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Effect of immune motivator Macrogard and *Spirulina platensis* on some growth, carcass and biochemical indices of stellate sturgeon *Acipenser stellatus*

Ammar Salehi-Farsani, Mehdi Soltani, Abolghasem Kamali, Mehdi Shamsaie

Department of Fisheries, Science and Research Branch, Islamic Azad University, Tehran, Iran. Corresponding author: A. Salehi-Farsani, ammar_1631@yahoo.com

Abstract. This study was carried out to survey the effect of immune motivator Macrogard and *Spirulina platensis* on some growth and biochemical and carcass composition of young cultured stellate sturgeon *Acipenser stellatus*. This investigation was designed using a completely random plan containing 0%, 0.1% and 0.5% Macrogard and *Spirulina* in 5 treatments with 3 repetitions. Young stellate fish with an average weight of 1100.14±65.38 g and density of 40 in each cement round pond were fed in 12 weeks with an experimental ration of 3% of body weight. In the beginning of the study, at the end of 6th week and 12th week, blood sampling was carried out. Also in the beginning and at the end of the experiment, sampling was done for carcass analysis. Growth factors including body weight and length, body weight index (BWI), sustainable growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER). Significant differences were observed in the fish fed by Macrogard and *Spirulina* (together) compared to control treatment and treatments fed by Macrogard or *Spirulina* ($p < 0.05$). No significant difference was observed in condition factor (CF) and hepatosomatic index (HSI) ($p > 0.05$). Obtained results showed significant differences between total protein, triglyceride, cholesterol and glucose in the studied treatments ($p < 0.05$), while about cortisol amount, there was no significant difference ($p > 0.05$). Significant differences were observed in approximate carcass including protein, fat and ash ($p < 0.05$). It can be concluded that, Macrogard and *Spirulina* can increase growth rate and improve some biochemical indices in stellate sturgeon. Also using these two additives together in amount of 0.1% and 0.5% for this fish in this weight range and in similar conditions is evaluated to be positive.
Key Words: feed conversion ratio, prebiotic, protein efficiency ratio, condition factor, triglyceride.

Introduction. Sturgeons which of called cartilaginous-bony fish, are from primary cartilaginous fish divided from bony fish about 200 million years ago remained in their present shape losing a lot of their characteristics (Keyvan 2003). About two centuries ago, these fishes were distributed in aquatic areas of many countries and their fishing, caviar production and commerce were very successful. But overfishing, weak fishing management, no protection, reduction of natural living and spawning areas, severe environmental pollution, building dams on rivers, eutrophication and etc. caused their natural habitats to be limited (Hung et al 1989). Nowadays, these valuable fish are distributed in freshwaters, brackish and marine waters of north hemisphere in some water basins of Asia, Europe and America. Main stocks of these fish are in basins of Caspian Sea, Black Sea and Aral Sea (Keyvan 2003). Flesh of these fish is very delicious, their caviar is incomparable, very expensive and are economically very important for countries owning their stocks. Because of high nutritious and economic values of flesh and caviar of sturgeons and also stock reduction of them in all natural habitats, artificial aquaculture of them was noticed by a lot of countries from many years ago and showed undeniable development. So that, in addition to culture of pure and fast growth species, in some countries, suitable hybrids are produced for more flesh and made a huge change in sturgeon aquaculture.

Prebiotic is known as a non-digestible food with positive effects. These materials activate fish growth or some bacteria of intestine which are effective on growth (Gibson & Roberfroid 1995). Prebiotic foods are carbohydrates which can be monosaccharide, oligosaccharide or polysaccharide (Ringo et al 2010). Macrogard is a kind of "Glucan" composed of glucose units. This glucan has 1,3 beta and 1,6 beta bonds between glucose units. This material is extracted from cell wall of "Yeast" *Saccharomyces cerevisiae*. Glucans are one of the important structural compositions of all fungi and Macrogard composition is not the only extracted glucan from them. *Spirulina* can be used in aquaculture or a completely protein source for aquatic animals. Related studies have shown that using *Spirulina* in culture of Sea bream (*Rhabdosargus sarba*) in an amount of 50% in their diet has a positive effect (El-Sayed 1994). *Spirulina*, a microscopic blue-green algae, is the most nourishing green food known in the world. This algae grows suspended in water, so does not need to build cell wall which limits access to intracellular nutrients. This algae has the most herbal protein with more than 60% and has the most absorbable one. Amino-acids in *Spirulina* are like egg and their quality is confirmed by FAO. This algae is a source of nutrients which are not available in other green foods. Linoleic acid, vitamins and sulfolipids are some of materials in this algae. Additionally, *Spirulina* is a good source of beta-carotene, carotenoids, vitamins and trace elements. *Spirulina* contains a significant amount of chlorophyll and other herbal pigments available in green plants. The amount of fat and carbohydrate is low in this algae and with no cholesterol. *Spirulina* is the simplest but the most nourishing food source in the world.

