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Growth Performance, Survival and Immunostimulation, of Beluga (*Huso huso*) Juvenile Following Dietary Administration of Alginic Acid (Ergosan)

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Abstract: A 60-day feeding trial was conducted to examine the effect of dietary alginic acid on growth performance, survival and immunostimulation in beluga, *Huso huso* juvenile. alginic acid supplemented at 0, 2, 4 and 6 g kg^{-1} diet (diets A, B, C and D, respectively). Each diet was fed to triplicate groups of Beluga with initial body weight of 41.7±1.8 g at 10 days intervals (1-10th, 20-30th and 40-50th with non-supplemented diet and 10-20th, 30-40th and 50-60th with supplemented diet). Control group fed non-supplemented diet at total period of the experiment. Final weight, final length, specific growth rate (SGR), condition factor (CF) and percent of weight gain in the fish fed supplemented diets were significantly higher than the control group ($p<0.05$). Food conversion ratio (FCR) in the fish fed diets C and D were statistically better than the other treatments ($p<0.05$). Survival was not different among all dietary treatments ($p>0.05$). Also, use of Ergosan resulted in significance differences in lymphocyte percentage, while there were no statistically significant differences in hematocrit (Hct), monocytes and myelocyte percentages, hemoglobin (Hb) concentration, number of erythrocytes (RBC), total leukocytes (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Neutrophils and eosinophils percentages in the control group were higher than the fish fed supplemented diet. These results indicated that diet C (4 g kg^{-1} alginic acid) had the best effect and dietary administration of alginic acid affected on some growth and immune system parameters in great sturgeon, *Huso huso* juvenile.

Key words: Immunostimulation, humoral immune system, Alginic acid, *Huso huso*

Introduction

Immunostimulants are biological extracts and synthetic chemicals which stimulate the immune response by promoting phagocytic cell function, increasing their bactericidal activity and/or non-specific cytotoxic cells and antibody production (Sakai, 1999). Different kinds of substances are known to act as immunostimulants but only a few are suitable for use in aquaculture (Raa *et al*., 1992; Siwicki *et al*., 1998). Alginic acid is derived from several genera of brown algae including *Macrocystis*, *Laminaria*, *Lessonia*, *Ascophyllum*, *Alaria*, *Ecklonia*, *Eisenia*, *Neroecystis*, *Sargassum*, *Cystoseira* and *Fucus*. Algin is distributed throughout the intercellular mucilage and cell walls of the aforementioned algal species (Chapman and Chapman, 1980) and is extracted from either fresh or dried material.

In fish, alginic acid enhanced the activity of head kidney phagocytes and their migration in the site of alginate injection by increasing their production of chemotactic factors and their sensitivity to them (Dalmo and Seljelid, 1995; Fujiki and Yano, 1997). Alginates have been shown to enhance oxygen transference through the cellular membrane of fish lymphocytes and macrophages, increasing metabolic activity, resulting in improved disease resistance and an enhanced capacity for repair of injured tissues (Nüssler and Thompson, 1992), extracts from *Laminaria hyperborean* enhanced spreading, pinocytosis, intracellular production of superoxide anion and acid phosphatase activity in salmon (*Salmo salar*) head kidney macrophages (Dalmo and Seljelid, 1995). In carp (*Cyprinus carpio* L.), sodium alginate enhances the migration of head kidney phagocytes to the site of injection, whilst concomitantly enhancing their phagocytic activity. Moreover, sodium alginate stimulates peritoneal leucocytes to produce chemotactic factors, in addition to augmenting the sensitivity of head kidney phagocytes to such factors (Fujiki and Yano, 1997). With specific reference to alginic acid, Miles *et al*. (2001) demonstrated that Striped Snakehead (*Channa striata*) injected intraperitoneally with 0.5 mg alginic acid, suspended in PBS, resulted in immunostimulation. Intraperitoneal injection of alginic acid at doses $\geq$ 2.5 mg kg^{-1} raised the number of neutrophils, degree of phagocytosis and respiratory burst activity and expression of interleukins (IL-1β) and chemokines (IL-8), but had no effect on lysozyme and antiprotease activity over a 7 days time period (Peddie *et al*., 2002).

