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Effects of super supplements on growth and some physiological indices in farmed juvenile Siberian sturgeon (*Acipenser baerii*)

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Abstract
This study was carried out with the aim of determining the effects of dietary super supplements on some physiological and growth indices in juvenile Siberian sturgeon (*Acipenser baerii*). A total of 180 fish after two weeks of adaptation, were randomly distributed in 15 fiberglass tanks (2000 liters). 12 fish were introduced to each tank. Super supplements at 2.5%, 5% and 10% were added to the diet and experiments were performed in triplicate. Samples were collected for the measurement of growth, blood, immune and osmotic indices during the rearing period. The results showed that the average final weight, final biomass, body weight increasing (BWI), specific growth rate (SGR), condition factor (CF) and daily growth rate (ADG) showed significant difference between control group with treated ones ($P<0.05$). According to the results, the use of super supplement improved growth and immune indices, particularly in the treatment containing 5% super supplement and adding it is recommended to the diet of *A. baerii*.

Keywords: Super supplements, hematological parameters, immune system, growth, siberian sturgeon

1. Introduction
During the last ten years, antibiotics are commonly being used for the management of aquatic animal diseases, as well as for increase in growth and improvement of feed conversion ratio [11]. Antibiotics affected the natural flora in fish and increase their resistance to them. Moreover, antibiotics are chemical matters that negatively affect on the environment [22]. It is also possible to antibiotics residual accumulated in aquatic animals [13]. Details of history of organic aquaculture and its standards were presented for the first time in the world [9]. Meanwhile, the first model of organic farming in 1994 was recorded in Australia and Germany for carp. It is obvious that considering to the organic aquaculture in the Asian markets is developed more than other parts of the world in recent years which its reason is the high per capita consumption of seafood in Asia and its historical background. The commercial value of organic products is 1.7 to 4 times compared to the conventional products [27]. So, if Iranian producers are able to produce and export organic products, the value of agricultural exports will be 2 to 4 times. With the production and export of organic products the added value of products will increase.

Organic aquaculture includes components that ensure that aquaculture activities are compatible with the environment and organism is in health and wellness conditions, today organic products in the aquatic animals industry by super supplements has found a special place among consumers, the use of chemicals, hormones, antibiotics and brooders that are genetically modified, is incompatible with the principles of organic aquaculture [27]. Super Supplements are nutrients that consisting of fat-soluble vitamins (Vit. A, D_3, E, K), essential minerals: calcium, phosphorus, iron, copper, magnesium, manganese, cobalt, zinc, iodine, selenium, chlorine, sodium, potassium, growth promoters: betafin, choline, carnitine, coated vitamin C, coated micro-ionized proteins (these are indigestible proteins), essential amino acids: lysine, methionine, cysteine, threonine and essential fatty acids: linolenic, arachidonic and linolenic that are used in fish nutrition. One of the most important and reliable indicators of health status and physiology of fish is measuring of haematological, immunity and osmotic parameters that affected by nutrition, environmental factors, and etc. [12]. Siberian sturgeon can be easily adapted to cultured conditions, is resistant to environmental changes, has a high growth rate and easily use available food sources. Because of low maturity age and rapid caviar reaching, this species is used in many studies and is one of the important species that is used in the aquaculture industry. Siberian sturgeon is a sturgeon species that has a high growth rate and is very suitable for aquaculture, but little research has been done on its nutritional requirements. In this study, we have tried to evaluate the impact of super supplements on physiology and growth parameters of Siberian sturgeon. The results of present study leads to commercial diet and improves the culture conditions [31].

2. Materials and Methods

In this study, 180 *Acipenser baerii* (with mean weight 680.89 ± 29.93 gr and mean length 63.79 ± 1.18 cm) were provided from the International Sturgeon Research Institute. After two weeks adaptation, fish were randomly introduced to 15 fiberglass tanks with a volume of 2000 litres. Fish were fed with Skretting diet based on table1:

Table 1: Skretting diet analysis based on presented specifications by Skretting Company

Ingredients	Crude Protein	Crude lipid	Carbohydrate	Ash	Fiber	Moisture
Percent (%)	46	17	18	8.5	2.5	10

In addition, super supplement from ATA Company with table 2-2 analysis and Oxy-tetracycline antibiotic 20% from Aras Baran Company were used. This study was conducted in five treatments with triplicates, including control without antibiotic, control with antibiotic and super supplement at 2.5%, 5%, and 10% for 90 days and three times per day [7, 42].

