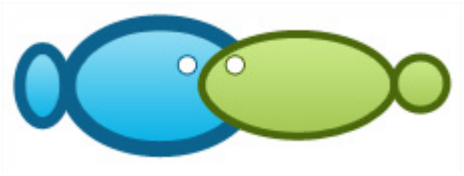

Effect of immune motivator Macrogard and *Spirulina platensis* on some hematological and immunophysiological parameters of stellate sturgeon *Acipenser stellatus*

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Abstract. This study was carried out to survey the effect of immune motivator Macrogard and *Spirulina platensis* on some hematological and immunophysiological parameters 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 specimens per 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. Haematological parameters including leucocyte count (WBC), erythrocyte count (RBC), haematocrit (HCT) and erythrocyte sedimentation rate (ESR) were measured. Significant differences were observed in the fish fed by Macrogard and *Spirulina* (together) compared to control treatment and treatments fed by Macrogard or *Spirulina* ($p < 0.05$). Obtained results showed that, Macrogard and *Spirulina* improve body and hematological indices and at the end of 12th weeks there were significant differences among these indices in the studied treatments. It can be concluded that, Macrogard and *Spirulina* can improve growth rate and improve some hematological and immunophysiological indices in stellate sturgeon. Also, using these two additives together in amount of 0.1% Macrogard and 0.5% *Spirulina* for this fish in this weight range and in similar conditions is evaluated to be positive.

Key Words: leucocyte count, prebiotic, erythrocyte count, haematocrit, treatment.

Introduction. Sturgeons are considered to be ‘‘living fossils’’ (Keyvan 2003). Primitive characteristics, such as a heterocercal tail and cartilaginous skeleton, have been maintained over approximately 100–200 million years, despite major environmental changes (Keyvan 2003; Baker et al 2005). 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 and eutrophication (Keyvan 2003; Baker et al 2005) caused their natural habitats to be limited (Hung et al 1989). The main stocks of these fishes are living in the Caspian Sea, Black Sea and Aral Sea basins (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 sturgeons flesh and caviar and their stock reduction in all natural habitats, artificial aquaculture was noticed by a lot of countries from many years ago (Keyvan 2003; Baker et al 2005).

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 (Gibson & Roberfroid 1995; Ringo et al 2010). *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 bluegreen 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 (El-Sayed 1994; Ringo et al 2010). 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 (El-Sayed 1994). 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 (El-Sayed 1994; Ringo et al 2010).

The present study was carried out to survey the effect of Macrogard and *Spirulina platensis* on some hematological and immunophysiological parameters 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, D3, E, B1, B2, B6, K3, 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.

Hematology. To estimate the effects of different diets on blood indices, before experiment, 20 stellate fish were caught randomly from the culturing tanks to measure hematological parameters including leucocyte count (WBC), erythrocyte count (RBC), hematocrit (HCT) and erythrocyte sedimentation rate (ESR). Also every 41 days (3 times totally) 2 fish were caught randomly from each tank to take blood samples. Blood samples were obtained through tail vein puncture and blood factors were measured using different experimental techniques in the laboratory. The blood samples were transferred to heparinized and nonheparinized tubes. In the laboratory blood was analyzed with routine method used in fish hematology (Blaxhall 1972). At the time of blood sampling, the appropriate smears were prepared for Giemsa staining. The smears were air-dried, fixed in 96% ethanol for 30 minutes and stained with Giemsa staining for 30 minutes. The smears were examined for leucocyte differential count under a compound microscope (Klontz 1994). The hematological parameters examined were erythrocyte count (RBC, $\times 10^{6}/mm^{3}$), hematocrit (HCT, %), ESR and leucocyte count (WBC $\times 10^{4}/mm^{3}$) and differential leucocyte count (Klontz 1994).

Serum biochemistry. 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. 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.

Statistical analysis. For analysis of all data SPSS version 17 and a software program of Excel 2007 were used. Biochemical data were analyzed with SPSS 17 for Windows using one-way analyses of variance (ANOVA) and significant means were subjected to a multiple comparison test (Duncan) at $p < 0.05$.

Results

Hematology. All the examined fish specimens were apparently healthy and there were no indications of infectious and parasitic diseases. Summaries of hematological values before feeding by experiment diets for 20 stellate sturgeon specimens shown in Table 3.

Table 3

Hematological profile of stellate sturgeon (mean ± SD) before feeding by experiment diets

WBC (n/mm^3)	*RBC* (n/mm^3)	*ESR* ($mm\ h^{-1}$)	*HCT* (%)	*Monocyte* (%)	*Lymphocyte* (%)	*Eosinophil* (%)	*Neutrophil* (%)
48200.78± 4120.23	1125631.54 ±71200.62	10.3± 0.098	26.34± 1.12	1.1± 0.5	66.50± 10.54	8.62± 1.45	22.95± 6.90

Summaries of hematological values in 6th weeks in young cultured stellate sturgeon with different levels of Macrogard and *Spirulina* are presented in Table 4.

