ARAŞTIRMA MAKALESİ   **RESEARCH PAPER**

Determination of Reference Values of Some Hematological and Immunological Parameters in Healthy Russian Sturgeon (*Acipenser gueldenstaedtii*)

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Abstract: This study aims to determine the reference intervals of some hematological and immunological parameters in healthy Russian sturgeon (*Acipenser gueldenstaedtii* Brandt & Ratzeburg, 1833) blood. These parameters are important indicators of fish physiological status and fish health. In the study, reference ranges of erythrocyte, leukocyte, lymphocyte, monocyte, eosinophil, neutrophil, basophil, and hematocrit values were determined for hematological parameters. In terms of immunological parameters, the reference intervals of TNF-α (tumor necrosis factor-alpha), IFN-γ (interferon-gamma), IL-1β (interleukin 1 beta), IL-6 (interleukin 6) and IL-8 (interleukin 8) values were determined. The reference ranges we have detected; erythrocyte $0.77–1.23 \times 10^6/mm^3$, leukocyte $9.21–16.96 \times 10^3/mm^3$, lymphocyte 43.35–59.78%, monocyte 4.61–11.39%, eosinophil 3.27–6.52%, neutrophil 12.16–18.41%, hematocrit 22.14–28.63%, TNF-α 27.59–49.83 pg/ml, IFN-γ 66.25–79.34 pg/ml, IL-1β 1.08–1.76 pg/ml, IL-6 11.2–19.7 pg/ml and IL-8 31.4–52.7 pg/ml. It is thought that the hematological and immunological reference intervals detected in the Russian sturgeons which will contribute significantly to the determination of the health and physiological situation of these fish.

Keywords: *Acipenser gueldenstaedtii*, hematology, immunology, sturgeon.

Sağlıklı Rus Mersin Balıklarında (*Acipenser gueldenstaedtii*) Bazı Hematolojik ve İmmünolojik Parametrelerin Referans Değerlerinin Belirlenmesi

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Öz: Bu çalışmanın amacı, sağlıklı Rus mersin balığı (*Acipenser gueldenstaedtii* Brandt & Ratzeburg, 1833) kanında bazı hematolojik ve immünolojik parametrelerin referans aralıklarını belirlemektir. Bu parametreler balığın fizyolojik durumu ve balık sağlığının önemli göstergeleridir. Çalışmada, hematolojik parametreler için eritrosit, lökosit, lenfosit, monosit, eozinofil, nötrofil, bazofil ve hematokrit değerleri referans aralıkları belirlenmiştir. İmmünolojik parametreler açısından ise, TNF-α (tümör nekroz faktör alfa), IFN-γ (interferon gama), IL-1β (interlökin 1 beta), IL-6 (interlökin 6) ve IL-8 (interlökin 8) referans aralıkları belirlenmiştir. Belirlediğimiz referans aralıkları; eritrosit $0,77–1,23 \times 10^6/mm^3$, lökosit % $9,21–16,96 \times 10^3/mm^3$, lenfosit % 43,35–59,78, monosit % 4,61–11,39, eozinofil % 3,27–6,52, nötrofil % 12,16–18,41, hematocrit % 22,14–28,63, TNF-α 27,59–49,83 pg/ml, IFN-γ 66,25–79,34 pg/ml, IL-1β 1,08–1.76 pg/ml, IL-6 11,2–19,7 pg/ml ve IL-8 31,4–52,7 pg/ml. Rus mersin balıklarında tespit edilen hematolojik ve immünolojik referans aralıklarının, bu balıkların sağlık ve fizyolojik durumlarının belirlenmesine önemli katkı sağlayacağı düşünülmektedir.

Anahtar kelimeler: *Acipenser gueldenstaedtii*, hematoloji, immünoloji, mersin balığı.