Table 2: Analysis of raw materials of Super supplement (ATA Company)

Value	unit	Composition	Value	Unit	Composition	Value	unit	0
2000	mg	Copper (Cu)	12000	Mg	L-Carnitin	300000	IU	Vit.A
12000	mg	Ferrous (Fe)	12000	Mg	Betaein	500000	IU	Vit.D3
7000	mg	Zinc (Zn)	20000	Mg	Choline	1000	mg	Vit.E
100	mg	Selenium (Se)	1400	Kcal/kg	Multi enzyme	600	mg	Vit.K
200	mg	Iodine (I)	2000	Mg	Net Protein	500	mg	Vit.B1
30	mg	Cobalt (Co)	10000	Mg	Arginine	400	mg	Vit.B2
5000	mg	Natrium (Na)	12000	Mg	Cystine	900	mg	Vit.B3
3000	mg	Chlor (Cl)	12000	Mg	Methionine	1400	mg	Vit.B5
2000	mg	Phosphorus (P)	20000	Mg	Lysine	500	mg	Vit.B6
70000	mg	Calcium (Ca)	1400	Kcal/kg	Gross Energy	300	mg	Vit.B9
10000	mg	Anti-Oxidant	2000	Mg	Lionoleic acid	500	μg	Vit.B12
6000	Mg	Manganese (Mn)				500	mg	Vit.H2
6000	Mg	Magnesium (Mg)				5000	mg	Vit.Srey C

Evaluation of growth trend was calculated based on the available references of mathematical equations [8].

- (ADG) (g/fish/day) (%) = $[w_t - w_i / w_i \times T] \times 100$ Average daily growth
- Percent body weight increase (PBWI) (%) = $[w_t - w_i / w_i] \times 100$
- Specific growth rate (SGR) (day) = $[\mathrm{Ln}W_t - \mathrm{Ln}W_i / T] \times 100$
- Feed Conversion Ratio = Feed intake (g)/ $w_f - w_i$

During experiment the physical and chemical parameters of water (temperature, pH, dissolved oxygen and total hardness of water) were measured and recorded on a daily basis by a multimeter (WTW Germany) (table 3).

Table 3: Physical and chemical parameters of water during 90 days.

Parameters	Quantity
Temperature (°C)	15.73±0.45
pH	7.33±0.14
Dissolved oxygen (mg/l)	6.87 ±0.29
Water hardness (mg/l) $CaCO_3$	236 ±2.44

Blood samples were collected in the middle (45 days) and the end (90 days) of experiment period using a 2 ml syringe under the caudal fin. Plasma separated from blood cells by using a centrifuge (Labofuge, 200, manufactured by Heraeus Sepatech Germany) at 3000 rpm for 10 minutes [29]. Measurement of blood, immune and osmotic parameters was conducted in physiology and biochemistry Department of international Sturgeon Research Institute, Rasht. Counting of white blood cells (WBC) and red blood cells (RBC) was done by haemocytometer (Neubauer) [7, 30]. Blood smears were prepared for differential count of white blood cells. Smears were fixed with methanol and stained with Giemsa and cells were counted under a light microscope [26]. Haematocrit was measured by haematocrit method [16]. The level of haemoglobin was measured by colorimetric method using a spectrophotometer at a wavelength of 560 nm [6, 16]. Haematological parameters including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were obtained from the following equations [16].

