

RESEARCH ON DETERMINATION OF CIRCULATING IMMUNE COMPLEXES IN THE BLOOD OF THE HIGH ECONOMIC VALUE FISH FARMED SPECIES IN ROMANIA

CERCETĂRI CU PRIVIRE LA DETERMINAREA COMPLEXELOR IMUNE CIRCULANTE ÎN SÂNGE LA PEȘTII DE CULTURĂ CU VALOARE ECONOMICĂ MARE DIN ROMÂNIA

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The range of paraclinical investigations applied in ichthyopathology aims at assessing those parameters that can define the pathological modifications and the physiological condition of the fish material, as well as the defense reaction of the unhealthy body. Modification in value of these indicators points out some metabolic perturbations in fish body. Fish have an immunitary system whose complexity and efficiency are directly proportional to their evolution level. That is why, in comparison to the superior vertebrates, the immune reactions of fish body to an antigenic attack are lower, the immune response is weaker and slower, and the quantity of antibodies to form is low. Under the influence of a specific antigen, the immunofforming cells synthetize the corresponding antibodies, determining occurrence of a specific antigen-antibody reaction, resulting in occurrence of the Ag-Ac immune complexes having a role in annihilating and destroying the respective antigens. Formation of immune complexes (CI) is a normal physiological process within the humoral immunity of bodies, representing one of the methods to remove from the body the substances identified as non-self.

Key words: Immune complexes (CI), immunity, antigen, antibody.

Introduction

The range of paraclinical investigations applied in ichthyopathology aims at assessing those parameters that can define the pathological modifications and the physiological condition of the fish material, as well as the defense reaction of the unhealthy body. Modification in value of these indicators points out some metabolic perturbations in fish body due to improper application of technologic norms, to being taken ill as a result of the attack of some pathogenic agents, to the harmfulness of the fish environment, to making use of a food with deficiency in the

vitamino-mineral and the protein level content, to the stress caused by repeated and incorrect handlings and to others.

Materials and Methods

From the methods to reveal *in vitro* the Ag-Ac reaction, the most applied one is the precipitate reaction. As a result of unifying the antigen with the antibody in liquid environment, immune complexes are formed that precipitate when the two reactants are found in certain proportions. The immune complexes that due to their characteristics enter and remain in the blood circulation are named **circulating immune complexes (CIC)**.

CIC are represented by small CI, formed in excess of antigen. Normally, the renal filtration is efficient for CIC, but when their number is highly increased due to the massive invasion of pathogens, they can deposit in the vascular walls and in the perivascular spaces, initiating or maintaining, at this level, different inflammatory processes.

The circulating immune complexes, due to their big size, can be precipitated through large molecular weight polymers, such as polyethyleneglicol (PEG), even when in small concentrations, this characteristic being the basis of the method of precipitation of CIC with PEG.

Principle: Polyethyleneglicol (PEG) can precipitate proteinic molecules found in solution depending on size, molecular mass and concentration.

In practice, use is made of PEG with molecular mass 6000 and with a concentration in the final mixture of 2.5-3.75%, area in which a selective precipitation of CIC is carried out.

Reagents: a) Boric pad (TB) with pH = 8.4
b) Solution PEG 6000 3.75%

Synonyms for PEG: Carbowax 6000; Macrogola 6000; Polidiol 6000.

Apparatus: Spekol with wave length of 450 nm, in vat of 1 cm, or any other analyzer having this wave length.

Working procedure:

Reagents	Specimen (P)	Martor (M)
PEG 3,75%	1,9 ml	-
TB pH = 8,4	-	1,9 ml
Serum	0,1 ml	0,1 ml
Stirring		
Incubation 24 hours in refrigerator (P and M)		
Reading (P against M) at Spekol on the pe wave length of 450 nm		

Calculation: $D = E_x \times 1000$, where:

D = the optical density of specimen in UF (photometric units)

E_x = extinction

Results and Discussions

From the specialized literature, it is known that the titre of antibodies present in blood is directly proportional to the number of circulating immune complexes being formed. This is why, while the concentration of antigen (the pathogenic agent that determined creation of antibodies in the body) is increased in circulation, the formed immune complexes become more and more numerous. The value registered upon determining CIC is directly proportional to the level of infestation of the body and it represents an important indicator of immunity.

