomass weight [15] for 8 weeks of culturing period and at 2 times (at 8:00 AM and 8:00 PM). Temperature and pH was measured by (WTW pH 330i, Weilheim Germany) and dissolved oxygen was measured by (WTW oxi 330o, Weilheim Germany) weekly during culturing period. Applied dietary in this research was Biomar® standard formulated dietary made in France, with diameter and length of 1.9 mm. After adding different levels of probiotic to diet, it has been analyzed based on standard method [2] (table1). For packing and preserving foods impenetrable plastic bags were used at 3˚c. After weighting and temperature balancing of food with environment, regarding considered treatments they were given to fishes. Tanks cleaned daily before feeding to exit unconsumed food and wastes from culturing tanks. Weight and length of all fishes of tanks were measured. At the end of culturing period growth indices such as average daily growth (ADG), body weight increase (BWI), percent of body weight increase (PBWI), specific growth rate (SGR), feed conversion ratio (FCR) and condition factor (K) were examined. Crude protein of food was measured by using standard Macro-kjeldahl method (model BAP40). For Nitrogen determination of food and by considering proper protein coefficient of the sample, the amount of protein percent was measured by the relation $Cp = \%N \times 6.25$. Crude fat was measured by using Soxhlet fat measuring device (model BOHER) and through solving fat in Ether and determining its amount through Soxhlet method. Percent of ash was measure through putting samples in electrical furnaces (Muffle furnances) and by the device (Heraeus) made in Germany at 550˚c for 4 hours [12]. Based on mentioned methods applied probiotic in this research was analyzed separately as percent of raw protein, raw fat, moisture, dry matter, ash and raw energy were 4.02%, 3.16%, 7.41%, 92/59%, 0.019% and 4108/47 Kcal/kg respectively.

For counting the number of LAB and also total viable bacteria available in intestine of Persian Sturgeon fed up with different levels of LAB at the end of period, 3 fishes were caught from each repeat randomly and transferred to the lab. After anesthesia of fishes by using Ms222 (200ppm) and hitting to the head, abdominal side of fishes were sterilized by using 70% alcohol [13,16]. In sterile condition abdominal side of fishes were cut and intestine were ejected. After exiting the ingredients of intestine, it was washed three times by sterile physiology serum and weighed. For homogenization they were transferred to sterile urine bottle. After preparing homogeny fluid by using sterile salty solution (0.87% w/V NaCl) dilution of 10^1 to 10^8 were provided from intestine. The volume of 0.1 ml was taken from provided dilution under condition of aseptic and transferred to the culturing field (TSA for determining total count of viable bacteria of intestine and MRS for determining the count of LAB) and distributed on the surface of plates [18,11]. Incubation of plates was done in aerobic condition for TSA and anaerobic condition in a jar for MRS at 30˚c. After incubation of bacteria of each plate based on colony forming unit (CFU) per gram intestine they were considered based on phenotype characteristics such as color, shape, size [17].

Table 1: Proximate composition of experimental diets

Basic dietary with 0.4 gr LAB kg^{-1} food	Basic dietary with 0.3 gr LAB kg^{-1} food	Basic dietary with 0.2 gr LAB kg^{-1} food	Basic dietary	Diet composition
50.48	49.55	50.65	50.22	Crude protein (%)
19.16	19.36	19.05	19.26	Crude fat (%)
4.31	4.27	4.17	4.55	Moisture (%)
95.69	95.73	95.83	95.45	Dry matter (%)
9.41	9.55	9.30	9.00	Ash (%)
4577.54	4597.30	4562.68	4539.71	Crude energy (kcal/kg)

This research has been done in the form of completely random design. Firstly normality of data was examined by kolmogorov-Smirnov test and homogeneity test of group was investigated by Levene's test. In case of homogeneity of data, for comparing means between dietary treatments one-way ANOVA analyze and for separating homogeneous groups Duncan test at the level of 5% significance was used. For heterogeneous data non-parametric kruskal-Vallis test was used and meaningfulness of groups was distinguished by using Mann-Whitney test at the level of 5% probability. Statistical software of SPSS version 19 for data analysis and Excel 2007 was used for design charts.