MCV (fl) = (Hematocrit / RBC) × 10

MCH (pg) = (Hemoglobin / RBC) × 10

MCHC (g /dL) = (Hemoglobin / Hematocrit) × 100

Measurement of immunoglobulin M and lysozyme level was performed using the Elisa reader (Awareness, USA, model 2100 - Stat fax) and turbidimetric method. Pars Azmoon test kit and turbidity method used for the measurement of complement C_3 and C_4. Automatic osmometer (Nr.9610003.Type-13, manufactured by Roebling, Germany) was used for measuring serum osmolality. Sodium and potassium ions were measured by Flame Photometer (C405) of Faster Electric and measurement of calcium ion was done by Pars Azmoon kit and Autoanalyzer (RA1000 model). Experimental data were examined by normality test. To determine significant differences one-way analysis of variance (Anova) was used and for non-normal data Kruskal - Wallis was used. One-way analysis of variance and Kruskal –

Wallis were used to determine significant differences in blood parameters were normal. Statistical analysis and drawing graphs was done through software SPSS 16 and Excel 2010 [3].

3. Results

3.1. Growth parameters

The mean of initial weight, biomass, length, final length and FCR showed no significant difference between control and other treatments (*P*>0.05). The mean of final weight at concentration 5% and 10% was significantly higher than the others (*P*<0.05). The mean final biomass, mean body weight increasing (BWI%), specific growth rate (SGR), daily growth rate (DGR) and condition factor (CF) in super supplement 5% and control treatment without antibiotic was significantly more than other treatments (*P*<0.05) (table 4).

Table 4: Growth parameters of various treatments in Siberian sturgeon during experimental period.

Growth parameters	Control Without antibiotic	Control with antibiotic	Super supplement 2.5%	Super supplement 5 %	Super supplement 10 %
Initial weight (g)	680.89 ±29.93ª	692.97 ±32.10ª	596.17 ±19.84ª	667.56 ±19.84ª	668.44 ±4.17ª
Final weight (g)	1839.27 ±21.13ª	1935.23 ±17.48ªᵇ	1983.13 ±15.96ªᵇ	2034.78 ±30.87ᵇ	2077.30 ±20.88ᵇ
Initial Biomass (g)	8170.67 ±359.27 ª	8315.67 ±385.22 ª	7154 ±238.12 ª	8010.67 ±357.07ª	8021.33 ±50.03ª
Final Biomass (g)	16577.82 ±1853.75ᵇ	13855.27 ±724.32 ªᵇ	11472.07 ±85.14 ª	16837.45 ±1023.44 ᵇ	13309.45 ±131.89 ªᵇ
Initial length(cm)	63.79 ±1.18 ª	63.58 ±0.66 ª	61.38 ±0.37 ª	64.01 ±0.94 ª	63.83 ±0.26 ª
Final length (cm)	70.93 ±0.58 ª	71.20 ±0.15 ª	70.67 ±0.13 ª	71.47 ±0.92 ª	71.14 ±0.21 ª
BWI (%)	99.68 ±7.14 ᵇ	66.52 ±1.25 ª	60.64 ±4.27 ª	113.97 ±8.05 ᵇ	65.93 ±1.86 ª
FCR	1.71 ±0.49 ª	2.02 ±0.03 ª	2.29 ±0.14 ª	1.60 ±0.51 ª	2.02 ±0.08 ª
SGR (day)	0.73 ±0.05 ᵇ	0.57 ±0.008 ª	0.53 ±0.029 ª	0.79 ±0.03 ᵇ	0.56 ±0.01 ª
GR	7.78 ±0.24 ᵇ	5.13 ±0.31 ª	3.99 ±0.16 ª	8.17 ±0.08 ᵇ	4.89 ±0.03 ª
CF	0.71 ±0.04 ᵇ	0.59 ±0.007 ª	0.54 ±0.007 ª	0.73 ±0.004 ᵇ	0.54 ±0.01 ª

3.2. Haematological indices

There was a significant difference in the total number of white blood cells in the control group with other treatments except for super supplement 5% (*P*<0.05). In the first phase of culture period, white blood cell differential count showed no significant differences between number of lymphocytes, monocytes and eosinophils in control compared to the other treatments (*P*>0.05). There was a significant difference between the neutrophils in control and concentration 10% of super nutrient (*P*<0.05). There was a significant difference in the number of red blood cells between the control with other treatments except for super supplement 5% (*P*<0.05). Hematocrit showed a significant difference between control and other treatments (*P*<0.05). The highest haematocrit level was observed in the control group including antibiotic (*P*<0.05). No significant differences were observed between the control group with the other treatments in the amount of haemoglobin level and blood indices (indices (Mean Corpuscular Volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration) (*P*>0.05) (table 5).