INTRODUCTION

Sturgeon, whose evolutionary past goes back 100 million years ago, is the anadrome and potamodromous species located in the northern hemisphere region (Bahmani, 1998; Tavakoli et al., 2019). Wild sturgeon populations have decreased significantly due to factors such as urbanization, dams preventing migration routes, overfishing and water pollution. The Russian sturgeon (*Acipenser gueldenstaedtii*, also known as diamond or Danube sturgeon) has recently been among the important species in sturgeon aquaculture applications (Duman, 2019a). Sturgeon is famous for its caviar obtained from the mature but unfertilized eggs of the female fish from a biological point of view, and it is also a valuable fish in terms of meat as they are large-bodied. Therefore, sturgeon culture is increasing rapidly in the world and Turkey (Duman, 2019b). Research for sturgeon has generally focused on protection biology, ecology, and reproduction areas, while less effort has been spent on sturgeon physiology (Baker et al., 2005). Blood is an accessible component in the body fluid system and is examined to assess the physiological state of the creature (Houston & Carlile, 1997). Hematological studies are accepted as one of the important biological features in aquaculture (Bahmani, 1999). Hematological parameters have a significant act in detecting the physiological response of fish to environmental conditions, also these parameters are between the most important indicators of pesticides, metals and toxic compounds for living creatures in aquatic environments (Van Vuren, 1986; Nussey et al., 1995). Cytokines are infection inhibitors that play an act in natural immunity and the development of hematopoietic cells, they also stimulate the immune function of cells (Duman & Şahan, 2018). In sturgeon breeding, it is important to examine the quantitative properties in the blood as it provides a reliable index of physiological conditions, especially in the development of fish (Alyakrinskaya & Dolgova, 1984). In the monitoring of the impact of physiological or sublethal stress caused by exogenous or endogenous changes in fish, changes of some blood parameters are used often (Cataldi et al., 1998). In sturgeon breeding, it is becoming increasingly important to evolve new methods to exactly determine the health of the fish during the production cycles and to diagnose diseases early. Changes in hematological and immunological values are frequently indicative of changes in physiological status and are used routinely to determine the health of domestic and wild animals, but it cannot be used due to the lack of reference ranges in many fish species, with the inclusion of sturgeon (Knowles et al., 2006).

This study aim is to determine the reference ranges of some hematological and immunological parameters such as erythrocyte, leukocyte, lymphocyte, monocyte, eosinophil, neutrophil, hematocrit, IFN-γ, TNF-α, IL-1β, IL-6 and IL-8 in healthy Russian sturgeon (*Acipenser gueldenstaedtii* Brandt & Ratzeburg, 1833).

MATERIAL AND METHOD

The research was carried out in a commercial fish farm that breeding Russian sturgeon (*Acipenser gueldenstaedtii*) in the city of Adana in Turkey. In this study, the principles of the local ethics committee were complied with. The fish with an average weight of 3670 ± 295.2 g and a mean length of 82.4 ± 9.3 cm were stocked in three concrete ponds (30 fish/pond, stocking density 13.7 ± 0.52 kg/m^2, n = 90). Supplied water flow rate for concrete ponds was 14 L min^{-1} and ponds dimension was 2×4×1 m. The fish were subjected to macroscopic (external examination by the eye) and microscopic (parasitological and bacteriological examination) health screening before being taken into ponds. Also, during the experiment, water temperature, oxygen, and pH values were measured every morning and evening by using a YSI 6600 CTD multi-parameter. In the trial, sturgeon feed (no: 4.5) was used and fish were fed at 0.75% of live body weight four times a day (Chebanov & Galich, 2011). After the two-week adaptation period, the fish were analyzed. The fish were anesthetized with 20 mL/L quinaldin sulfate (Şahan et al., 2017) and 2 ml blood was drawn from the caudal vein of each fish using a heparinized syringe for hematological analyzes (Blaxhall & Daisley, 1973; Trenzado et al., 2003).