Table 4

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

Haematological parameters	*M0S0*	*M0S1*	*M1S0*	*M1S1*	*M5S5*
WBC (n/mm^3)	46200.13± 4243.12	48800.35± 4025.48	49280.89± 5239.36	50200.67± 4774.79	54230.98± 5225.28
RBC (n/mm^3)	1065331.14± 61200.62	1085741.59 ±72200.62	1115653.13 ±5610.65	1195441.79 ±51200.62	1215322.34 ±71280.62
ESR ($mm\ h^{-1}$)	10.57±1.01	8.42±0.78	11.35±0.91	9.67±0.68	8.3±0.79
HCT (%)	24.41±0.78	25.12±1.78	23.79±2.08	21.43±1.06	22.76±1.53

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

According to these results, in 6th week in young cultured stellate sturgeon fed by different levels of Macrogard and *Spirulina*, the maximum WBC and RBC were in M5S5 treatment, the maximum ESR was in M1S0 and the maximum of HCT was observed in M0S1 treatment. The least amount of WBC and RBC were in M0S0, minimum of ESR and HCT were observed in M5S5 and M1S1 treatments, respectively. About these factors, there was no significant difference among different treatments ($p>0.05$).

Summaries of smear and flow cytometry WBC differential counts in 6th weeks in young cultured stellate sturgeon with different levels of Macrogard and *Spirulina* are presented in Table 5.

Table 5

Average of flow cytometry-microscope white blood cell (WBC) differential counts (average and standard deviation) obtained in 6th weeks in young cultured stellate sturgeon fed by different levels of Macrogard and *Spirulina*

Indices	*M0S0*	*M0S1*	*M1S0*	*M1S1*	*M5S5*
Monocyte (%)	1.61 ± 0.27^{a}	1.45 ± 0.56^{a}	1.51 ± 0.48^{a}	0.84 ± 0.33^{b}	0.79 ± 0.36^{b}
Lymphocyte(%)	68.65 ± 1.21^{b}	68.21 ± 2.32^{b}	66.43 ± 1.86^{b}	73.81 ± 1.08^{a}	76.98 ± 2.10^{a}
Eosinophil (%)	6.35 ± 1.21^{a}	5.05 ± 1.21^{a}	2.85 ± 1.21^{b}	4.41 ± 1.21^{ab}	2.65 ± 1.21^{b}
Neutrophil(%)	22.65 ± 0.91^{a}	21.84 ± 1.01^{ab}	23.41 ± 0.81^{a}	20.35 ± 1.41^{b}	21.03 ± 1.22^{ab}

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

According to comparing the smear and flow cytometry WBC differential counts, in 6th weeks in young cultured stellate sturgeon fed by different levels of Macrogard and *Spirulina* (Table 5), the maximum percentage of Monocyte and Eosinophil were in control treatment (M0S0), the maximum Lymphocyte (%) and Neutrophil (%) were in M1S0. Also the least percentage of Monocyte was in M5S5, minimum of Lymphocyte (%) in M1S0, minimum of Eosinophil (%) in M5S5 and minimum of Neutrophil (%) observed in M1S1. About these indices, there was significant difference among different treatments ($p < 0.05$).

Summaries of hematological values in 12th weeks in young cultured stellate sturgeon with different levels of Macrogard and *Spirulina* are presented in Table 6.

Table 6

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

Haematological parameters	*M0S0*	*M0S1*	*M1S0*	*M1S1*	*M5S5*
WBC (n/mm^3)	46763.13± 5213.89^{b}	48906.56± 4826.42^{b}	49280.21± 4235.65^{b}	50200.91± 5641.23^{ab}	54200.84± 4935.78^{a}
RBC (n/mm^3)	1065331.12± 54675.12	1085641.59 ±72200.65	1114621.89 ±56103.74	1115761.35 ±66137.98	1215842.76 ±51280.32
ESR ($mm\ h^{-1}$)	10.67±1.01^{a}	8.4±0.79^{ab}	11.36±0.91^{a}	9.21±0.68^{a}	6.32±0.79^{b}
HCT (%)	21.25±1.06^{b}	23.47±2.09^{ab}	22.29±1.53^{b}	24.31±0.78^{a}	25.12±1.18^{a}

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

According to these results, in 12th weeks in young cultured stellate sturgeon fed by different levels of Macrogard and *Spirulina*, the maximum WBC, RBC and HCT were in M5S5 treatment and the maximum ESR was observed in M1S0. Also the least amount of WBC and RBC were in M0S0, minimum of ESR in M5S5 and minimum of HCT observed in M1S1. About these factors, there were no significant differences between RBC and HCT treatments ($p > 0.05$), but there were significant differences between ESR and RBC treatments ($p < 0.05$)

Summaries of smear and flow cytometry WBC differential counts in 12th weeks in young cultured stellate sturgeon with different levels of Macrogard and *Spirulina* are presented in Table 7.