The present study was carried out to survey the effect of Macrogard and *Spirulina platensis* on some growth indices, biochemical and carcass composition of young cultured stellate sturgeon *Acipenser stellatus*.

Material and Method. This study was carried out in Gerdab Falard Farm in Chaharmahal-o-Bakhtiari Province nearby Sendijan River. In this farm, during experiment, there were 700 stellate sturgeons with an average weight of 87.56±1.06 g. For culturing operations, 15 concrete ponds were used, each pond with a volume of 4000 liters. Water level was 100 cm, required water was provided from Sendijan River. Water flow of each pond was 5 L s^{-1}. During this experiment, water temperature, dissolved oxygen and pH were 19.34±4.14^{o}C, 6.53±0.75 mg L^{-1} and 7.62±0.08, respectively. Random research design contained 5 treatments in 3 repetitions (Table 1), including control treatment (M0S0) which contains fish fed by basic diet (Table 2). Treatment number 1 contains fish fed by food with 0.1% Macrogard (M1S0), treatment number 2 contains fish fed by food with 0.1% *Spirulina* (M0S1), treatment number 3 contains fish fed by food with 0.1% Macrogard and 0.1% *Spirulina* (M1S1), treatment number 4 contains fish fed by food with 0.5% Macrogard and 0.5% *Spirulina* (M5S5). Each pond included 40 fish and each treatment had 3 repetitions. Fish were fed in 12 weeks, 4 times a day (at the hour 2, 8, 14 and 20) in amount of 3% of body weight. Experimental period was for 12 weeks, from June to September 2013. Dietary compounds and approximately analytical results are shown in Table 1.

Table 1

Dietary compounds (%) used in experiment

Dietary compounds	*Diet*				
	M0S0	*M0S1*	*M1S0*	*M1S1*	*M5S5*
Fish meal (%)	46	46	46	46	46
Wheat flour (%)	19	19	19	19	19
Dried milk (%)	6	6	6	6	6
Soybean meal (%)	11	11	11	11	11
Corn gluten (%)	7	7	7	7	7
Fish oil (%)	5	5	5	5	5
Yeast (%)	3	3	3	3	3
Mineral-vitamin supplements	3	3	3	3	3

Table 2

The approximate composition of the basic diet

	Moisture	*Ash*	*Protein*	*Fat*	*Fiber*
The approximate composition (%)	10.20±0.20	21.30±5.30	45.00±0.90	13.30±0.20	10.20±0.20

Method of making diet. To preparing the diet, all compounds (including fish meal, wheat flour, soybean meal, dried milk, corn gluten and yeast) were powdered by mill device and mixed together by mixer for 20 minutes. Then the additives including salt and mineral-vitamin supplements were added to the mixture in a low amount and also Macrogard and *Spirulina* were added based on the required amount at the same time and were mixed together for 15 minutes. Mineral-vitamin supplements contain vitamins A, C, D_3, E, B_1, B_2, B_6, K_3, Nicotinamide, and minerals including copper, iron, zinc, manganese. Then, using a meat grinder, the mixed food was exchanged into pellets with 8 mm length and 6 mm diameter. Finally, pellets were dried in dryer device in 30^{o}C for 24 hours and were packed and marked in black polyethylene plastics and were kept in tight closed containers until consumption time in a freezer in -20^{o}C. One hour before food distribution, the diets were kept in the room temperature. After equilibration, concentrate foods were weighed using a digital scale and were distributed in fish ponds according to treatments. It is worth noting that making food was carried out weekly. Approximate analysis of diet was done according to standard methods of AOAC (1995). Wet materials were dried in an oven (Memmert, made in Germany) to estimate dried material's weight. Raw protein with Kjeldahl system (Buchi, made in Swiss), Raw fat with Soxhlet system (Buchi, made in Swiss) and amount of ash with weighing after burning in 550^{o}C for 6 hours in a furnace (Gallenkamp, made in U.K.) a bomb calorimeter (Parr, made in U.S.A.) was determined to know the amount of energy. Fish were weighed and measured every 3 weeks individually and diet was determined according to new biomass of each container. Growth indices of fry fish such as body weight index (BWI), sustainable growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF), hepatosomatic index (HIS) and survival rate were calculated according to standard formula (Hung et al 1993; Haghighi et al 2009; Luo et al 2010): BWI = (final body weight-original body weight/original body weight) × 100, SGR = (ln final body weight-ln original body weight/number of days) × 100, FCR = total feeds consumed/number of kilogram live weight produced, PER = gain in body mass (g)/protein intake (g), CF = (body weight/body lenght^3) × 100, HIS = (liver weight/body weight) × 100, Survival rate = (final number of fish/original number of fish) × 100.

Blood sampling. To estimate the effects of different diets on blood indices, before experiment, 20 stellate fish were caught randomly from the culturing tanks to measure biochemical parameters (total protein, cortisol, cholesterol, glucose and triglyceride). Also every 41 days (3 times totally) 2 fish were caught randomly from each tank to take blood samples.

