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COMPARATIVE STUDIES ON LEUCOCYTES OF SOME FRESHWATER FISH SPECIES

Bazı Tatlı Su Balığı Türlerinin Lökositleri Üzerinde Karşılaştırmalı Çalışmalar

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Özet : Balık kanının bileşimi, balıkların normal ve patolojik biyolojilerinin kesin bir şekilde anlaşılması bakımından temel oluşturduğu için, gelecekte hematoloji balık hastalıklarının teşhis edilmesinde önemli bir yer alacaktır. Bundan dolayı, çalışmada Türkiye'de ekonomik değeri olan beş tatlı su balığı türünün (Gökkuşağı alabalığı, Yayın, Tatlı Su Kefali Aynalı ve Pullu Sazan) lökosit formülü ve lökosit tiplerinin indentifikasyonları yapıldı.

Lökositlerin ayırımında ve oranlarının belirlenmesinde May Grünwald - Giemsa karışık boyama yöntemi kullanıldı .Lökositlerin çekirdekleri normal olarak hazırlanan Giemsa boyası ile yeteri kadar boyanmadıklarından, boya hazırlanırken her ml distile suya 1.5 damla Giemsa ana mahlül ilave edildi.

Her lökosit tipinin ortalama yüzdeleri ile ayırımları balık türleri için ayrı ayrı yapıldı.

Bu araştırma makalesinde verilen bazı balıklara ait lökosit oranları ile resimlerinin, karşılaştırmalı hematoloji ile uğraşanlar açısından, tatlı su balıklarının kan değerlerinin daha iyi tanınmasında yardımcı olacağı sonucuna varıldı.

Summary : Since the composition of fish blood is fundamental to a comprehensive understanding of the normal and pathological biology of fish, haematology will play an important role in the diagnosis of fish diseases in the future. Therefore, economically important some freshwater

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fish species (Salmo gairdneri Rich., Silurus glanis L., Cyprinus carpio L. and Leuciscus cephalus L.) in Turkey have been studied for identification of leucocyte types and leucocyte formula.

May Grünwald - Giemsa mixed stain was used for the differential leucocyte count and identification of leucocytes of fish species. Since, the nucleus of leucocytes was not stained sufficiently with Giemsa stain, 1.5 drops of concentrated Giemsa stain were aded in 1 ml of distilled water.

The mean percentage and the identification of each type of leucocyte present was calculated for each species.

It is concluted that, the differential leucocyte count and their pictures of some fishes given in this paper will help the comparative haematologist to understand better the circulating blood values of freshwater hishes.

Introduction

Knowlodge of the composition of fish blood is fundamental to a comprehensive understanding of the normal and pathological biology and biochemistry of fish. Such information is particularly important to interprete diseases during their early stages and other environmental factors. For these reasons, fish blood is further studied for toxicological understanding and enviromental monitoring using fish, and for determining possible indicators of physiological changes in fishery management and disease investigations (29).

The analysis and differentiation of leucocytes have wide application in human haematological studies providing indices of metabolic abnormalities and disease conditions. In fish, the significance of blood cells and their biological function has not been widely investigated yet. Workers disagree on the indentification of different leucocytes largely because of the difference in number and morphology of these cells between species and even within a species.

In the future, haematology will have an important place in the diagnosis of fish diseases. To establish a reliable diagnostic blood for identifying systematic and metabolic dysfunctions more information is needed.

This study was undertaken to present the normal leucocyte formula of five freshwater fish species. Rainbow trout, European catfish, scaly carp, mirror carp and chub were chosen because they have a world wide distribution and they have important economical values in almost every part of the world.

The categorisation of fish leucocytes has let to contradictory reports. Leucocytes respond to various stressors, including infections and chemical irritantes (30). Many workers reported that the enviromental conditions such as age, sex, seanson, diet and temperature could effect the numbers of circulating leucocytes (10, 11, 19, 31, 35). Ellis (11), stated that the different interpretations by scientists have resulted from the use of different methods of preparing the blood smears.