The sturgeons (Acipenseridae) are an ancient group of chondrostean Fishes with fossil records dating back to

the lower Jurassic period (Findeis, 1997). Unfortunately over fishing, degradation of habitat and spawning grounds along with environmental pollution have caused these species to be included in the IUCN Red List of endangered species and in 1997 all sturgeon fishes were included in the CITES Appendices. Beluga (*Huso huso*) is one of the most important species of sturgeon in Caspian sea. It is the largest species of Acipenseriforms reaching a length of size six meters and a weight of more than one tone (Berg, 1948). On the other hand owing to its fast grow capacities and high tolerance to adverse environmental condition is a valuable species for artificial rearing and the production of them for meat and caviar has been increasing recently in Iran (Bahmani, 1998).

Thus, the aim of this study was determination of growth performance, survival and cellular immunity system activity in beluga (*Huso huso*) juvenile fed dietary alginic acid.

Materials and Methods

Experimental diets: The formulation of the experimental diet is given in Table 1. Four diets were each supplemented with 0 (diet A), 2 (diet B), 4 (diet C) and 6 (diet D) g kg^{-1} dry diet of alginic acid (Schering-Plough Aquaculture, UK). Fish meal and soybean meal were used as sources of protein. Fish oil, soybean oil and lecithin were used as lipid source. Wheat and corn flour were used as carbohydrate source. The diets were prepared by thoroughly mixing the dry ingredients with oil and then adding cold water until a stiff dough resulted. This was placed into a grinder for through mixing and extruded through a 2.0 mm diameter strand. Food was stored at 4°C until used. Control diet was prepared in the same way without the addition of alginic acid. Analysis of the test diets showed in Table 2.

Experimental design: Beluga (*Huso huso*) juvenile with mean body weight of 41.7±1.8 g obtained from Shahid Marjani proliferation and culture center for Sturgeon fish, Gorgan, Iran. They were randomly distributed into 12 tanks at a density of 15 fish per tank (300 l), where three tanks were assigned to each diet. Each experimental diet was fed at feeding rates of 5 % fish weight per day. Feeding rate was adjusted to the actual fish biomass in each treatment. The fish were fed four times per day, 7 days per week, for 60 days and they fed at 10 days intervals (1-10th, 20-30th and 40-50th with non-supplemented diet and 10-20th, 30-40th and 50-60th with supplemented diet). Water of the experimental tanks replaced every 12 h to prevent accumulation of ammonia and other toxic metabolites and uneaten food were removed from the bottom. Supplemental aeration was also provided to maintain dissolved oxygen levels near saturation. Water temperature was 24/3±2.3°C during the feeding periods. Diurnal light/dark cycle was controlled at 13:11 h. Total fish weight in each tank was measured every 10 days for more accurate feeding rate adjustment.

Table 1: Ingredient composition (percent dry weight) of the experimental diets

Ingredients	(%)
Fish meal	450
Wheat flour	80
Corn flour	80
Soybean meal	200
Fish oil	100
Soybean oil	60
Lecithin	5
Vitamin mix	15
Mineral mix	10

Table 2: Composition analyze of the experimental diets

	Experimental diets			
Ingredients	A	B	C	D
Crud protein (%)	44.5	43.7	44.6	43.2
Crud lipid (%)	19.8	20.1	20.5	19.6
Ash (%)	13.4	13.9	13.7	13.1
Fiber (%)	2.2	2.3	2.5	2.3
Moisture (%)	8.7	8.9	9.2	9.1

Analysis: At the end of the feeding trial, all fish were weighed and percent weight gain (body weight gain×100/initial body weight), feed conversion ratio (dry feed fed/body weight gain), specific growth rate ({[Ln final weight-Ln initial weight]×100}/ days), condition factor ({[body weight/body length (cm^3)] × 100}) and percent of survival were calculated. To study the immunity system factors the blood was collected from the caudal vein of individual fish (six fish per each dietary treatment) employing heparinized syringes at the end of the experimental period. Determination of erythrocytes (RBC) and total leukocytes (WBC) was conducted according to Martins *et al.* (2004), hemoglobin (Hb) rate according to Collier (1944), hematocrit (Hct) according to Goldenfarb *et al.* (1971), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) according to Wintrobe (1934).