Method of serum preparation. After transferring Eppendorf tubes containing 4 ml blood to laboratory, separation of serum from blood cells was carried out by centrifuge (Model 200 Labofuge, Heraeus sepatch Company, made in Germany) in 3000 rpm for 5 minutes. Then using Pastor Pipette, serum was transferred to marked Eppendorf and was maintained in -20^{o}C until measurement (Pottinger & Carrick 2001), also serologic studies was done.

Determine the amount of cortisol. Cortisol hormone was measured using Radio immune assay (RIA) by labeling with radioactive element I^{125} by automatic Gama counter device model: L. K. B. made in Finland and by Immune tech (made in France) in ng mL^{-1}.

Determine the amount of glucose. Measurement of glucose of serum with enzymic method (glucose oxidase-peroxidase; GOD-POD) was carried out (Teuscher & Richterich

1971; Braham & Tinder 1972). So spectrophotometer (model RA-1000, Technicon company, made in U.S.A.) and Man kates (made in Iran) were used and the amount of glucose was determined in mg dL^{-1}.

Determine the amount of cholesterol. The determination of the amount of cholesterol was carried out by enzymatic method (Allain et al 1974). So, spectrophotometer device (model RA-1000, Technicon company, made in U.S.A) and Man kates (made in Iran) helped to measure cholesterol in mg L^{-1}.

Determine the amount of triglyceride. The determination of the amount of triglyceride was done by the method of Annoni et al (1982). So spectrophotometer device (model RA-1000, Technicon company, made in U.S.A.) and Man kates (made in Iran) were used to determine the amount of triglyceride in mg dL^{-1}.

Determine total protein of serum. To do it, "Refractmeter" device (model SPR-Ne, made in Japan) was used. This device has a scaled plate from 0 to 10 and moving eyepieces. Before putting serum sample on the plate, full scaled plate is blue; but upon pouring a few drops of blood serum on the special plate of device, a part of scaled plate will be white that represents amount of total protein: in g dL^{-1}.

Approximate carcass analysis. In the beginning of experiments and after adaptation period, 20 fish were determined to measure the amount of raw protein, raw fat, carbohydrate, moisture and ash. Also, at the end of experiments and after last blood sampling, 4 specimens of each treatment and in total 20 specimens were sent to laboratory for carcass analysis and compounds determination (raw protein, raw fat, moisture, ash and carbohydrate) to compare these compounds with pre-experiment.

AOAC (1995) methods were used to mentioning analyses. Samples (whole fish) were analyzed after grinder. To determine moisture, 105^{o}C oven for 18 hours, and for ash 450^{o}C muffle Furnaces for 4 hours were used. Fat experiment by Soxhlet method (model Boher, made in Germany), Protein by Kjeldahi method (model BAP40, made in Germany) were used. Carbohydrate amount was obtained by subtracting resulting numbers of protein, fiber, fat, moisture and ash from 100.

The obtained results were compared by one way ANOVA test and comparisons among averages of treatments were carried out using SPSS 16.

Results

Growth parameters. Average of some growth indices (average ± standard deviation) obtained in the 3th and 6th weeks in young cultured Stellate sturgeon with different levels of Macrogard and *Spirulina* are presented in Table 3.

Table 3

Average of some growth measurements (average and standard deviation) obtained in 3th and 6th weeks in young cultured stellate sturgeon fed by different levels of Macrogard and *Spirulina*

Growth indices	*M0S0*	*M0S1*	*M1S0*	*M1S1*	*M5S5*
Initial weight (g)	1100±68.65	1100±85.93	1100±84.78	1100±74.53	1100±45.83
Final weight (g)	1365.29±63.49^{b}	1378.97±59.75^{b}	1398.02±50.42^{b}	1486±69.11^{a}	1510±71.22^{b}
Initial length(cm)	74.30±1.31	74.32±1.29	74.45±1.25	74.92±1.16	74.67±1.11
Final length (cm)	79.30±1.31	79.32±1.16	79.45±1	80±1.22	80.51±1.46
FCR	5.01±0.31^{a}	4.85±0.21^{a}	4.54±0.11^{a}	3.5±0.33^{b}	3.30.12^{b}
PER	0.44±0.005^{b}	0.46±0.042^{b}	0.49±0.055^{c}	0.63±0.010^{a}	0.67±0.028^{a}
BWI (%)	24.11±2.25^{b}	25.36±2.91^{b}	27.09±2.75^{b}	35.14±2.28^{a}	37.27±3.38^{a}
CF	0.27±0.010	0.28±0.005	0.28±0.016	0.29±0.014	0.29±0.015
SGR (% day)	0.52±0.019^{b}	0.55±0.010^{b}	0.58±0.005^{b}	0.73±0.010^{a}	0.77±0.005^{a}
HIS (%)	3.35±0.32	3.24±0.33	3.36±0.36	3.33±0.31	3.48±0.32

* Columns without letters show that there is no significant difference in that parameter.