Results:

Temperature, dissolved oxygen and pH during culturing were 24 centigrade degree, 5.6 ppm and 7.8 respectively. Regarding the results of culturing LAB on TSA agar it was distinguished that foods were well coated with different concentration of LAB and the count of LAB was based on Logarithm of colonial forming unit per gram food in treatments of B (0.2), B (0.30 and B (0.4) and control that was 7.47±0.00, 7.69±0.00, 7.90±0.00 and 0.00±0.00 respectively. The results of indices of growth has been shown in table (2). At the beginning of study there wasn't significant difference between treatments regarding weight and length (P>0.05). Based on multiple-range test of Duncan for pair wise comparisons between means of treatments and control at the end of period, Indices of growth including final weight, BWI, ADG, PBWI, SGR and K factor showed that significant difference between B (0.2) and B (0.4) treatments (P≤0.05) and B (0.2) was higher than other treatments. FCR showed significant difference between B(0.2) and B(0.4) and the lower FCR has been observed in control and B(0.2) treatment (P≤0.05). Based on this test in final length there hasn't been observed significant difference between treatments and control (P>0.05) but the highest final length was in treatment B(0.2). Survival at the end of period in all treatment was equal and 100% and there wasn't significant difference (P>0.05). As it was shown in table 3 in considering intestinal microbiota of fishes fed up with different concentration of LAB in basic diet there wasn't significant difference at the count of total viable bacteria (TVB) in different treatments (P>0.05). However in control treatment the total count of TVB was more than other treatments. Control, B (0.2) and B (0.4) treatments has had significant difference in LAB count between together (P≤0.05). The lowest and the highest LAB count has been seen in control and B (0.4) treatment respectively.

Table 2: Growth performance of Persian Sturgeon in the 8-week feeding trial with different levels of LAB (N=78 per treatment)

B0.4	B0.3	B0.2	control	
28.30 ± 0.77	28.27 ± 0.63	28.57 ± 0.63	26.44 ± 1.64	Initial weight(g)
20.27 ± 0.18	20.37 ± 0.16	20.45 ± 0.16	19.98 ± 0.28	Initial length(cm)
120.26 ± 6.09 ^{a}	127.11 ± 1.75 ^{ab}	153.65 ± 0.35 ^{d}	134.16 ± 1.77 ^{bc}	Final weight(g)
32.31±0.29	32.29±0.24	32.72±0.26	32.04±0.26	Final lenghth(cm)
5.40 ± 0.20 ^{a}	5.82 ± 0.13 ^{ab}	7.29 ± 0.04 ^{d}	6.80 ± 0.19 ^{c}	ADG(%)
91.96±5.45 ^{a}	98.83 ± 1.88 ^{abc}	125.08 ± 0.42 ^{e}	107.72 ± 0.83 ^{cd}	BWI(g)
324.39 ±12.14 ^{a}	349.65 ± 8.28 ^{ab}	437.76 ± 2.61 ^{d}	408.16 ± 11.50 ^{c}	PBWI(%)
2.40±0.04 ^{a}	2.50 ± 0.03 ^{ab}	2.80 ± 0.00 ^{d}	2.70 ± 0.03 ^{cd}	SGR(% day^{-1})
0.87 ± 0.02 ^{c}	0.84 ± 0.01 ^{bc}	0.77 ± 0.00 ^{a}	0.80 ± 0.02 ^{ab}	FCR
0.35 ± 0.00 ^{a}	0.37 ± 0.00 ^{ab}	0.43 ± 0.00 ^{c}	0.40 ± 0.00 ^{bc}	K

Data in the same column with different letters are significant differ (P≤0.05) among different treatments. Values are mean ± S.D

Table 3: total number of viable bacteria (TVC) and LAB (based on log CFU/gr intestine) in intestinal microbiota of persian Sturgeons 8-week feeding trial with different levels of LAB

B0.4	B0.3	B0.2	control	
4.16 ± 0.67	4.77 ± 0.47	4.27 ± 0.89	6.69 ± 0.16	TVC
3.74 ± 0.07 ^{e}	3.27 ± 0.13 ^{cd}	2.78 ± 0.10 ^{b}	0.00 ± 0.00 ^{a}	LAB