The morphology of the blood cells of various species of fish has been given in detail by many authors (11, 18). Molnar (28), Antipova (1), Ellis (10), Kocabatmaz and Ekingen (21, 22) reported leucocyte number of various fish species. McCarthy et al. (27), gave the percentages of thrombocytes as 1 - 6 % (1.8 % mean) in rainbow trout. Wedemeyer et al. (37), found that the number of thrombocytes increases in fish that are stressed or injured. Casillas and Smith (4), found an average of 21.000 thrombocytes per microlitre of blood in trout prior to stress and 43.000 in minutes after a short periot of stress.

Antipova (1), reported that neutrophils and eosinophils are in insignificant quantities and infrequantly, neutrophiles are found only in the one - year - old carps (0 - 0.32 %). Ellis (11) reported this, if true, is an exceptional case. The number of circulating neutrophils reported in fishes vary in considerable ranges though they comprise a smaller proportion than in mammals due to the greater proportion lymphocytes and the presence of thrombocytes in fish blood (11). Kocabatmaz and Ekingen (21) gave neutrophiles range as 17 - 66 % (34.7 % mean) for European catfish and 2 - 22 % (9.7 % mean) for rainbow trout.

Basophils filled with granules were not found in any of the blood smear examined (23). As with eosinophils, reports on the presence of basophils in the blood of fishes vary (11). Drzawina (8) reported the rare presence of orthobasophilic granulocytes in the blood of a few species of the 68 samples studied. Basophil leucocytes have been reported in goldfish (36, 38). Loewenthal (26), described their relative proportion amongest blood leucocytes as 9 % in Cyprinus carpio. Haider (14), also described them in the blood of carp, but Hines and Yashouv (17) and Hines and Spiral (17) failed to find basophils in the Israeli strain of this species. Some workers have not been able to find basophils in the blood of perch (40), plaice (12), brown trout (3), rainbow trout (20, 21), European catfish (21, 22) and Channel catfish (3). In tilapia, basophils were occasionally seen in the blood smears (12).

The entire literatures concerning eosinophils in fish are contradictory and have claims over their presence or absence in many fish species.

Eosinophils have usually been reported to be rare in the fish blood and many of the description of eosinophils in teleosts refer to cells found in the haemogostic tissues (11, 15), Yuki (41), Klontz (20) reported that eosinophils are absent in the blood of rainbow trout. Hines and Yashou (17), reported that hey are absent in the Israeli strain of carp. Durand (9) has not found this cell in the blood of any of the freshwater fish he examined. Blaxhall and Daisley (2) did not found them in the blood of brown trout. Antipova (1), found them in two - year - old carp. Lester and Daniels (24) reported that eosinophils are rare or absent from the blood of many teleosts. Loewenthall (25), claimed that comprised 8 % of the total leucocyte population. Chiller et al. (6) and Ellis (11) have observed them in rainbow trout. Cannon et al. (3) have not seen them in the blood of Channel catfish.

Antipova (1) gave the percentages of eosinophils in the blood of mirror carp as 0 - 0.10 %; Kocabatmaz and Ekingen (21) found 0 - 1 % in European catfish and did not find any in rainbow trout.

The literature is extremely confused on the presence of monocytes in fishes. Many studies on tleost blood indicate the absence of these cells (2, 5, 7, 15, 32, 36). Yuki (41) described blood monocytes in rainbow trout. Weinreb (38) reported the presence of blood macrophages in normal rainbow trout under experimental conditions of inflammation. Williams and Warner (39), Ferguson (13) and Ellis (10, 12) desribed monocytes in the blood of various tleosts. The difference of monocytes and lymphocytes were discribed by Cannon et al. (3) who worked with ligt and ultrastructural study.