Statistical analysis: Data were analyzed by one-way analysis of variance (ANOVA). When ANOVA identified differences among groups, multiple comparisons among means were made with Duncan's new multiple range tests. All variances were checked for normality and homogeneity. All percentage values were transformed using arcsine transformation. Data are presented as treatment means±SD. The values of P less than 0.05 ($p<0.05$) were considered significantly different.

Results

Growth factors are summarized in Table 3. Addition of alginic acid to beluga diet led to statistically significant

Table 3: Response of beluga juvenile to the various test diets after 60 days of feeding

	Treatment			
Parameters	A	B	C	D
Initial weight (g fish^{-1})	41.4±2^{a}	41.6±2^{a}	42.2±1.8^{a}	41.6±1.7^{a}
Final weight (g fish^{-1})	135.2±7.7^{a}	144.4±5^{b}	156.2±6.4^{c}	154.7±6.6^{c}
Initial length (cm)	23.5±1.2^{a}	23.9±1.1^{a}	23.3±0.9^{a}	23.3±0.8^{a}
Final length (cm)	35.2±0.8^{a}	35.7±0.7^{b}	36.7±0.5^{c}	36.7±0.5^{c}
FCR[1]	2.21±.06^{a}	2.08±.08^{b}	1.82±.04^{c}	1.83±.07^{c}
SGR[2]	1.96±0.15^{a}	2.06±0.1^{ab}	2.18±0.12^{b}	2.18±0.13^{b}
W.g[3] (%)	226.6±5^{a}	247.2±21^{ab}	270.9±21^{b}	259.6±16^{ab}
CF[4]	0.29±0.02^{a}	0.3±0.01^{ab}	0.32±0.01^{b}	0.31±0.02^{b}
Survival	95.3±2.9^{a}	96.1±2.3^{a}	96.5±2.2^{a}	96.7±2.3^{a}

[1]Feed conversion ratio, [2]specific growth rate, [3]percent weight gain, [4]condition factor.
Note: Means in the same column with different superscripts are significantly different ($p<0.05$)

differences among the experimental groups in units of growth performance. Fish fed diets C and D had highest final weight, final length and weight gain ($p<0.05$). Food conversion ratio was significantly improved in the fish fed diets B, C and D than in the fish fed the diet A (control). The results in specific growth rate followed the same pattern as in the other growth performance indicators. There were no statistically differences between A and B groups in some of the growth parameters (Table 3). Condition factor in the Fish fed diets C and D was significantly higher than the fish fed the control diet but there was no comparable difference between group B and the other treatments in this parameter. Survival was not different among all dietary treatments ($p>0.05$).

Tables 4 and 5 show the statistics obtained in the analysis of variance of hematology of the fish at the end of the experiment. Adding alginic acid to the diet had no effect on hematocrit (Hct) percent, hemoglobin (Hb) concentration, number of erythrocytes (RBC), total leukocytes (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Conversely, supplementation with alginic acid caused an increase in Lymphocytes percent in groups B, C and D ($p<0.05$). The fish that received diet without alginic acid supplementation (0 g kg^{-1}) showed a higher number of Neutrophils. Myelocyte and Monocytes percentage exhibited no statistically different among all dietary treatments but number of Eosinophils in the fish fed diet A (control) was significantly higher than the fish fed supplemented diets.