Table 5: Results of hematological indices in the first culture phase of Siberian sturgeon.

Haematological indices	Control Without antibiotic	Control with antibiotic	Super supplement 2.5%	Super supplement 5%	Super supplement 10%
WBC (n/mm³)	5700 ±416.33 ª	6600±305.50 ªᵇ	8633.333 ±425.57 ᵇ	5800 ±818.53 ª	8533.333 ±1523.51 ᵇ
Lymphocyte (%)	72 ±0.57 ª	69.33 ±0.88 ª	67.66 ±1.85 ª	71.33 ±0.33 ª	65 ±2.64 ª
Neutrophil (%)	24.33 ±0.33 ª	26 ±0.57 ª	27.33 ±0.88 ª	24.33 ±0.33 ª	31±2.08 ᵇ
Eosinophil (%)	1 ±0.00 ª	0.66 ±0.66 ª	0.66 ±0.33 ª	0.66 ±0.66 ᵇ	2±1.00 ª
Monocyte (%)	2.66 ±0.66 ª	4 ±0.57 ª	4.33 ±0.88 ª	3.66 ±0.88 ª	3.66 ±0.88 ª
RBC (n/mm³)	424666.7 ±10 ªᵇ	447333.3 ±8089.77 ᵇ	431333.3 ±19632.74 ªᵇ	387333.3 ±13295.78 ª	435333.3 ±19376.39 ªᵇ
Hct (%)	27 ±0.57 ª	28.33 ±0.33 ᵇ	27 ±1.15 ªᵇ	24.33 ±0.88 ªᵇ	27.33 ±1.20 ªᵇ
Hb (gr/dl)	7.16 ±0.14 ª	7.40 ±0.05 ª	7.10 ±0.32 ª	6.43 ±0.26 ª	7.16 ±0.33 ª
MCV (fl)	635.33 ±1.45 ª	633.333 ±4.66 ª	625.66 ±1.76 ª	627.66 ±3.38 ª	627.66 ±0.88 ª
MCH (pg)	168.33 ±0.66 ª	165.33 ±1.66 ª	164.33 ±1.20 ª	166 ±1.15 ª	164.33 ±0.88 ª
MCHC (%)	26.33 ±0.33 ª	26 ±0.000 ª	26.33 ±0.33 ª	26.66 ±0.33 ª	26.00 ±0.00 ª

According to table 5, there is a significant difference between the total numbers of white blood cells in the control group with the other treatments exception of super supplement 2.5% (*P*<0.05). White blood cell differential count showed that there was no significant difference between number of lymphocytes, neutrophils, monocytes and eosinophils in control with other treatments (*P*>0.05). RBC showed no significant difference in control group and the other treatments (*P*>0.05). Significant differences were observed in the haematocrit level between control compared with the other treatments and the maximum level was observed in control with antibiotic and super supplement 2.5%, which was significantly more than the other treatments (*P*<0.05). No significant difference was observed in the amount of haemoglobin and other blood indices (Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC)) between the control with the other treatments (*P*>0.05) (table 6).

Table 6: Results of blood indices of Siberian sturgeon in the final culture phase.

