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Research Article

EFFECT OF *LACTOCOCCUS LACTIS* AND *SPIRULINA PLATENSIS* ON SOME GROWTH PERFORMANCESOF SOUTH CASPIAN STELLATE STURGEON (*ACIPENSER STELLATUS*) JUVENILES

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ABSTRACT
In this research the effect of alga *Spirulina platensis* and bacteria *Lactococcus lactis* on growth indices in juvenile fishes of sturgeon (*Acipenser stellatus*) was studied and compared together. For this aim 210 juvenile fishes with average weight of 10.75±0.07 gr and density of 10 fishes in each fiber glass tank with the approximate volume of 1000 liter were kept for 60 days. Considering research includes 6 experimental treatments and control treatment done by 3 repeat. Treatments 1, 2 and 3 include 0.2, 0.3, and 0.4 gr of spirulina algae per kilogram diet and treatments 4, 5 and 6 include probiotic at the three levels of 0.2, 0.3 and 0.4 gr of bacteria (10^{10}CFU/g^{-1}) per kilogram diet during experiment. Biometry of fishes was done once every 10 days. The result showed that final average weight, daily growth, body weight increase in first treatment comparing to other treatments and control treatment has devoted the highest amount to itself($P<0.05$). Statistical considerations denotes lack of meaningful difference in factor of food conversion ratio among treatments ($p>0.05$). Factor of situation or fatness coefficient showed the highest amount in third treatment than other treatments and control treatment. Obtained data showed that adding spirulina algae and probiotic to the main ration of juvenile fishes of sturgeon causes improvement of output of growth and efficiency of food. Also for comparing algae with probiotic, spirulina algae especially at the level of 0.2 gr per kilogram diet has shown better performance at enhancing growth parameters than probiotic ($p<0.05$).

Keywords: *Acipenser stellatus, Growth Performances, Spirulina platensis, Lactococcus Lactis*

INTRODUCTION
Sturgeon with the scientific name of *Acipenser Stellatus* is one species of fishes that because of producing caviar it has high economic value and the caviar is counted as an important source of currency income for producing countries. It is migrating fish to the river and lives in clay, sand region and clay sediment of western and southeast of Caspian Sea. It feed lavae of insects, crustacean and aquatic invertebrates and small fishes (Abbasi *et al.*, 2009).
Acquiring information about favorable diet of fishes is important for growers of fishes. Extreme feeding leads to the decrease of water quality increase of disease, death of fishes, low capacity and efficiency of production and nutrition (Hung *et al.*, 1989b).
Spirulina is a kind of green-blue ague that is counted as the most primitive form of life on the earth. This alga is 3.5 billion years old. Nutrition value of spirulina depends on condition of growing and preserving it that has 65% protein,15% carbohydrate, 6.5% fat,9% minerals,3% water and 1.5% fiber also it has vitamins B12, B1, B3, B6, C, D,E and minerals including potassium, calcium, ferrous, phosphorus, sodium and also necessary fatty acids of GLA (Gamalinolenic acid). Spirulina has 18-22 that constitutes the most complete plant protein.
Basically consuming spirulina is effective at making high immunity in the body of creatures. Spirulina is important source of carbohydrate such as glycogen that is absorbed through body and causes high energy. Using spirulina instead of antibiotics causes decrease of pollution, reduction of care cost and increase of

efficiency of growing systems (Sakari, 1999). Various studies has been done about using spirulina for affecting growth and backwardness, color and immunity in human, animals and aquatics that denotes positive effect of this combination on above indices (Jaime-ceballos *et al.*, 2006).

Palmegiano *et al.*, (2005), have applied spirulina algae as the food source in nutrition of the fish *Acipenser baeri* and considered its effect on this fish. These researchers stated that if problems related to the high cost of production are met this alga can be a good replacement as part of fish food.

On the one hand using probiotic can be known as one of positive achievements of researchers that as the indices of probiotics cause increase of production at the level unit (Ghashghaei, 2004). Probiotics in many species are effective for enhancing growth, attracting nutrients, increasing immunity and enhancing survival.

Nowadays significant interest has been created in using lactic acid bacteria as probiotic at increasing resistance against diseases, increasing growth and immunity at culturing fishes.

