EFFECT OF LACTOCOCCUS LACTIS AND SPIRULINA PLATENSIS ON SOME GROWTH PERFORMANCES OF 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 of 3.5-5% of live mass weight in 3 tims 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, 0.09mg/l,0.04mg/l,0.01mg/l,187mg/l.

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

<table>
<thead>
<tr>
<th>Experimental diets</th>
<th>Treatment</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomar + Spirulina platensis 0.2 gr/kg food</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Biomar + Spirulina platensis 0.3 gr/kg food</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Biomar + Spirulina platensis 0.4 gr/kg food</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Biomar + Lactococcus lactis0.2 gr/kg food(10^{10} CFU/g^{-1})</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Biomar + Lactococcus lactis0.3 gr/kg food (10^{10} CFU/g^{-1})</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Biomar + Lactococcus lactis 0.4 gr/kg food (10^{10} CFU/g^{-1})</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
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

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

(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 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

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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 platensis and bacteria of Lactococcus lattis 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 1 that 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 10³cfu/ml.

Chiu et al., (2010) has reported the best weight increase and efficiency of nutrition with probiotic Saccharomyces cerevisiae in concentration of 10⁷cfu/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 3.8x10⁸cfu/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%.
Research Article

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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