Haematological indices	Control without antibiotic	Control with antibiotic	Super supplement 2.5%	Super supplement 5%	Super supplement 10%
WBC (n/mm^3)	5666.667 ±185.59 ^{a}	8200±519.61 ^{c}	5500 ±57.73 ^{a}	6833.333 ±491.03 ^{ab}	7433.333 ±949.26 ^{b}
Lymphocyte (%)	69.66 ±4.91 ^{a}	67.00 ±0.57 ^{a}	71.33 ±3.17 ^{a}	69.66 ±0.33 ^{a}	67.33 ±1.20 ^{a}
Neutrophil (%)	23.33 ±1.20 ^{a}	28.33 ±0.33 ^{a}	22.33 ±1.33 ^{a}	25.66 ±0.33 ^{a}	27.33±0.88 ^{a}
Eosinophil (%)	0.66 ±0.33 ^{a}	0.33 ±0.33 ^{a}	0.33 ±0.33 ^{a}	0.33 ±0.33 ^{a}	1.00±0.00 ^{a}
Monocyte (%)	3.00 ±0.57 ^{a}	4.66 ±0.33 ^{a}	2.66 ±0.66 ^{a}	4.33 ±0.33 ^{a}	4.66 ±0.33 ^{a}
RBC (n/mm^3)	412333.3 ±25075.44 ^{a}	41333.3 ±15452.44 ^{a}	435000 ±12858.2 ^{a}	408666.7 ±7535.10 ^{a}	38700 ±4932.88 ^{a}
Hct (%)	25.66 ±1.20 ^{ab}	27.33 ±0.88 ^{b}	28 ±1.15 ^{b}	26.33 ±0.88 ^{ab}	24.33 ±1.20 ^{a}
Hb (gr/dl)	6.66 ±0.44 ^{a}	6.76 ±0.27 ^{a}	7.00 ±0.20 ^{a}	6.53 ±0.12 ^{a}	6.13 ±0.14 ^{a}
MCV (fl)	623 ±9.29 ^{a}	661.66 ±3.28 ^{a}	643.66 ±6.17 ^{a}	643.66 ±7.62 ^{a}	626.33 ±13.64 ^{a}
MCH (pg)	161.66 ±0.88 ^{a}	163.33 ±1.20 ^{a}	159.66 ±2.18 ^{a}	159.66 ±0.66 ^{a}	157.66 ±1.20 ^{a}
MCHC (%)	26.72 ±0.57 ^{a}	24.66 ±0.33 ^{a}	25.00 ±0.57 ^{a}	24.66 ±0.33 ^{a}	25.33 ±0.33 ^{a}

As shown in table 7, the difference in the amount of immunoglobulin M was not significant in control with the other treatments with the exception of super supplement 5% treatment (*P*<0.05). C_3 showed significant difference in control with the other treatments (*P*<0.05), but significant differences were not observed in C_4 concentration between treatments (*P*>0.05). Difference was not significant in the amount of serum electrolytes and osmolality of Siberian sturgeon in the first culture phase between control group with any of the treatments (*P*>0.05) (table 7).

Table 7: Immune and osmotic indices in Siberian sturgeon at the first culture phase.

Immune and osmotic Super indices	Control without Super antibiotic	Control with antibiotic	Super supplement 2.5%	Super supplement 5 %	Super supplement 10 %
IgM (mg/dl)	23.66 ±1.45 ^{a}	35±2.08 ^{b}	31.33 ±6.88 ^{b}	22.33 ±2.96 ^{a}	43±5.67 ^{c}
Lysozyme (µg/ml)	22.66 ±1.20 ^{ab}	25.66 ±0.88 ^{ab}	22.66 ±2.90 ^{ab}	17.33 ±1.85 ^{a}	27.66 ±4.66 ^{b}
C_3 (µg/ml)	32.66 ±2.72 ^{b}	38.66 ±2.02 ^{c}	37.66 ±3.71 ^{c}	26.66 ±3.71 ^{a}	40.33±9.68 ^{d}
C_4 (µg/ml)	16.66 ±1.76 ^{a}	26 ±2.51 ^{a}	25.33 ±3.48 ^{a}	19 ±2.30 ^{a}	29±6.80 ^{a}
Ca^{2+} (µg/ml)	9 ±0.58 ^{a}	8.8 ±0.88 ^{a}	8.73 ±1.85 ^{a}	8.46 ±0.33 ^{a}	8.93 ±2.65 ^{a}
Na^+ (mg/dl)	126.33 ±1.20 ^{a}	135.67 ±1.20 ^{a}	127 ±1.15 ^{a}	128.67 ±0.33 ^{a}	130 ±0.58 ^{a}
K^+ (mg/dl)	2.6 ±0.12 ^{a}	2.87 ±0.03 ^{a}	2.63 ±0.20 ^{a}	3.03 ±0.09 ^{a}	3±0.1 ^{a}
Osmolarity (mOsml/l)	298 ±1.52 ^{a}	311 ±6.55 ^{a}	297 ±1.15 ^{a}	298 ±2.64 ^{a}	299.66 ±0.88 ^{a}