Shenavar *et al.*, (2012) considered identification of lactic acid bacteria of intestine of juvenile fish named *Acipenser persicus* and their efficiency on some factors of growth and immune physiology. The result of this study showed that consumption of *L.lactis* can be effective at improvement of growth indices, efficiency of food and health of *Acipenser persicus* as a probable probiotic.

Parseh *et al.*, (2011) in a research considered the performance of probiotic on growth indices in rainbow trout fishes and concluded that adding probiotic to the degree of 0.5-1 % can have positive and significant effect on growth indices of such species.

Heo *et al.*, (2013), have considered the effect of probiotic *Lactococcus Lactis* Subsp.Lactis 12 on growth and immune response in the fish *Paralichthys olivaceus*.

The goal of this study was comparing the effect of *spirulina platensis* and *Lactococcus Latis* (JF 831150) on some growth factors in juvenile fishes of sturgeon named *Acipenser stellatus*.

MATERIALS AND METHODS

This research has been done in Shahid Dr. Beheshti Sturgeon Fish Propagation and Rearing complex in 25 southeast kilometers of Rasht city beside Sefidroudriver since August 2014 to November 2014. For this aim 210 juvenile fishes of sturgeon with average weight of 10 gr and density of 10 fishes in each tank of fiber glass were kept for 60 days. Considering research includes 6 experimental treatments and control treatment was done by three repeat.

3 groups of algae of *Spirulina platensis* with concentration of 0.2, 0.3, and 0.4gr per kilogram food and 3 experimental bacteria of *Lactococcus lactis* with similar concentration were considered. Feeding was done by Biomar food made in France based on weight mass of juvenile fishes in each tank to the degree of3.5-5% of live mass weight in 3 tirns of morning, at noon and in the evening. For attaching algae and powder probiotic the soya bean oil the degree of 50cc per kilogram fish food was used and in control group oil without algae and probiotic was added to the diet. Biometry of fishes had been done every 10 days for considering growth and determination of the degree of proper food proper to the increase of biomass.

Average water temperature, soluble oxygen, percent of oxygen saturation, PH, $No3^-$ ،$No4^+$ ،NH3 and total hardness during the whole period of growing was measured in order of 15.7 c, 9.4 mg/l,7.4, 0.009mg/l,0.04mg/l,0.01mg/l,187mg/l.

Table 1: Experimental ratio applied for nutrition of juvenile fishes in different treatments

Experimental diets **Biomar**	**Treatment** **Control**
Biomar + *Spirulina platensis* 0.2 gr/kg food	1
Biomar + *Spirulina platensis* 0.3 gr/kg food	2
Biomar + *Spirulina platensis* 0.4 gr/kg food	3
Biomar + *Lactococcus lactis*0.2 gr/kg food(10^{10} CFU/g^{-1})	4
Biomar + *Lactococcus lactis*0.3 gr/kg food (10^{10} CFU/g^{-1})	5
Biomar + *Lactococcus lactis* 0.4 gr/kg food (10^{10} CFU/g^{-1})	6

And finally after finishing growth period indices such as average daily growth (ADG), body weight increase (BWI), special growth rate (SGR), food conversion ratio (FCR) and condition factor (CF) were calculated based on standard formula (Ferguson *et al.*, 2010).

Data Analysis

For considering normal distribution of data in groups and repeats kolmogrov-smirnov test was used for confirmation of treatments. In case of normality of data for statistical comparison between groups in treatments one-way Anova test was used and after that the test of Homogeneity of Variance was used for comparing groups together. Also for comparing 2 groups independent sample t-test was used.

Averages compared based on ±SE mean presented at the level of 95% for meaningfulness of differences. All statistical analysis was done by using Spss software edition 17 and for drawing charts the software Excel 2007 was used.

RESULTS AND DISCUSSION

Result

Result of considering mean of total length and weight and also the result of evaluating growth indices including ADG, BWI, SGR, and CF has been mentioned in table 2.