Table 7

Average of flow cytometry-microscope white blood cell (WBC) differential counts (average and standard deviation) obtained in 12th weeks in young cultured stellate sturgeon fed by different levels of Macrogard and *Spirulina*

Indices	*M0S0*	*M0S1*	*M1S0*	*M1S1*	*M5S5*
Monocyte (%)	2.81±0.27^{a}	1.55±0.56^{ab}	1.41±0.48^{ab}	0.74±0.23^{b}	0.22±0.16^{b}
Lymphocyte(%)	68.15±1.26^{b}	70.21±2.32^{b}	70.43±1.86^{ab}	73.81±1.08^{a}	75.08±2.10^{a}
Eosinophil (%)	7.35±1.22^{a}	7.95±1.21^{a}	8.85±1.21^{a}	1.41±1.21^{b}	1.05±0.71^{b}
Neutrophil(%)	21.55±0.91^{ab}	18.84±1.72^{b}	17.91±0.94^{b}	23.15±1.41^{a}	23.73±1.22^{a}

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

According to comparing the smear and flow cytometry WBC differential counts, in 12th weeks in young cultured stellate sturgeon fed by different levels of Macrogard and *Spirulina* (Table 7), the maximum percentage of Monocyte was in control treatment (M0S0), the maximum of Eosinophil (%) was in M1S0 and maximum of Lymphocyte (%) and Neutrophil (%) were in M5S5. Also the least percentage of Monocyte was in M5S5, minimum of Lymphocyte (%) in M0S0, minimum of Eosinophil (%) in M5S5 and minimum of Neutrophil (%) observed in M1S0. About these indices, there were significant differences among different treatments ($p < 0.05$).

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 (Papp et al 1995). Results of the present study showed that, Macrogard and *Spirulina* improve body and hematological indices. About differences between hematological values at the end of 6th weeks in young cultured stellate sturgeon with different levels of Macrogard and *Spirulina* there were no significant differences among the white blood cells in the studied treatments. However the maximum of white blood cells were observed in M5S5 and minimum of them were observed in control treatment. But at the end of 12th weeks in young cultured Stellate sturgeon with different levels of Macrogard and *Spirulina* there were significant differences between the white blood cells in M5S5 and control treatments. Also at the end of 12th weeks in young cultured Stellate sturgeon with different levels of Macrogard and

Spirulina there were significant differences among the leucocyte cells in the studied treatments. The maximum of white blood cells was observed in M5S5 and minimum of them were observed in control treatment. The percentage maximum of lymphocyte and neutrophil and the percentage minimum of monocyte and eosinophil were observed in M5S5, respectively. Also the percentage minimum of lymphocyte and neutrophil and the percentage maximum of monocyte and eosinophil were observed in control treatment. These results are agreed with previously published reports (e.g. Sado et al 2008). In this study in end of 6th weeks in young cultured Stellate sturgeon with different levels of Macrogard and *Spirulina* there were no significant differences in percentage of HCT among the studied treatments, but the percentage maximum of HCT was observed in M5S5 and the percentage minimum of it was observed in control treatment. However at the end of 12th weeks there were significant differences between percentage of HCT in M5S5 and control treatments.

Papp et al (1995) adding vitamin C into diet of hybrid *Acipenser* with an average weight of 11.9±2.1, observed after 8 weeks that also the least percentage of white blood cells were observed in control treatment.

Usage of prebiotics for improvement of hematological indices in different fish species requires more research to explain inconsistent results. Differences in results of this study with findings of scholars is likely related to the cultured species, size, age, production stage, period of adaptation and culture, health, environmental conditions and culture system, feeding behaviors, physiologic features, raw materials used in diet preparation and their quality and quantity (Ringo et al 2006; Refstie et al 2006; Sado et al 2008).

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 fish species. 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 rainbow trout, viscosity of total protein of plasma is more in male compared with the 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 plasma protein is not affected by diet vitamin levels and no significant interaction was observed between vitamins C and E.

Conclusions. Results of this study on cultured stellate sturgeon showed that using the amount of interaction of *Spirulina* and Macrogard in the 6th week of culture did not have a significant difference in hematological and immunophysiological parameters between treatments but in the 12th week of culture was observed a positive effect on some hematological and immunophysiological indices.

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