According to these results, the maximum FCR was in control treatment (M0S0), the maximum CF in M1S1 and M5S5 and the maximum of other parameters: BWI, FE, PER, GR, final weight (W_2) and final length (L_2) were observed in M5S5 treatment. The least amount of BWI, FE, PER, GR, CF, PER, W_2 and L_2 were in M0S0, minimum of HSI in M0S1 and minimum of FCR observed in M5S5. About FCR, BWI, FE, PER there were significant differences between treatments ($p < 0.05$). About other factors, there was no significant difference between treatments ($p > 0.05$).

Average of some growth indices (average ± standard deviation) obtained in the 9th and 12th weeks in young cultured stellate sturgeon with different levels of Macrogard and *Spirulina* are presented in Table 4.

According to the obtained results, the maximum FCR was observed in control group, maximum BWI, FE, PER, GR, W_2 and L_2 were observed in M5S5, maximum CF in M1S1 and M5S5 treatments and maximum HSI were observed in M1S1. The minimum of BWI, FE, PER, GR, HIS, CF, W_2 and L_2 were in M0S0. While the minimum of FCR was observed in M5S5. About FCR, BWI, PER, W_2 and SGR, there were significant differences among the treated fishes ($p < 0.05$). But about the other factors, there was no significant difference between treatments ($p > 0.05$).

Table 4

Average of some growth indices (average ± standard deviation) obtained in 9th and 12th weeks in young cultured stellate sturgeon fed by different levels of Macrogard and *Spirulina*

Growth indices	*M0S0*	*M0S1*	*M1S0*	*M1S1*	*M5S5*
Initial weight (g)	1365.29±63.49	1378.9±59.75	1398.02±50.42	1486.57±69.11	1510±71.22
Final weight (g)	1679.30±58.15[b]	1736±70.67[b]	1770±48.33[b]	1994±83.97[a]	2054±80.35[a]
Initial length(cm)	79.30±1.26	79.32±1.16	79.45±1	80±1.22	80.51±1.46
Final length (cm)	80.02±0.98	82.25±1.01	82.20±0.94	84.40±1.11	84.60±1.21
FCR	4.92±0.21[a]	4.81±0.35[a]	4.62±0.21[a]	3.60±0.31[b]	3.41±0.11[b]
PER	0.45±0.004[b]	0.46±0.044[b]	0.48±0.056[c]	0.61±0.059[a]	0.65±0.026[a]
BWI (%)	25±2.15[b]	25.57±2.82[b]	26.67±2.35[b]	34.10±2.18[a]	36.07±3.38[a]
CF	0.30±0.015	0.31±0.005	0.31±0.018	0.33±0.010	0.33±0.010
SGR (% day)	0.54±0.10[b]	0.57±0.005[b]	0.57±0.10[b]	0.71±0.015[a]	0.75±0.005[a]
HIS (%)	3.33±0.32	3.35±0.33	3.39±0.36	3.63±0.31	3.58±0.32

* Columns without letters show that there is no significant difference in that parameter.

Carcass analysis. The approximate carcass analysis in 10 fish in the beginning of culture period is shown in Table 5.

Table 5

Results of carcass analysis of stellate sturgeon before starting to feed by experimental diets (n=10)

Carbohydrate (%) from dried material	*Fat (%) from dried material*	*Protein (%) from dried material*	*Ash (%) from dried material*	*Moisture (%) from dried material*
33.74±5.67	28.81±4.49	33.49±6.12	3.46±0.26	76.87±5.52

The approximate analysis of carcass at the end of the experimental period showed that the minimum amounts of carbohydrate and protein were in M0S0 and the minimum amounts of fat, ash and moisture were in M5S5 treatment. While the maximum amount of fat was observed in M1S0, the maximum amounts of carbohydrate and protein were in M5S5, the maximum amount of ash was in M0S0 and the maximum amount of moisture was observed in M0S1 treatment (Table 6). About carbohydrate and moisture, there was no significant difference among the treatments ($p > 0.05$). But about fat, protein and ash, there were significant differences between the studied treatments ($p < 0.05$).

Table 6

Results of carcass analysis of stellate sturgeon at 12th week (n=20)

The amount of S and M (mg kg^{-1})	*Carbohydrate (%) from dried material*	*Fat (%) from dried material*	*Protein (%) from dried material*	*Ash (%) from dried material*	*Moisture (%) from dried material*
M0S0	32.14±2.58	26.52±1.59^{a}	33.27±1.12^{b}	3.46±0.46^{a}	74.39±5.67
M0S1	33.99±1.39	23.34±3.67^{ab}	36.19±2.72^{b}	3.27±0.54^{a}	76.56±6.12
M1S0	34.24±2.37	28.63±2.19^{a}	41.33±1.83^{ab}	2.47±0.87^{b}	75.71±7.76
M1S1	33.39±5.66	22.54±3.08^{ab}	53.42±2.92^{a}	2.06±0.16^{b}	74.11±8.34
M5S5	35.64±2.77	18.11±2.32^{b}	56.76±1.92^{a}	2.01±0.00^{b}	73.17±5.52

* Columns without letters show that there is not significant difference in that parameter.