There was significant difference in IgM level between the treatment super supplement 5% with other treatments (*P*<0.05). At the final phase of culture, there are no significant differences in the amount of lysozyme and C_3 between control group and other treatments (*P*>0.05). There was significance difference in the amount of C_4 in control with the other treatments (*P*<0.05). Serum electrolytes and osmolality changes showed no significant difference between control with the other treatments at the final phase of culture (*P*>0.05) (table 8).

Table 8: Immune and osmotic indices of Siberian sturgeon in the final culture phase.

Immune and osmotic indices	Control without antibiotic	Control with antibiotic	Super supplement 2.5%	Super Supplement 5%	Super supplement 10%
IgM (mg/dl)	3.17 ^{a} ±30.33	4.58 ^{a}± 34	1.85 ^{a} ±30.66	5.03 ^{b} ±39	5.19 ^{a}±31
Lysozyme (µg/ml)	4.50 ^{a} ±34	1.52 ^{a} ±35	6.65 ^{a} ±32	5.23 ^{a} ±31.66	4.93 ^{a} ±34
C_3 (µg/ml)	3.17 ^{a} ±34.33	7.21 ^{a} ±44	1.76 ^{a} ±34.33	1.45 ^{a} ±30.66	3.84 ^{a}±40.33
C_4 (µg/ml)	1.45 ^{a} ±21.66	^{b} 5.03 ±33	2.02 ^{ab} ±24.66	3.28 ^{ab} ±28.66	3.05 ^{b}±34
Ca^{2+} (µg/ml)	4.91 ^{a} ±8.10	0.58 ^{a} ±7.7	3.18 ^{a} ±8.30	0.33 ^{a} ±7.7	1.20 ^{a} ±8.93
Na^+ (mg/dl)	1.20 ^{a} ±128.33	2.19 ^{a} ±127.33	2.60 ^{a} ±129.33	0.88 ^{a} ±129.33	0.89 ^{a} ±131.33
K^+ (mg/dl)	0.15 ^{a} ±2.50	0.14 ^{a} ±2.23	0.03 ^{a} ±2.03	0.09 ^{a} ±2.23	0.14 ^{a}±2.23
Osmolarity (mOsml/l)	1.52 ^{a} ±298	6.55 ^{a} ±311	1.15 ^{a} ±297	2.64 ^{a} ±298	0.88 ^{a} ±299.66

4. Discussion

In the present study, the most effect of super supplements on growth parameters such as mean final weight, final biomass, increased body weight (BW%), specific growth rate (SGR), daily growth rate (GR) and condition factor (CF) was observed in treatment containing super supplement 5%. In this study the duration of feeding storage was short. This also helps to keep the food quality which is of the basic standards in organic fish production [27]. studied the impact of organic culture with 2.5 and 5% super supplement on some growth parameters in Common Carp (*Cyprinus carpio*) and found that that average final weight, weight gain, condition factor, specific growth rate and average daily growth showed significant differences [27] (*P*<0.05).

The results of experiments for evaluation of different levels of betaine and methionine, as an attractant, in the growth parameters of juvenile beluga showed that adding supplementation in the diet causes increasing in weight, specific growth rate and condition factor [36]. Reported that simultaneous use of vitamin C and probiotic; *Bacillus subtilis* in Ruho (*Labeo rohita*) can significantly increase growth parameters. Positive role of mineral supplements has been proven in nutrition of aquatic animals to have a normal and healthy life; fish provide these minerals from food and water. In this study, an increase in growth parameters such as BWI %, SGR, GR and CF observed in the treatment super supplement 5% compared to other treatments, especially control with antibiotic may be due to increased food intake and improved digestion process because of various nutrients, especially vitamins and minerals in super supplement that stimulates growth in fish, probably due to a change in the composition of nutrients in the treatment super supplements 5%. Various factors such as the composition of nutrients in the diet, method of feeding, environmental factors such as water temperature and dissolved oxygen, fish size [17], and the combination of ingredients [33] affected feed conversion ratio (FCR).