Table 2: Comparing average final weight, final length, ADG, BWI, SGR, CF and CF in control with different treatments at the end of period

Experimental groups							Parameter
Treat 6	**Treat 5**	**Treat 4**	**Treat 3**	**Treat 2**	**Treat 1**	**Control**	
44.23±1.91^{ab}	46.13±0.58^{ab}	45.03±^{ab}0.66	46.63±0.74^{ab}	41.06±^{ab}0.70	49.2±^{b}1.1	37.23±^{a}1.82	Final weight (gr)
28.25±0.64^{ab}	28.40±0.26^{ab}	28.49±^{ab}0.33	28.23±0.66^{ab}	27.68±^{ab}0.48	29.21±^{b}0.63	±^{a}1.002 26.92	Final length (cm)
5.35±0.20^{ab}	5.27±0.46^{ab}	5.26±^{ab}0.29	5.47±0.50^{ab}	4.81±^{ab}0.52	6.01±^{b}0.53	4.09±^{a}0.64	ADG (gr/day)
±12.45^{ab} 321.02	±27.55^{ab} 316.79	315.61±^{ab}17.92	±30.53^{ab} 328.54	±^{ab}31.73 288.6	±^{b}32.02 361.05	±^{a}38.70 245.47	BWI (%day^{-1})
0.56±0.03^{ab}	0.58±0.04^{ab}	0.57±^{ab}0.033	0.59±0.048^{ab}	0.50±^{ab}0.05	0.64±^{b}0.06	0.44±^{a}0.07	SGR (%day^{-1})
1.22±0.07	1.19±0.02	1.30±0.02	1.15±0.04	1.32±0.13	1.21±0.04	1.43±0.16	FCR
0.19±0.003^{ab}	0.20±0.007^{ab}	0.19±0.003^{ab}	0.21±0.002^{a}	0.19±^{ab}0.01	0.20±0.002^{ab}	0.19±0.004^{b}	CF (%)

(In common latin letters in row show significant statistical difference at dunkan test at the level of 5%)

According to Duncan multi-dimensional test for two by two comparison of groups together, average final weight and final length in treatment 1 was meaningfully more than control group and statistical difference was observed (P<0.05). Also other treatments have had higher weight and length mean than control group but there wasn't observed significant statistical difference between treatments 1 with other experimental treatments (table 2).

Also ADG, BWI and SGR in treatment 1 were more than control group and significant statistical difference wasn't observed (P< 0.05). Other treatments have had higher average daily growth than control. For comparing FCR based on Duncan multi-dimensional test and for two by two comparison of groups together and with control, significant statistical difference hasn't been observed (P>0.05) (table 2).

Condition factor (CF) in treatment 3 was more than control group and significant statistical difference was observed (P<0.05). Also other treatments have had higher than control, but there hasn't been observed significant statistical difference between treatments 3 with other experimental treatments (table 2).

Discussion and Conclusion

For business and efficient production of sturgeon, strong management, proper condition of growth, feeding suitable nutrition that has cheaper and effective ingredient that has optimum growth and least

amount of FCR, seems necessary (Hung & Kutes, 1987). Now the main problem at business aquatic growing is improvement of formulated nutrition for increasing growth and enhancement of health of fishes. Researchers believe that increasing efficiency of aquatic production of depends on constructive ingredients of nutrition such as protein, fat, vitamins, minerals, price and being available (Chebanov & Billard, 2001).

Nowadays Japanese grower have founded 5 fitting keys at consumption of spirulina in aquatic diet that includes the degree of better growth, improving quality at fish color taking, degree of more survival, decreasing drug consumption, decreasing environmental waste. Spirulina can be as part of food supplement or replaces completely protein in nutrition of aquatic culturing (FAO, 2008).

One the one hand probiotics having lactic acid bacteria lead to the increase of the degree of aliveness of hostess in confrontation with pathogens (Gatesoupe, 1999). Also it has been observed that some probiotics increase appetite, improve health and lead to the total increase of weight that is probably due to increasing for digestion (Gatesoupe & Ringo, 1998).

In current research the algae *Spirulina* platesis and bacteria of *Lactococcus latis* as two factors of stimulating growth for increasing growth criteria were added to nutrition of fishes and their efficiency were compared together. The results denotes that using algae and probiotic in all experimental than control group has shown positive function at enhancing growth of juvenile sturgeon. All ration supplemented with algae and probiotic lead to growth output and better consumption of food comparing to basic diet in control group.