Antipova (1) found the percentages of monocytes as 0.65 - 28.00 % in mirror carp, Kocabatmaz and Ekingen (21), reported their percentages as 1 - 9 % (3.7 % mean) for Silurus glanis and 0 - 6 % (3 % mean) for Salmogairdneri. According to Cannon et al. (3), in Channel catfish, their relative numbers per 100 white blood cells is 8.

Although, lymphocytes acconted for most of the cell population (3), the number of these cells in blood can vary between individuals at a single species according to the conditions under which the sample of blood is taken viz on the physiological conditions of the fish (20).

McCarthy et al. (27), found their percentages as 85 - 99 % (mean: 93.5 %) in rainbow trout, Kocabatmaz and Ekingen (21) 76 - 91 % (87.3) in rainbow trout and 32 - 81 % (61.0) in European catfish, Antipova (1) 61.6 - 99.15 % in mirror carp.

Materials and Methads

Freshwater fishes used in this experiment were: Rainbow trout (Salmo gairdneri Rich.), European catfish (Silurus glanis L.), scaly and mirror carp (Cyprinus carpio L.) and chub (Leuciscus cephalus L.). Each time three fish samples brought to the laboratory from the Fisheries Research Station, The School of Fisheries. Fırat University and kept in a bucket containing 50 litres of water to minimize the stress. The blood sample was taken through the cardiac puncture (21) and collected in a tube containing EDTA, Then blood smears were obtained. Ten slides were prepared from each blood sample and then were stained with May Grünwald - Giemsa mixed stain method (33). We added 1.5 drops of concentrated Giemsa stain for 1 ml of distilled water.

The classification and percentages of cell were determinated under light microscope from four hundred blood cells of each blood sample. The identification of cells was made according to the pictures of Ezzat et al. (12) and the the photographs were taken by trinocular research microscope.

Results and Discussion

Classifications and percentages of leucocytes from five freshwater fish species used in the study are shown in Table 1. Comparative pictures of cell types from these fishes are shown in Plate 1.

As Ellis (11) stated, the environmental and physiological factors are known to effect many parameters of the blood and such factors should be considered in a haematological study. However, reports lack in information concerninig these factors so that comparison with the results of other workers is difficult.

Many workers have confused lymphocytes and thrombocytes in fish and some reports have apparently ignored the presence of thrombocytes. The difficulty in distinguishing lymphocytes and thrombocytes in smear preperations may be overcome by disregarding the stress in fish during sampling, only those smears where the thrombocytes are preserved in the spiked or oval form should be used for determining the proportion of thrombocytes to lymphocytes. Lone nucleus types are virtually impossible to distinguish from the dissorted lymphocytes (11).

Weinreb (38) found that the lymphocyte predominant neutrophils were scarce and eosinophils and basophils only occassionally seen in rainbow trout. Although, Steucke and Schoettger (34) were unable to detect any differences in cell counts in blood taken from different parts

of the fish, Lester and Budd (23) reported, in counting the blood cells there were several potential sources of error. Blood samples taken from different regions of circulatory system gave different result. For these reasons Cannon et al. (3) have some questions on; «whether small lymphocytes can be distinguished from thrombocytes, if do teleost have neutrophils (heterophils) or eosinophils, basophils and monocytes, whether heterophils and eosinophils can be distinguished.»

These studies showed that fish own all these types of cell but because of the numerous enviromental and physiological factors it is quite difficult to detect and see all of them in one fish. Their numbers also alter in circulatory blood.

Table 1. Leucocyte formula (%) in various freshwater fishes

Fish species	Lymphocyte	Monocyte	Neutrophil	Eosinophil	Basophil
Salmo gairdneri	62.4	1.3	36.3	—	—
Mirror carp	88.8	0.6	9.8	0.8	—
Scaly carp	86.7	0.9	11.5	0.7	0.2
Silurus glanis	85.1	0.9	14.0	—	—
Leuciscus cephalus	64.0	1.2	32.3	0.4	2.1