Biochemical parameters (total protein, triglyceride, cortisol, cholesterol and glucose). Obtained results at the beginning of culture period in 20 fish are shown in Table 7.

Table 7

Biochemical measurements of stellate sturgeon's blood serum before starting to feed by experimental diets (n=20)

Total protein (g dL^{-1})	*Triglyceride (mg dL^{-1})*	*Cortisol (ng mL^{-1})*	*Cholesterol (mg dL^{-1})*	*Glucose (ng mL^{-1})*
6.57±0.54	593.05±284.05	57.10±2.90	3.67±0.38	129.57±17.14

Obtained results of blood serum at the 12th week are shown in Table 8. According to this table, the maximum amount of triglyceride was observed in control treatment, the maximum amounts of total protein and cortisol were in M5S5, maximum amount of cholesterol was in M1S0 and the maximum amount of glucose was measured in M1S1 treatment. While, the minimum amount of cortisol was in M1S0, minimum amounts of glucose and total protein were in control treatment, minimum amounts of cholesterol and triglyceride were observed in M5S5 and control treatments. Statistical analysis showed that there are significant differences between treatments about biochemical factors including total protein, glucose, triglyceride and cholesterol ($p < 0.05$) and about cortisol there was no significant difference ($p > 0.05$).

Table 8

Blood serum analysis of Stellate sturgeon's at the 12th week (n=20)

Amount of M and S (gr kg^{-1})	*Total protein (g dL^{-1})*	*Triglyceride (mg dL^{-1})*	*Cortisol (ng mL^{-1})*	*Cholesterol (mg dL^{-1})*	*Glucose (mg mL^{-1})*
M0S0	4.29±0.34^{b}	613.08±284.85^{a}	49.70±4.32	35.27±3.22^{a}	122.19±14.39^{b}
M0S1	5.84±0.39^{a}	534.15±214.56^{b}	52.42±3.56	31.72±5.43^{a}	146.87±13.18^{a}
M1S0	4.51±0.12^{b}	563.61±184.15^{ab}	47.87±2.25	35.18±6.29^{a}	143.81±15.65^{a}
M1S1	5.97±0.57^{a}	514.47±192.08^{b}	51.43±3.17	28.14±4.38^{ab}	153.29±16.36^{a}
M5S5	6.37±0.23^{a}	506.23±164.54^{b}	52.54±2.78	19.56±3.65^{b}	139.57±14.49^{ab}

* Columns without letters show that there is no significant difference in that parameter.

Discussion. One of the primary purposes of aquaculture is producing food and restructure of stocks. The main goal of nutrition studies is to exchange fish food into flesh in a short time with profits and economic benefits (Hughes 1991). Results of the present study showed that, Macrogard and *Spirulina* improve growth and nutrition indices. About FCR, BWI, PER, W_2, and SGR there were significant differences among the studied treatments and about the other factors, despite the lack of significant differences, they improved them. Carcass raw protein of the fish fed by 1 and 5 g kg^{-1} Macrogard and *Spirulina*, was significantly more than the control group. These results are agreed with previously published reports. Staykov et al (2007) reported that, using MOS 0.2% in

rainbow trout (*Oncorhynchus mykiss*) diet increases body weight significantly and reduces FCR and mortality in comparison with control treatment. Torrecillas et al (2007) reported that when a suitable relationship exists between MOS and nutrition levels in European sea bass (*Dicentrarchus labrax*) fed by MOS in two levels: 2% and 4%, a significant increase happens in body weight and total length. Enrichment of live food like rotifer and *Artemia* with MOS 0.2% increases ability to tolerate stress caused by low salinity in cobia (*Rachycentron canadum*) larvae (Salze et al 2008). In another research, Andrews et al (2009) found that diet with MOS 1%, 2% and 4%, improves WG, SGR and FCR in fingerlings of Rohu (*Labeo rohita*).

Over a study, Li & Gatlin (2004) adding 1% and 2% prebiotic kind Grobiotic^{TM} AE and 1-2% prebiotic Brewer's yeast into diet of hybrid striped bass (*Morone chrysops* × *M. saxatilis*) with an average weight of 91.4 g, observed after 7 weeks that growth operation and nourishing and survival efficiency in groups fed by these supplementary increased significantly compared with control group. Mahious et al (2006b) studied on Siberian sturgeon (*Acipenser baeri*) and African catfish (*Clarias gariepinus*) and found that diets enriched by mentioned prebiotics, caused to improve growth; thus the special growth rate is more in Siberian sturgeon with experimental diet containing inulin and oligofructose compared with control group and about African catfish (*Clarias gariepinus*) they found that diets enriched by mentioned prebiotics caused to growth improvement; thus, special growth rate is more in Siberian sturgeon with experimental diets containing inulin and oligofructose than control group and in African catfish, the most of this index was in treatments fed by oligofructose, inulin and cellulose respectively. Diet containing 20 g kg^{-1} oligofructose, a fructooligosaccharid (FOS) produced by slight enzymatic hydrolysis with hot water extracted from roots of chicory, led to further growth in Turbot (*Psetta maxima*) fish larva. But only usage of inulin with amount of 20 g kg^{-1} did not have any effect on growth (Mahious et al 2006a). Growth, nourishing efficiency and survival rate improved in to experiments done on rainbow trout. These fish were fed by food containing 2 g kg^{-1} mannanoligosaccharide (Staykov et al 2007; Grisdale-Helland et al 2008). El-Sayed (1994) showed that using *Spirulina* (50% in diet) had a positive effect in growth of sea bass (*Rhabdosargus sarba*). If this algae is used in amount of 75%, growth will reduce significantly but FCR had no difference with the time common diets were used. But if usage of the algae is 100%, FCR will reduce in shrimp farms. A lot of additives are added into diet of shrimp, but *Spirulina* is the only macro algae with a lot of benefits for growth rate of shrimp. Using *Spirulina* had a significant effect on crayfish (*Macrobrachium rosenbergii*) growth and survival rate improvement (Nakagawa & Gomez-Diaz 1975). Existence of *Spirulina* in abalone (*Haliotis midae*) food increases its growth rate significantly compared with cases there is no *Spirulina* in the diet (Stott et al 2004).