Blood is one of the physiological parameters of aquatic animals as a fluid tissue and is one of the most important biological liquids and its composition is changed under influence of various physiological and pathological states [20, 32]. Blood parameters are indicators for determining the health status of aquatic animals [37]. Based on the obtained results in the first phase, the largest number of white blood cells observed in treatments including super supplements 2.5%, 10% and control with antibiotic. In the final phase of culture, the highest number of white blood cells was observed in the control with antibiotic and super supplements 5% and 10%. The number of red blood cells can have a significant effect on the balance of whole energy of the body [34].

In a study, the effect of super supplements on the blood and immune indices of grass carp (*Ctenopharyngodon idella*) was investigated and sampling carried out in four phases and found that the fish blood immunoglobulin were significantly different between treatments and control in the second and third phases of sampling [25]. Vitamins in super supplements such as vitamin C is one of natural anti-stress compounds in fish that has numerous roles [43], including the stimulation of the immune system component such as alternative complement pathway and serum lysozyme activity [39].

Studied the effect of different levels of vitamin supplement containing vitamins C and E on the immune parameters of Caspian trout, and reported an increase in immunoglobulin and lysozyme activity [35]. Investigated various doses of vitamin C on hybrid sturgeon (*A. ruthenus & A. baerii*) and concluded that lysozyme activity showed significant differences in fish were fed by different levels of vitamin C [18].

Therefore, vitamin C has a positive effect on blood serum lysozyme activity in fish. The changes in lysozyme as one of the most non-specific immune parameters is so special that will increase production of the enzyme lysozyme. Increasing in the production of the enzyme lysozyme is due to the presence of vitamin C in vitamin supplement. In the first phase of culture, there was a significant difference between the amount of lysozyme in the control group compared with super supplement 5 and 10%.

But at final phase of culture, significant differences were not observed in the amount of lysozyme between controls with other treatments. Level of C_3 has a significant difference in the control group with the exception of super supplement 5%, compared with other treatments. At the final phase of culture, significant differences were not observed in the level of C_3 in the control group and other treatments.

Level of C_4 in control group with the other treatments showed no significant difference, but there was a significant difference in the C_4 level in control group compared with other treatments but in the final phase of culture. Complements are the most important defense factors that are about 35 soluble proteins, which play a key role in the innate and adaptive immunity [10].

The most important biological functions of the complement system consists of removing microorganisms through participation and cooperation in the processes of phagocytosis, inflammatory response, clearing immune complexes, induction and enhancement of antibody responses [15, 21]. Many immunostimulants increased complement activity in the fish serum [24]. Changes in the serum complement to the protection of non-specific immune system in fish are very important and high levels of C_3 and C_4 indicated the health of the fish [41]. studied the simultaneous use of vitamin C and probiotic *Lactobacillus rhamnosus* on immune system of rainbow trout (*Oncorhynchus mykiss*) which increased the serum lysozyme activity and complements (C_3 and C_4) [38].

Osmotic indices influenced by environmental factors, especially dissolved oxygen, salinity, temperature and so on. Many of the fish showed interactions in order to maintain its generation by changing the environmental conditions [2]. Considering to the mentioned factors, Super supplements not affected on serum osmolarity and electrolyte indices. Investigated the effect of feeding ratio on some biochemical parameters of carp (*Cyprinus carpio*) fingerlings. The results of this study showed that the frequency and percentage of feeding had no effect on the level of serum calcium in carp fingerlings [23].

In general, it can be concluded from this study that the use of super supplement in the diet improves the considered parameters. Treatment super supplement 5% compared to treatment with antibiotic showed better growth and immunity. Therefore, the use of super supplement in the diet as an additive stimulates the growth and immune system. It is recommended that it should be used in combination with diet for Siberian sturgeon. With regards to the persistence of chemical substances, especially drugs and antibiotics in the bodies of farmed fish, it seems that it is essential to be considered the production of organic food products due to the importance of the food healthy and safety in the society.

5. References

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