Data in the same column with different letters are significant differ (P≤0.05) among different treatments. Values are mean ± S.D

Discussion:

Many studies has been done on the ability of probiotics to increase growth indices in aquatics [22,4,21,15,1,9,3]. Manipulation of intestinal microbiota through applying probiotics can be considered as valuable mechanism for increasing growth factors and rate of survival in fishes [23]. Probiotics are effective on improvement of nutrition by detoxification of harmful substances and change of the nature of indigestive compounds by hydrolytic enzymes includes Amylase, Protease and Vitamin production such as Biotin and Vitamin B12 [5]. The survey of El-haroun and *et al.* [5] indicated that using some probiotic include *Bacillus licheniformis* and *Bacillus Subtilis* for 120 days on Nile tilapia (*Oreochromis niloticus*), has positive effects on some growth factors such as final length, PBWI, FCR, SGR, protein efficiency ratio and efficiency of food (P≤0.05). Askarian *et al.* [3 showed that enrichment of chironomids by using native Sturgeon probiotic such as *Lactobacillus mesenteroides* and feeding them to Sturgeon fishes in weight of 40-50 gr can cause meaningful increase (P≤0.05) in SGR of Persian Sturgeon (*Acipenser persicus*) and also in

study of Shenavar *et al., Lactococcus lactis* bacteria was used for evaluating its effects on growth factors of Persian Sturgeon and showed that native bacteria of *Lactococcus lactis* cause significant increase ($P \leq 0.05$) in SGR. Also they reported that regarding the effect of bacteria *Lactococcus lactis* on some main factors of growth it seems that probably this bacteria can be considered as stimulator of growth in Persian Sturgeon. These researches confirm this research due to using native intestinal bacteria of Persian Sturgeon in our survey and affect on all growth factor except final length, by adding 0.2 gram probiotic per kilogram diet it became decrease.

In parallel survey applying some probiotics such as *Enterococcus faecium* (ZJ4) in diet of Nile tilapia (*oreochromis niloticus*and) and Indian carp (*labeo rehita*) causes increase on growth factors [22,7]. In applying probiotics and their effects on growth factors of some species of fish has been seen some differences that may be due to difference at probiotic type or vector [6]. Therefore in some studies there wasn't observed effectiveness of probiotic consumption on some growth factors [6,14,15] unlike current research. Applying *Bacillus subtilis* ،*Enterococcus faecium, Bacillus licheniformis* in rainbow trout didn't have any effect on growth factors and only treatment of *E .faecium* in FCR was effective [14]. Using *Pediococcus acidilactici* bacteria in Nile tilapia (*Oreochromis niloticus*) diet for 32 days didn't affect on growth factors such as SGR and FCR [6]. On the other hand consuming *Pediococcus acidilactici* in rainbow trout didn't show positive effect on growth factors except condition factor (K) [15].

In this survey using native LAB bacteria in diet of Persian Sturgeon shows improvement on final weight, BWI, ADG, PBWI, SGR, FCR and K and B (0.2) treatment showed significant difference with other treatments ($P \leq 0.05$) although there was no significant difference between treatments and control in final length ($P > 0.05$) but the level of 0.2 g/kg food bacteria showed better condition than other treatments. Using native intestinal LAB in diet of Persian Sturgeon was effective on final length also. Another study showed that any reduction of pathogenic bacteria in intestine can improve efficiency of food and indices of growth in Nile tilapia (*Oreochromis niloticus*) and its mechanism is not distinguished yet [14]. In this study by adding LAB probiotic on diet TVC have not had significant difference between treatments and control ($P > 0.05$) but the count of TVC in control (without probiotic) was more than other treatments that may due to used probiotic in diet in other treatments. The maximum count of intestinal LAB in level of 0.4 g Bacteria/kg food treatment shows that by using more LAB in foods can increase the count of intestinal LAB in fishes.

Therefore in Sturgeon species comprehensive studies about effectiveness of probiotics on growth indices and food value is required.

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