Erythrocyte and leucocyte cells were counted using Natt-Herrick solution and Thoma hemocytometer (Kocabatmaz & Ekingen, 1984). Cyan-methemoglobin and micro-hematocrit methods were used for Hemoglobin and hematocrit values (Kocabatmaz & Ekingen, 1984; Stolen et al., 1994). Peripheral blood smears were stained with a mixture of May-Grünwald and Giemsa subsequently, the percentage of lymphocyte, monocyte, neutrophil, and eosinophil cell types were identified using a microscope (Blaxhall, 1972; Fujimaki & Isoda, 1990). Catalog no. MBS702530 fish ELISA Kit for IFN-γ, catalog no. MBS024441 fish ELISA Kit for TNF-α, catalog no. MBS700230 fish ELISA Kit for IL-1β, catalog no. MBS702353 fish ELISA Kit for IL-6 and catalog no. MBS700055 fish ELISA Kit for IL-8 were used in the cytokine analyses. In these analyses, the enzyme-linked immunosorbent assay (ELISA) kits were used which suitable for fish and the results of TNF-α, IFN-γ, IL-1β, IL-6, and IL-8 cytokine levels were evaluated based on the principles of the double-antibody sandwich method (Voller et al., 1978). In the statistical analysis of the results, the non-parametric estimation method with the SPSS

statistical package program was used for the reference interval determination (Düzgüneş et al., 1983).

RESULTS AND DISCUSSION

In this study with healthy Russian sturgeon (*Acipenser gueldenstaedtii),* some hematological (such as erythrocyte, leukocyte, lymphocyte, monocyte, eosinophil, neutrophil, hematocrit), and immunological (such as IFN-γ, TNF-α, IL-1β, IL-6, IL-8) parameter levels were determined. Furthermore, basic data on this subject was created. In terms of hematological parameters, the mean erythrocyte, leukocyte, lymphocyte, monocyte, eosinophil, neutrophil, and hematocrit values were determined as $1.07 \times 10^6/mm^3$, $12.09 \times 10^3/mm^3$, 52.35%, 9.17%, 4.21%, 16.31%, and 26.48%, respectively. Also, in terms of immunological parameters, the mean IFN-γ, TNF-α, IL-1β, IL-6, and IL-8 values were determined as 70.95 pg/ml, 40.26 pg/ml, 1.57 pg/ml, 16.46 pg/ml and 44.58 pg/ml, respectively. Reference ranges of hematological analyzes are given in Table-1, also reference ranges of immunological analyzes are given in Table-2. The lowest and highest values of the measured water parameters (O_2, pH and temperature) are given in Table 3.

Table 1. Reference intervals of hematological parameters in Russian sturgeon *(Acipenser gueldenstaedtii).*

Hematological parameters	Reference intervals
Erythrocyte ($\times 10^6/mm^3$)	0.77 – 1.23
Leukocyte ($\times 10^3/mm^3$)	9.21 – 16.96
Hematocrit (%)	22.14 – 28.63
Lymphocyte (%)	43.35 – 59.78
Monocyte (%)	4.61 – 11.39
Eosinophil (%)	3.27 – 6.52
Neutrophil (%)	12.16 – 18.41

Table 2. Reference intervals of immunological parameters in Russian sturgeon (*Acipenser gueldenstaedtii*).

Immunological parameters	Reference intervals
TNF-α (pg/ml)	27.59 – 49.83
IFN-γ (pg/ml)	66.25 – 79.34
IL-1β (pg/ml)	1.08 – 1.76
IL-6 (pg/ml)	11.2 –19.7
IL-8 (pg/ml)	31.4 – 52.7

Table 3. In the measured water parameters, lowest and highest values of O_2, pH and water temperature.

O_2 (mg/L)	pH	Temperature (°C)
6.5 – 7.8	8.1 – 8.6	17.3 – 19.5

Sturgeon has a complex life cycle and can tolerate various environmental conditions with its strong morphological structure. However, almost all sturgeon species in the world are threatened and their generations have been extincting in today. Hematological and immunological parameters are an effective way to examine physiological and pathological changes in fish. Bahmani et al. (2001) investigated the hematological indices of Persian sturgeon (*Acipenser persicus*) and beluga (*Huso huso*) species in different growth stages (1, 2 and 6 years old).