In contrast to these studies, Pryor et al (2003) observed that there is no difference between final weight, fork length, CF, SGR and FCR in sturgeon *Acipenser oxyrinchus* fed by 0.3% MOS and fish of control group. Also Grisdale-Helland et al (2008) found in a study that with a diet of 1% MOS no difference will appear in food digestion and growth of Atlantic salmon (*Salmo salar*) and body protein reduction is obvious. Papp et al (1995) checked the effects of different levels of vitamin C (0, 100, 1000 and 2000 mg kg^{-1}) in hybrid sturgeon (*Acipenser ruthenus* × *A. baeri*) with the average weight of 11.9±2.1 g in the beginning of experiment. After 8 weeks of culture and in the average temperature of 22-23^{o}C, fish reach 5 times the initial weight (45-54 g) and no significant difference was observed. Akrami et al (2009) with replacement of inulin in levels of 1, 2 and 3% with cellulose for diet of control treatment in beluga sturgeon (*Huso huso*) reported that inulin had no positive effect on some growth and nutrition indices like final weight, special growth rate, protein efficiency ratio and FCR and stated that inulin is not a suitable supplement for rainbow trout. Food enrichment of Beluga (*Huso huso*) with 1, 2 and 3% inulin, showed a reverse relationship among some indices like WG, SGR, PER, ER, FE, PR and enrichment of inulin surface. Also, growth indices in fish fed by inulin were less than control group.

Studies carried out on rainbow trout by Cowey et al (1981) and channel catfish (Wilson et al 1984) showed that vitamin E supplement of diet had no effect on the weight gain.

Usage of prebiotics for improvement of growth indices in different species of fish requires more research to explain inconsistent results. Differences in results of this study with findings of scholars is likely related to cultured species, size, age of cultured species, production stage, period of adaptation and culture, health conditions of environment and culture system, feeding behaviors, physiologic features, raw materials used in diet preparation and their quality and quantity, different methods of adding inulin into diet (some mix inulin with oil and add it to commercial diets) and probably special microbial flora able to use inulin as substrate. Some others e.g. Ringo et al (2006) had replaced dextrin with inulin, Mahious et al (2006a, b) cellulose with inulin and Refstie et al (2006) extruded wheat with inulin. Sado et al (2008) reported that complexity in carbohydrate structure in cell wall of yeasts, different subspecies of yeasts, fermentation and preparation methods can be effective on results.

Biochemistry of fish blood is a suitable method for fish biology and pathology assessment. Numerous factors affect the amount of biochemical factors and electrolytes of blood serum and cause them to change. Some of these factors are: method of sampling and storage of samples, stress, fish sex, fish age, spawning season, water thermal changes, fish feeding method, water pH, life cycle and environment of fish, method of transfer the samples, using anesthetics or anti-clotting drugs. Fluctuations of biochemical indices of blood serum as biological indices, are affected by stressing and environmental factors like fishing, handling, transportation, maintenance, high density, physicochemical characteristics of water, method of fish feeding and diet compositions. Most biochemical indices of blood serum are very sensitive against these factors and their changes are dependent to amount of each factor (Stoskopf et al 1983).

Different studies are carried out by scholars to measure biochemical indices of fish blood in natural environmental conditions, stage of sexual maturity, sex, temperature of culture environment and contamination with pathogens in different species of fish. Sometimes, results shown positive effects and sometimes the reports on these factors are not to be effective on biochemical indices of blood. Morales et al (1990) found in a study on effects of handling stress on total biochemical indices of rainbow trout that, handling stress caused to increase amount of protein, glucose and cortisol of plasma after one hour and reach its peak after 2 hours. Also it was obvious that in juvenile of rainbow trout, viscosity of total protein of plasma is more in male compared with female (Rehulka et al 2005). This can be a sign of more protein metabolism in males.