Discussion

In this study, dietary administration of alginic acid significantly improved growth parameters in the fish fed supplemented diet. Growth factors were more favorable in the fish fed diets B, C and D than the fish fed diet A. Between diets containing of alginic acid, diet C had better influences on the growth factors, while survival was not different between dietary treatments at the end of the examination. In the case of crustaceans, incorporation of alginic acid into diets at a concentration of 0.5% (w/w) enhanced the growth of the shrimps (*Litopenaeus vannamei*) over the 15 day experimental period (Montero-Rocha *et al*., 2006). A similar effect has been reported using "kelps" which include the brown algae *Ascophyllum nodosum*, *Sargasumm* spp and *Laminaria digitata*, as dietary supplements (Cruz-Suarez *et al*., 2000). Peddie *et al.* (2005) demonstrated that dietary administration of alginic acid increased growth and survival of juvenile Chinook salmon (*Onchorhynchus tschawytscha*).Conversely, dietary yeast β-glucan (Macrogard) and alginic acid (Ergosan) had no effect on sea bass *Dicentrarchus labrax* (Bagni *et al*., 2005) and *Dentex dentex* (Efthimiou, 1996) growth performance.

The fish defense system is similar to that described in mammals. Leukocytes are one of the main parts of the cellular immunity system and fluctuation of them is increasingly used as indicators of stress response in fish (Stoskopf, 1993). In response to stressors in the aquatic environment, an overall drop in WBCs could indicate immunosuppression. An overall increase could mean infection or response to stressors (Adams, 2002). In the present study, supplementation with alginic acid did not have influence on hematocrit (Hct) percent, hemoglobin (Hb) concentration, number of erythrocytes (RBC), total leukocytes (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Of the humoral immune parameters measured, a higher number of lymphocytes were observed in the blood of fish that received alginic acid supplemented diets. On the other hand, number of neutrophils and eosinophils in the control group were higher than the fish fed supplemented diet. alginic acid exhibited no significant effect on the total number of Monocytes and Myelocyte compared to control group.

Dietary administration of alginic acid had a significant effect on serum complement activity in sea bass, *Dicentrarchus labrax* (Bagni *et al*., 2005). Also, Marino (2003) demonstrated immunomodulatory effect of alginic acid on non-specific immunity in sea bass under

Table 4: Statistics of the hematological data in beluga juvenile at the end of the feeding trial

	Treatments			
Variable	A	B	C	D
HCT (%)	20.5±3[a]	19.8±2.7[a]	21.6±2.6[a]	21.6±1.5[a]
Hb (%)	6±0.6[a]	5.7±0.9[a]	5.8±0.9[a]	6.1±0.5[a]
RBC ($\times 10^4$ mm^{-3})	65.5±10.7[a]	62.6±10.3[a]	64.6±6.1[a]	62±7[a]
WBC ($\times 10^3 mm^{-3}$)	21±2.2[a]	20±3.2[a]	22.5±2.1[a]	22±3.3[a]
MCV (fL)	314.9±37[a]	322.8±15[a]	338.3±57[a]	351.5±29[a]
MCH (pg)	93.7±11.2[a]	93.1±7[a]	91.3±16[a]	100.2±9.5[a]
MCHC (%)	29.3±1[a]	28.7±0.7[a]	27.6±7[a]	28.5±1[a]

Note: Means in the same column with different superscripts are significantly different ($p<0.05$)

Table 5: Statistics of the hematological data in beluga juvenile at the end of the feeding trial

	Variable				
Treatments	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Myelocyte (%)
A	15.6±6.4[a]	65.8±6.3[a]	0.5±0.5[a]	14.8±3.9[a]	3.1±2.7[a]
B	6.6±2.8[b]	80.6±3.8[b]	0.5±0.5[a]	9.8±3.1[b]	2.3±1.5[a]
C	8.8±2.5[b]	79.6±4.7[b]	0.5±0.5[a]	9.5±3.6[b]	1.6±1[a]
D	8.8±4.6[b]	79.3±3.2[b]	0.33±0.5[a]	9.5±3.7[b]	2±1.6[a]