Shenavar *et al.,* (2012) by considering lactic acid bacteria of intestine of the fish *Acipenser persicus* claimed that consumption of *L.Lactis* can be effective at improvement of growth index, efficiency of food and health of the fishes as a probable probiotic.

Faramarzi *et al.,* in 2011 due to using probiotic (Bacillus sp) in nutrition of Larvae of the fishes reported positive results about growth parameters comparing control group.

Similar results were reported by Abdel-Tawwab *et al.,* (2008) about spilulinaalgae (*Arthrospira platensis*) as stimulator of growth and immunity in new-born juvenile fishes of Tilapia (*Oreochromis niloticus*).

Also Palmegiano *et al.,* (2005) have used algae of Spirulina as the nutrition in diet of sturgeon (*Acipenserbaeri*) and considered its effect on growth of this fish. This scientist state that if problems related to high cost of production is met this algae can be a good replacement as part of fish's food.

Based on the obtained result the highest final average weight(49.2±1.1), daily growth(6.01±0.53), body weight increase(361.05±32.02) and special growth rate(0.64±0.06) was observed in treatment 1that meaningfully was more than other treatments and control and has had statistical meaningful difference($P<0.05$). Although there wasn't statistical meaningful difference between other treatments and control, the least degree of growth in all cases was observed in control group.

Since the degree of dose used for probiotics was lactic acid bacteria type in the range 0.2-0.5gr per kilogram food (Soltani, 1999) therefore related dose(0.2, 0.3, 0.4gr per kilogram food) for *Lactococcus Lactis* were chosen on this way that after doing test meaningful difference hasn't been observed between chosen doses($P>0.05$).

Faramarzi *et al.,* (2011) have considered the effect of probiotic *lactobacillus acidophilus* on growth indices in rainbow trout. These researchers reported the best average weight, SGR and FCR with concentration of 10^3cfu/ml.

Chiu *et al.,* (2010) has reported the best weight increase and efficiency of nutrition with probiotic *Saccharomyces cerevisiae* in concentration of 10^7cfu/kg^{-1} in the fish *Epinephelus coiodes*.

In another experiment that was done by Bagheri *et al.,* (2008) on rainbow trout, probiotic Bacillus spp at the level of 3.8×10^9cfu/g^{-1} showed the best result about growth parameters in this fish.

Since mentioned study was comparative so doses related to *Spirulina platensis* was considered similar to *lactococcus lactis* that in the experiment concentration of 0.2gr of algae per kilogram food has devoted the best result about growth parameters to itself.

Palmegiano *et al.,* (2005) have considered the effect of spirulina algae on growth of sturgeon (Acipenserbaeri). These researchers reported the highest degree of growth at the level of 50%.

Abdel-Tawwab (2008) obtained optimum growth in Tilapia fish (*Oreochromis niloticus*) due to using algae (*Arthrospira platensis*) with concentration of 5gr per kilogram food. These scientist stated proper level of this algae 5-10gr per kilogram food.

Also in another research that was done on the fish *Oplegnathus fasciatus* the highest increase of weight was reported with spirulina algae the species *Spirulina pacifica* at 5% level (Kim *et al.*, 2013).

Conclusion

Generally it is concluded that adding spirulina algae and probiotic to main ration of sturgeon juvenile fish causes improvement of growth input and efficiency of food. Also for comparing algae with probiotic, spirulina algae especially the level of 0.2gr per kilogram food (treatment 1) has shown better performance than probiotic at enhancing growth parameters.

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REFERENCES

Abbasi K, Valipour A, Haghighi D, Sarpanah A and Nezami Sh (1999). Dictionary of fishes of Sefidroud River and Anzali pond, publication of the center of fisheries research center of Guilan 113.

Abdel-Tawwab M, Ahmad MH, Abdel-Hadi YM and Seden MEA (2008). Use of Spirulina (*Arthrospin platensis*) as a growth and immunity promoter for Nile Tilapia, *Oreochromis niloticus*(L.) fry challenged with pathogenic *Aeromonas hydrophila*. *8th International Symposium on Tilapia in Aquaculture*.