They found that erythrocyte count is in the range of 495×10^3 - 735×10^3 cells ml^{-1}, leukocyte count is in the range of 31.6×10^3 - 57.7×10^3 cells ml^{-1}, the lymphocyte is in the range of 54.5 - 67.5%, neutrophil in the range of 22.63 - 33.86%, eosinophil in the range of 6.60 - 13.7% and monocyte in the range of 0.6 - 2.25% for the beluga species. They also found these values for Persian sturgeon as 240×10^3 - 452.5×10^3 cells ml^{-1}, 13.43×10^3 - 46.48×10^3 cells ml^{-1}, 73.25 - 82.70%, 12.3 - 20%, 2.25 - 6.5% and 0.2 - 2.5%, respectively. The erythrocyte, lymphocyte and eosinophil values for beluga were found approximately similar, leukocyte high, monocyte low to the values we detected in our study. For Persian sturgeon, leukocyte, neutrophil and eosinophil values were similar to our findings in our study. Besides, erythrocyte and monocyte values were low, and lymphocyte levels were higher than our study. In a study on hematological reference intervals for cultured shortnose sturgeon (*Acipenser brevirostrum*), was determined PCV as 26 - 46%, RBC count as 0.65 - $1.09 \times 10^6/\mu L$, WBC count as 28376 - 90789/μL, large lymphocytes as 2122 - 10435/μL, neutrophils as 3758 - 33592/μL, monocytes as 0 - 7137/μL and eosinophils as 0 - 1544/μL (the fish ranged from 3- to 5-years old and sexually immature) (Knowles et al., 2006). They reported that these reference intervals values will be beneficial for the early detection, diagnose and monitoring of disease in cultured shortnose sturgeon. Köksal et al. (1999) performed hematological examinations on Siberian sturgeon (*Acipenser baeri*), they found hematocrit value as 25.28 - 36.54%, erythrocyte count as 1.14 - $1.60 \times 10^6/\mu L$, leukocyte count as 19 - $23 \times 10^3/\mu L$, lymphocyte ratio as 93 - 95% and monocyte ratio as 1 - 2%. Additionally, Ghiasi et al. (2014) examined the hematological values to sterlet sturgeon (*Acipenser ruthenus*), In the control group it was noticed that RBC count as $1.32 \pm 0.05 \times 10^5$, WBC count as $62.3 \pm 7.5 \times 10^3$, Hct level as $27.0 \pm 1.3\%$, lymphocytes as $91.7 \pm 1.4\%$, neutrophils as $6.8 \pm 1.2\%$, eosinophils as $0.8 \pm 0.3\%$ and monocytes as 0.3 ± 0.1. Also, Bucur et al. (2009) evaluated hematological parameters of North American sturgeon (*Polyodon spathula*) in weighing between 200.4±40.28 - 1193.7±57.56 g cultured in ponds. They detected the hematocrit (Hct) value between 24.5 - 41.6% and the red blood cell count (RBC) between 1.046 - $2.252 \times 10^6/\mu L$. In another research, Docan et al. (2017) examined the hematological changes of healthy Russian sturgeon (*Acipenser gueldenstaedtii*) and they determined RBC count as $0.45\pm0.1 \times 10^6/mm^3$, also Hct value as 14.82±2.73%. In a different study, hematological parameters of *Huso huso* (5-month-old) in the environment where the environmental salinity was 0 ppt were investigated. Hematological parameters were found as erythrocyte count $86.0\pm7.5 \times 10^4/mm^3$, leukocyte count $18.2\pm2.8 \times 10^3/mm^3$, lymphocyte value $57.0 \pm 4.0\%$,