Note: Means in the same column with different superscripts are significantly different ($p<0.05$)

stress. Intraperitoneal administration of both glucans and alginic acid has been shown to activate leucocyte responses that can represent, in turn, a prelude of multifaceted inflammatory-like immune response (Jorgensen *et al*., 1993) and (Peddie *et al*., 2002). Conversely, intraperitoneal injection of Ergosan did not elicit an immunostimulatory effect on humoral immune parameters (antiprotease, lysozyme and complement), whereas neutrophil migration into the peritoneal cavity, phagocytosis, respiratory burst and cytokine expression are increased (Peddie *et al*., 2002). Although the role of the neutrophils in inflammation is still a topic of debate (Dalmo *et al*., 1997), it is likely that they are a major player in phagocytic and respiratory burst mediated bactericidal activity. Typically, in the un-stimulated peritoneal cavity of *O. mykiss*, neutrophils are relatively uncommon, with macrophages the predominant leucocyte population present (Afonso *et al*., 1997). In the present study, the effect of alginic acid treatment did not show a statistical significance in some of the blood parameters. This seemed to be due to that were no physical, chemical or bacterial stresses in this study to more stimulation of humoral immunity. Nevertheless, Water temperature was slightly high (28°C), notably at the final days of the trial. High numbers of neutrophils in the control group might be due to this high temperature. The range of the heterophil or neutrophil numbers in fish blood is quite wide, but this cell type is usually not the predominant leukocyte in peripheral circulation (Stoskopf, 1992). An increase in WBC and neutrophil counts appeared directly or indirectly related to temperature. Fathead minnows, *Pimephales promelas,* were shown to have increased numbers of leukocytes during their spawning season during a time of increased temperatures, where the count tripled (Thomas *et al*., 1999). In stressed channel catfish *Ictalurus punctatus*, neutrophils approximated 30% of the circulating leukocytes and in non-stressed catfish levels were about 4% (Ellsaesser and Clem, 1986). In fish, an increase of neutrophils due to stress responses is frequently associated with a decreased overall leukocyte number (Slicher, 1961) and may be seen in instances of chronic stress (Adams, 2002). Numbers of the neutrophil cell type, in response to increased circulating cortisol, is often indicative of stressful conditions or infectious disease (Ellsaesser *et al*., 1985; Ellsaesser and Clem, 1986). Hybrid Russion sturgeon (*Acipenser gueldenstaedtii* x *Huso huso*) fingerlings fed increased dietary protein and lipid showed a distinct decrease in neutrophil numbers and an increase in lymphocytes (Gershanovich and Kiselev, 1993). Significant differences were found in the differential leukocyte counts between stellate sturgeon *Acipenser stellatus* and beluga *Huso huso* sturgeons at age ~200 days, where 68.0-73.5% were lymphocytes, 21.8-25.1% were neutrophils and 3.0-4.6 were eosinophils (Palikova *et al*., 1999). In 6-year-old Persian sturgeon *Acipenser persicus*, the leukocyte count was 10.3% with 20% neutrophils and in the 6-year-old beluga the leukocyte count was 7.9% with 33.9% neutrophils (Bahmani *et al*., 2001). Furthermore, number of eosinophils was higher in group A than the fish fed supplemented diets, whereas dietary alginic acid affected lymphocytes counts in fish fed supplemented diets. The study of leukocyte in sturgeons is in its first trials (Palikova *et al*., 1999) and with attention to the role of them in health of fish we need more studies on the fluctuation of leukocyte and other blood parameters and their characteristics in these important species. Based on the results obtained in this study it can be concluded

that dietary supplementation of alginic acid can affect on growth performance and some humoral immunity system in beluga (*Huso huso*) juvenile. Further studies of the mechanisms by which alginic acid induces immunostimulatory effects and whether this leads to increased disease resistance are needed in sturgeon.

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