Bagheri T, Hedayati SA, Yavari V, Alizade M and Farzanfar A (2008). Growth, Survival and Gut Microbial Load of Rainbow Trout (*Onchorhynchusmykiss*) Fry Given Diet Supplemented with Probiotic during the Two Months of First Feeding. *Turkish Journal of Fisheries and Aquatic Sciences* **8** 43-48.

Chebanov M and Billard R (2001). The culture of sturgeon in Russia: production of juveniles for stocking and meat for human consumption. *Aqua Living Resource* **14** 375-381.

Chiu CH, Cheng CH, Gua WR, Guu YK and Cheng W (2010). Dietary administration of the probiotic, *Saccharomyces cerevisiae*P13, enhanced the growth, innate immune responses, and disease resistance of the grouper, *Epinephelus coioides*. *Fish & Shellfish Immunology* **29** 1053-1059.

Faramarzi M, Jafaryan H, Patimar R, Iranshahi F, LashkarBoloki M, Farahi A, Kiaalvandi S, Ghamsary M and Makhtoumi NM (2011). The Effects of Different Concentrations of Probiotic *Bacillus* spp. And Different Bioencapsulation Times on Growth Performance and Survival Rate of Persian Sturgeon (*Acipencer persicus*) Larvae. *World Journal of Fish and Marine Sciences* **3**(2) 145-150.

Faramarzi M, Kiaalvandi S, Lashkarboloki M and Iranshahi F (2011). The Investigation of *Lactobacillus acidophilus* as Probiotics on Growth Performance and Disease Resistance of Rainbow Trout (*Oncorhychus mykiss*). *American-Eurasian Journal of Scientific Research* **6**(1) 32-38.

Ferguson RMW, Merrifield DL, Harper GM, Rawling MD, Mustafa S, Picchietti S, Balcazar JL and Davies SJ (2010). The effect of *Pediococcus acidilactici* on the gut microbiota and immune status of on-growing red tilapia (*Oreochromis niloticus*). *Journal of Applied Microbiology*, ISSN 1364-5072.

Gatesoupe FJ (1999). The use of probiotic in aquaculture. *Aquaculture* **180** 147-165.

Gatesoupe FJ and Ringo E (1998). Lactic acid bacteria in fish: a review. *Aquaculture* **160** 177-203.

Heo WS, Kim YR, Kim EY, Bai SC and Kong IS (2013). Effects of dietary probiotic, *Lactococcuslactis*subsp. Lactis 12, supplementation on the growth and immune response of olive flonder (Paralichthysolivaceus). *Aquqculture* **376-379** 20-24.

Hung SSO and Lutes PB (1987). Optimum feeding rate of hatchery produced juvenile white Sturgeon (*Acipenser transmontanus*) at 20 C. *Aquaculture* **65** 307-317.

Jaime-Ceballos B, Hernandez-Llamas A, Garcia T, Perez-Jar L and Villareal H (2006). Substitution of *Chaetoceros mulleri* by *Spirulina platensis* meal in diets for *Litopenaeuss chmitti* larvae. *Aquaculture* **266** 215-220.

Kim SS, Rahimnejad S, Kim KW and Lee KJ (2013). Partial Replacement of Fish Meal with *Spirulina pacifica* in Diets for Parrot Fish (*Oplegnathus fasciatus*). *Turkish Journal of Fisheries and Aquatic Sciences* **13** 197-204.

Palmegiano GB, Agradi E, Forneris G, Gai F, Gasco L, Rigamonti E, Sicuro B and Zoccarato I (2005). Spirulina as a nutrient source in diets for growing sturgeon (*Acipenser baeri*). *Aquaculture Research* **36** 188-195.

Parseh A, Tahmasebi A, Jamalzadeh HR and Alizadeh M (2011). Considering performance of probiotic *Pediococcus acidilactici* on growth indices and parameters of hematology of rainbow trout fish.The book of papers summary. *The First National Congress of Aquatic Culturing in Iran, Bandar e anzali* 452.

Sakai M (1999). Current research status of fish immunostimulants. *Aquaculture* **172** 63-92.

Shanavar AR (2012). Identification of lactic acid bacteria of Iranian juvenile sturgeon intestine (*Acipenser persicus*) and their efficiency on some growth factors and immunophysiology, Ph.D thesis of health and aquatics, Tehran university, veterinary college.