neutrophil value 28.6±3.7%, eosinophil value 13.3±2.5% and monocyte 1.0±1.0% (Zarejabad et al., 2010). In another study with beluga (*Huso huso*), Hoseinifar et al. (2011) were found erythrocyte count as 74.16±16.92 × 10^4/μL, leukocyte count as 20.18±0.99 × 10^3/μL, hematocrit value as 19.71±1.93%, lymphocyte value as 68.51±2.17%, neutrophil value as 21.96±2.49%, monocyte value as 4.66±1.02% and eosinophil value as 4.41±1.73%. In addition to Matsche et al. (2014) determined hematological parameters reference intervals of Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) sampled from three different regions as follows; RBC count (× 10^{12} cells/L) 0.90 - 1.24, hematocrit value (%) 21 - 26, WBC count (× 10^9 cells/L) 21.0 - 33.7, lymphocytes (× 10^9 cells/L) 12.5 - 22.7, neutrophils (x 10^9 cells/L) 1.6 - 7.9, monocytes (× 10^9 cells/L) 0.3 - 1.0 and eosinophils (× 10^9 cells/L) 0 - 4.6.

The hematocrit values mentioned above by Köksal et al. (1999), Knowles et al. (2006), Bucur et al. (2009), Ghiasi et al. (2014) and Matsche et al. (2014) were found similar to the value we found in our study. The erythrocyte count in our research is similar to the values reported by Köksal et al. (1999), Knowles et al. (2006), Bucur et al. (2009), Zarejabad et al. (2010), Hoseinifar et al. (2011). In terms of leukocyte count, Köksal et al. (1999), Zarejabad et al. (2010) and Hoseinifar et al. (2011) reported close results to our study. Also, Zarejabad et al. (2010) in terms of lymphocyte value and Hoseinifar et al. (2011) in terms of eosinophil and neutrophil value reported similar results to our study. The data similar to our study in terms of monocyte levels were stated by Knowles et al. (2006) and Hoseinifar et al. (2011).

Differences in hematological parameters of fish may be due to varying environmental factors such as changes in the physicochemical parameters of the water, season, water temperature, stock density and photoperiod. Furthermore, many other factors affect hematological parameters in fish such as age, gender, stress, the content of feed, maturity, hypoxia and disease (Affonso et al. 2002; Ruchin 2007; Bucur et al. 2009; Sadati et al. 2011; Nesrin et al. 2018; Duman 2019b; Verep & Yıldırım, 2020).

It has been reported that activated macrophages increase IFN-γ production which plays an important role in the immune system and also stimulates cytotoxic and suppressor T cells (Mishima et al., 2004). In some studies conducted by Yin et al., (1997), Tafalla et al., (2001) in different types of fish such as catfish (*Clarias gariepinus*) and turbot (*Scophthalmus maximus*), they found that the immune response occurred rapidly with increasing TNF-α levels. In a different study with sea bream (*Sparus aurata*), it has been noticed that in fish stimulated using concanavalin A (Con A) and phorbol myristate acetate (PMA), TNF-α activates macrophages against infection and increases respiratory activity, phagocytosis and nitric oxide production (Mulero & Meseguer, 1998). To increase the effectiveness of cells participating in the immune response; cytokines, synthesized by stimulated lymphocytes, monocytes and macrophages, and some other somatic cells, regulate immunity and events, including systemic response to cell growth, healing and injury. Bacterial antigens which especially affecting T lymphocytes, increase the release of cytokines such as IL-1, IL-6 and IL-8 (Akdoğan & Yöntem, 2018). Additionally, Laing et al. (2002) reported that interleukin-8 in rainbow trout (*Oncorhynchus mykiss*) has important effects on understanding and controlling the processes of inflammation and other immune functions. In fish, cytokines have a direct relationship with microbial interactions and the vital condition of the fish. Also, cytokines are one of the important indicators of non-specific immune response and they are considered to be important immune-activators involved in antigenic stimulation.

CONCLUSION

It is thought that hematological and immunological reference intervals are important in terms of early diagnosis, detection of diseases, treating diseases and reducing mortality in sturgeon culture. The first time to determine the reference values of these parameters is important, which are considered as indicators of fish health and stress fact for the Russian sturgeon. More research is recommended on the biology, physiology and development of these fish, which are very valuable due to their caviar.

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