Some researchers studied the effects of diet containing additives such as vitamins C and E on biochemical indices of blood serum in different fish species. Results of these studies in some cases, show positive and some times report these factors not to be effective on these indices; as Sealey & Gatlin (2002) in a research about effects of interaction of vitamins C and E of diet on these indices of striped bass (*Morone chrysops* × *M. saxatilis*) found that after 10 weeks of culturing, level of total protein of plasma is not affected by vitamin levels of diet and no significant interaction was observed between vitamins C and E.

Conclusions. Results of this study on cultured stellate sturgeon showed that the amount of total protein of blood serum, triglyceride, cholesterol and glucose in the 6^{th} week of culture did not have a significant difference between treatments. Continuing culturing process in 12^{th} week, a significant difference was observed in total protein of blood serum, triglyceride, blood cholesterol and glucose between different treatments. The least interaction of *Spirulina* and Macrogard was a positive factor on increasing the amount of these factors. High levels of these two materials had more effect on the amount of total protein. While about the amount of cortisol, there was no significant difference among treatments.

References

Akrami A., Hajimoradloo A., Matinfar A., Abedian Kinari A. M., 2009 Effect of dietary prebiotic inulin on growth performance, intestinal microflora, body composition and hematological performance of juvenile Beluga *Huso huso* (Linnaeus, 1758). Journal of the World Aquaculture Society 40:771-779.

Allain C. C., Poon L. S., Chan C. S., Richmand W., Fu P. C., 1974 Determination of total cholesterol by enzymatic method. Clinical Chemistry 20:470–475.

Andrews S. R., Sahu N. P., Pal A. K., Kumar S., 2009 Hematological modulation and growth of *Labeo rohita* fingerlings: effect of dietary mannan oligosaccharide, yeast extract, protein hydrolysate and chlorella. Aquaculture Research 41:61-69.

Annoni G. G., Bottasso R. M., Ciaci D., Donato M. F., Tripoli A., 1982 Clinical investigation of triglycerides. J Res Lab Med 9:115.

Association of Official Analytical Chemists, 1995 Official methods of analysis of AOAC International. 16th ed. AOAC International, Arlington, Virginia, USA,1298 pp.

Braham D., Trinder P., 1972 Methods for determination of blood glucose level by spectrophotometer. Analyst 97:142-143.

Cowey C. B., Adron J. W., Walton M. J., Murray J., Youngson A., Knox D., 1981 Tissue distribution, uptake and requirement for alpha-tocopherol of rainbow trout (*Salmo gairdneri*) fed diets with a minimal content of unsaturated fatty acids. Journal of Nutrition 111:1556–1567.

El-Sayed A. F. M., 1994 Evaluation of soybean meal, *Spirulina* meal and chicken offal meal as protein sources for silver seabream (*Rhabdosargus sarba*) fingerlings. Aquaculture 127:169-176.

Gibson G. R., Roberfroid M. B., 1995 Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition 125:1401-1412.

Grisdale-Helland B., Helland S. J., Gatlin D. M., 2008 The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of atlantic salmon (*Salmo salar*). Aquaculture 283:163-167.

Haghighi D. T., Saad C. R., Hosainzadeh Sahafi H., Mansouri D., 2009 The effect of dietary lipid level on the growth of kutum fry (*Rutilus frisii kutum*). Iranian Journal of Fisheries Sciences 8(1):13-24.

Hughes S. G., 1991 Use of lupine flour as a replacement for full-fat soy in diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 93:57-62.

Hung S. S. O., Lutes P. B., Conte F. S., Storebakken T., 1989 Growth and feed efficiency of white sturgeon (*Acipenser transmontanus*) subyearlings at different feeding rates. Aquaculture 80:147–153.

Hung S. S. O., Lutes P. B., Shqueir A. A., Conte F. S., 1993 Effect of feeding rate and water temperature on growth of juvenile white sturgeon (*Acipenser transmontanus*). Aquaculture 115:297-303.

Keyvan, A. 2003. Iranian sturgeons in the Caspian Sea (systematic, biology, artificial propagation, biomass evaluation and conservation, fishing and production of caviar). Iranian Fisheries Company, Tehran, 400 pp. [in Persian].

Li P., Gatlin D. M., 2004 Dietary brewers yeast and the prebiotic Grobiotic™AE influence growth performance, immune responses and resistance of hybrid striped bass (*Morone chrysops* × *M. saxatilis*) to *Streptococcus iniae* infection. Aquaculture 231:445-456.

Luo G., Xu J., Teng Y., Ding C., Yan B., 2010 Effects of dietary lipid levels on the growth, digestive enzyme, feed utilization and fatty acid composition of Japanese sea bass (*Lateolabrax japonicus*) reared in freshwater. Aquaculture Research 41:210-219.

Mahious A. S., Gatesoupe F. J., Hervi M., Metailler R., Ollevier F., 2006a Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, *Psetta maxima* (Linnaeus, C. 1758). Aquaculture International 14(3):219-229.