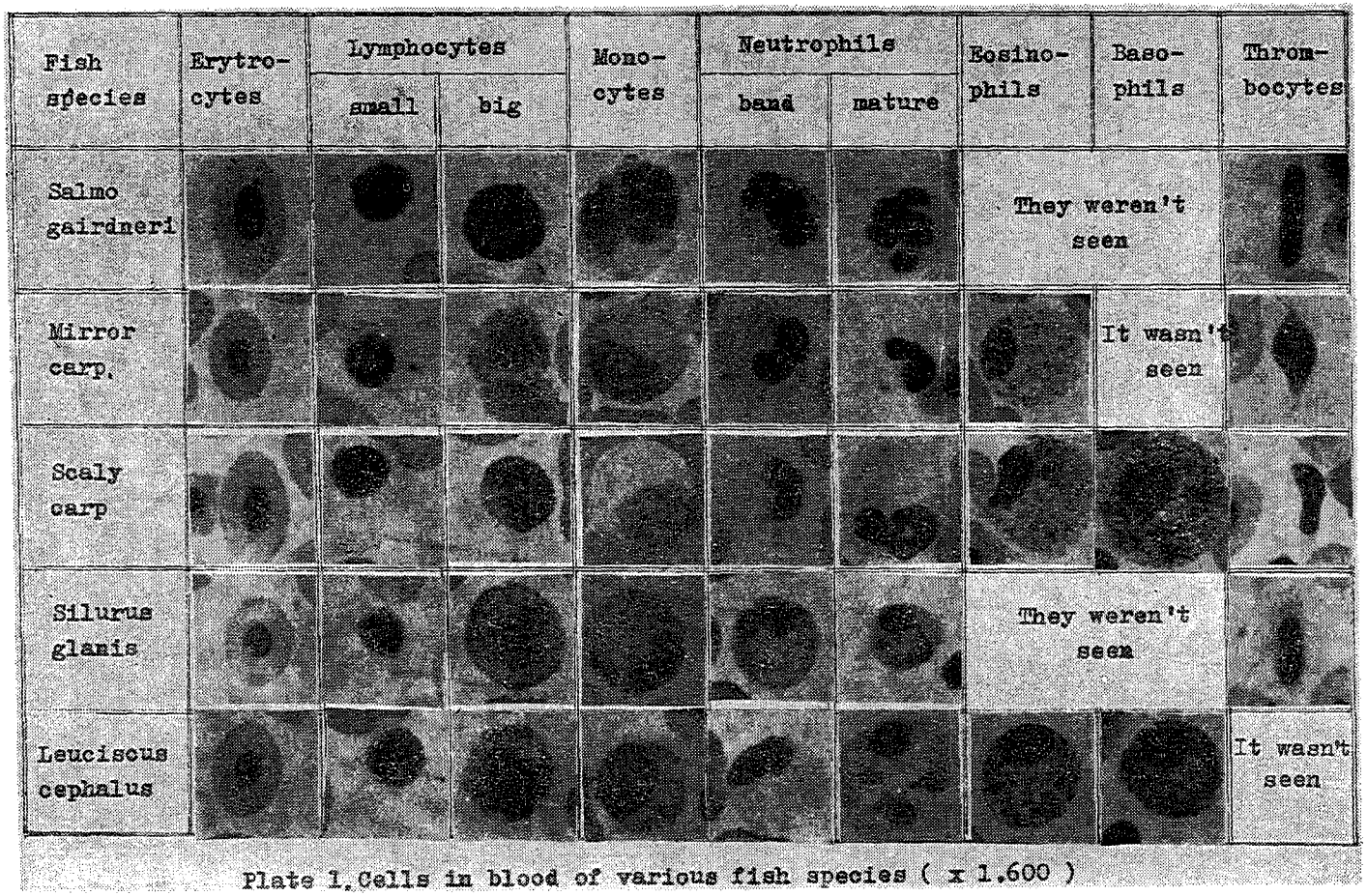

Plate 1. Cells in blood of various fish species (x 1.600)

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