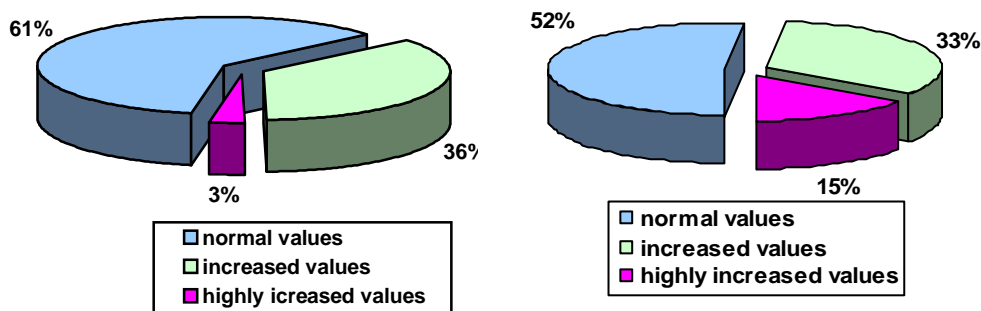
According to the results obtained, the normal values were considered to be comprised in the interval **1-100 UF**. Pathogenic values are met in a series of so called diseases by circulating immune complexes or as a secondary result of a disease. The clinic consequences are the edema, the tissue destructions, the inflammations and the hemorrhagic wounds.

From the pathogenic processes with increased values of CIC, the most important ones are the infectious and the parasitic.

The determinations made on fish individuals from fish cultured species revealed normal, increased and highly increased values of CIC (graph no.1).

At Pleaşa Fish Farm, from the total number of examined fish individuals, 61% registered values comprised in the interval of normal values, 36% presented increased values and 3% presented highly increased values (graph no.1.a).

At the Research and Development Centre Brateş, from the total number of examined fish individuals, 52% registered values comprised in the interval of normal values, 33% registered increased values and 15% presented highly increased values, especially the carp individuals that presented at the macroscopic clinic examination a high level of infestation with protozoans and bacteriosis in different stages (graph no.1.b).

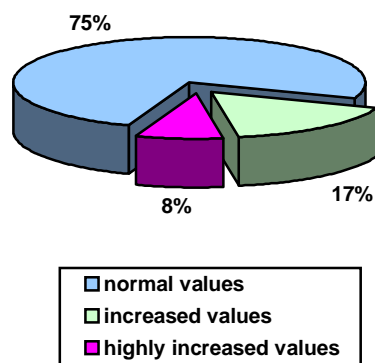


a) Ferma Pleaşa

b) Baza Cercetare Dezvoltare Brateş

Graph no.1 Representation in percentage of the results registered upon determination of CIC at carp species (*Cyprinus carpio*)

In case of the sturgeons under examination, from the total number of fish individuals, 75% registered values comprised in the interval of normal values, 17% registered increased values and 8% presented highly increased values (graph no.2), especially the fish individuals having had low total proteins, below 2 g/dl, albumins below 21.6% and the ratio albumins/globulins below 0.28.



Graph no.2 Representation in percentage of the results registered upon determination of CIC at sturgeons

Conclusions

1. The titre of antibodies present in blood is directly proportional to the number of circulating immune complexes being formed. This is why, while the concentration of antigen is increased in circulation, the formed immune complexes become more and more numerous.

2. In case of fish that presented wounds on the body surface and other external marks of disease and that had low biochemical indicators (level of proteins below 2 mg/dl, glycemy below 40 mg/dl and the ratio A/G below 0.30), indicating a disease, values of the circulating immune complexes (CIC) were registered, expressed in UF, 3-4 times higher than the values considered as normal.

3. The existence of CI indicates the presence of an immune response or the past of an immune response, because CI represents an efficient method to remove the antigen from the body or it demonstrates inefficiency of removing the antigen, especially in case of a long term existence of CI.

4. The value that is registered upon determination of CIC is directly proportional to the level of infestation of the body and it represents an important indicator of immunity.

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