Mahious A. S., Van Loo J., Ollevier F., 2006b Impact of the prebiotics, inulin and oligofructose on microbial fermentation in the spiral valve of Siberian sturgeon *Acipenser baerii*. World Aquaculture Society, Florence, Italy, May 3–9, pp. 564-565.
Morales A. E., Garcia-Rejon L., de la Higuera M., 1990 Influence of handling and/or anaesthesia on stress response in rainbow trout: effect on liver primary metabolism. Comparative Biochemistry and Physiology 95A:87–93.
Nakagawa H., Gomes-Diaz G., 1975 Usefulness of *Spirulina* sp. meal as feed additive for giant freshwater prawn, *Macrobrachium rosenbergii*. Suisanzoshuku 43:521-526.
Papp Z. G., Jeney Z., Jeney G., 1995 Comparative studies on the effect of vitamin C feeding of European catfish (*Silurus glanis* L.) and sturgeon hybrid (*Acipenser ruthenus* L. × *Acipenser baeri* L.). Journal of Applied Ichthyology 11(3-4):372–374.
Pottinger T. G., Carrick T. R., 2001 Stress responsiveness affects dominant-subordinate relationships in rainbow trout. Hormones and Behavior 40:419-427.
Pryor G. S., Royes J. B., Chapman F. A., Miles R. D., 2003 Mannanoligosaccharides in fish nutrition: effects of dietary supplementation on growth and gastrointestinal villi structure in gulf of Mexico sturgeon. North American Journal of Aquaculture 65:106-111.
Refstie S., Bakke-McKellep A. M., Penn M. H., Sundby A., Shearer K. D., Krogdahl A., 2006 Capacity for digestive hydrolysis and amino acid absorption in Atlantic salmon (*Salmo salar*) fed diets with soybean meal or inulin with or without addition of antibiotics. Aquaculture 261(1):392-406.
Rehulka J., Minarik B., Adamec V., Rehulkova E., 2005 Investigation of physiological and pathological levels of total plasma in rainbow trout, *Oncorhynchus mykiss* Walbaum. Aquaculture Research 36(1):22-32.
Ringo E., Sperstad S., Myklebust R., Mayhew T. M., Olsen R. E., 2006 The effect of dietary inulin on bacteria associated with the hindgut of arctic charr (*Salvelinus alpines* L.). Aquaculture Research 37:891-897.
Ringo E., Olsen R. E., Gifstad T. O., Dalmo R. A., Amlund H., Hemre G. I., Bake A. M., 2010 Prebiotics in aquaculture: a review. Aquaculture Nutrition 16:117-136.
Sado R. Y., Bicudo A. L. J. D. A., Cyrino J. E. P., 2008 Feeding dietary mannan oligosaccharides to juvenile Nile tilapia *Oreochromis niloticus* has no effect on hematological parameters and showed decreased feed consumption. Journal of the World Aquaculture Society 39(6):821-826.
Salze G., McLean E., Schwarz M. H., Craig S. R., 2008 Dietary mannan oligosaccharide enhances salinity tolerance and gut development of larval cobia. Aquaculture 274:148-152.
Sealey W. M., Gatlin D. M., 2002 Dietary vitamin C and vitamin E interact to influence growth and tissue composition of juvenile hybrid striped bass *Morone chrysops* × *M. saxatilis* but have limited effects on immune responses. Journal of Nutrition 132:748–755.
Staykov Y., Spring P., Denev S., Sweetman J., 2007 Effect of mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). Aquaculture International 15:153-161.
Stoskopf M., Neely E., Mangold B., 1983 Avian hematology in clinical practice. Modern Veterinary Practice 64:713-717.
Stott A. E., Takeuchi T., Koike Y., 2004 Performance of a new artificial abalone hatchery culture system in terms of settlement of larvae and growth and survival of post-larvae *Haliotis discuss*. Fish Sci 70:1070-1081.
Teuscher A., Richterich P., 1971 Enzymatic method of glucose. Schweizerische Medizinische Wochenschrift 101:345-390.
Torrecillas S., Makol A., Caballero M. J., Montero D., Robaina L., Real F., Sweetman J., Tort L., Izquierdo M. S., 2007 Immune stimulation and improved infection resistance in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. Fish Shellfish Immunol 23:969-981.
Wilson R. P., Bowser P. R., Poe W. E., 1984 Dietary vitamin E requirement of fingerling channel catfish. Journal of Nutrition 114:2053–2058.

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Authors:
Ammar Salehi-Farsani, Department of Fisheries, Science and Research Branch, Islamic Azad University, Tehran, Iran, e-mail: ammar_1631@yahoo.com
Mehdi Soltani, Department of Fisheries, Science and Research Branch, Islamic Azad University, Tehran, Iran, e-mail: msoltani@ut.ac.ir
Abolghasem Kamali, Department of Fisheries, Science and Research Branch, Islamic Azad University, Tehran, Iran, e-mail: Kamali.abolghasem@gmail.com
Mehdi Shamsaie, Department of Fisheries, Science and Research Branch, Islamic Azad University, Tehran, Iran, e-mail: drshamsaie@gmail.com

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