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PRELIMINARY HEMATOLOGIC STUDIES ON *POLYODON SPATHULA* (WALBAUM, 1792) REARED IN CONTROLLED SYSTEM

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A preliminary morphological and Biochemical study was carried out the North American sturgeon blood, Polyodon Spathula (Walbaun, 1972), brought to Romania starting with 1992 until1999, for the Fish Culture Research Development Center, Nucet-Dambovita, situated on the Ilfov Brook, the Targoviste plane surface. The study was carried out during the years of 1995-1996 and 2007, on 1 and 2 years ald Polyodon Spathula, originated from the USA waters, also on descendents (obtain by artificial breeding on CCDP-Nucet), reared in ponds on polyculture with cyprinids. The researches relieved the mean domain of variability for the hematological parameter studies as follows: (Hb) hemoglobin 3.8 – 9.9 g/dl; (Hct) haematocrit 24.5 – 41.6 %; (RBC) red blood cell count 1.046 – 2.252 x 10^6/µl; (TSP) total serum proteins 1.24 – 3.7 g/100 ml; and glycemia 7.5 – 19.96 mg/100 ml.

Keywords: Polyodon spathula, sturgeon, hematologic indexes, physiological state, pararasites, ponds.

1. Introduction

Considering that Romania has large areas of equipped fishy, and the North American sturgeon *Polyodon spathula* is a large size fish, feeding exclusively on natural food, this event is of a special interest not only for aquaculture studies (taking into account the economic advantage standpoint), but also for the food industry as such, being able to offer a fairly cheap, ecologic, higher quality and healthy-safety food-product.

Polyodon spathula (Walbaum, 1792) – paddlefish – Order *Acipenseriformes*, Family *Polyodontidae*, is a freshy water primitive sturgeon originated from North America, a plankton feeder, mainly on zooplankton, aquatic terrestrial insects (Ruelle & Hudson, 1977; Rosen & Hales, 1981), chironomidae and grate quantities of vegetal scrap (Melcencov, 1991) but also phytoplankton, organic detritus and plant seeds (Zoology of vertebrates collective – prof. dr. Lotus Mester, Biology Faculty of Bucharest University, (Stoicescu, 1995). Can reach a total length of (TL) – 2.2 m and a body mass of 91 kg, (Cech, 2004) the most common body mass being of 4.5 – 7 kg. Sexual maturity is reached at 10 to 13 years (Graham, 1986).

On CCDP Nucet, a preliminary hematological study began, in 1995, regarding the determination of some biochemical indexes necessary for the establishment of sanguine metabolic profile on *Polyodon spathula* species, known the fact that the changes, on that lavel, act as a high sensitivity sensor as far as the integrity of environmental conditions are concerned, especially of nutritional status of cultured fishes as well as for wild ones (Molnar, 1970). The changes of the hematological indices depend on the fish species, age and cycle of sexual maturity and well as their health condition (Blaxhall, 1972). Moreover, the hematological tests and the analyses of the serum constituents have shown useful information on the detection and diagnosis of any metabolic disturbance/diseases in fishes species standing as limiting factors of fishy production, (Aldrin,1982; Jamalzadeh, 2009) braking their growth

rhythm and depreciating the food value and the commercial appearence, not in the least mass mortality.

Any disturbance of environmental condition, extent or duration of certain activities, quantity and quality of food availability included, may became a fish stress factor, diagnosed as such studying the fish main hematological (index) value.

Such were our hematological researches in 1995 that came to complete the biological, morphological and ethological fish species studies. The hematological investigations were also continued in 2007, the biological material for own study being this time represented by the progeny acquired by artificial breeding, on CCDP Nucet.

Polyodon spathula$_1$ (second summer) and *Polyodon spathula$_2$* (third summer), were reared in ponds, on polyculture with cyprinids. The hematological researches were made on three great moments: after wintering time, in full rearing season (in summer) and in autumn. A comparative appreciation of following hematological indexes was made: Hb, Hct, RBC, PST and glycemia.

The erythrocyte constant MCV, MCH and MCHC afford relations of extension on form and charge with hemoglobin of red cells. They allow determining the morphological type (normocyte, macrocyte and microcyte anemia) and chromic type (normochrom, hyperchrom and hypochrom anemia) providing useful indications regarding the etiological diagnosis.

Knowing the sanguine physiological constancies, the research aimed to determine the reacting and growth capacity of polyodon species in the new habitat, as reflected by its body weigh, waist and external appearance (attributes that ultimately define the commercial appearance), associated with detection alternations occurred in hematological structure under the normal level or any other pathological conditions correlative with stocking formulations.

2. Material and Methods

In the year 1995, after wintering, at the stocking of rearing ponds with *Polyodon spathula$_1$*, a number of 17 exemplaries (of the 1994 brood stock generation) have been taken for hematological investigations and the level of (Hb), (Hct) and (RBC) was determined, correlated with the fish leucocytes formula. The low quantity of blood sampled in spring didn't allow to make the blood biochemical examination namely PST determination and glycemia index.

On account of fair weather conditions, *Polyodon spathula$_1$* could has been retained in the wintering pond until 1995 may the 16th, when it was stocked in two experimental variants, in the rearing ponds:

- *Polyodon spathula$_1$* (300 ex/ha) with cyprinid breeders (*Cyprinus carpio, Hypophthalmichthys molitrix* and *Ctenopharyngodon idella*), in pond no. 4 Nucet (5 ha);
- *Polyodon spathula$_1$* (300 ex/ha), with cyprinids (*C.carpio, H. molitrixi* and *Ct. idella,* all at the age of 1 year), in pond no. 5 (2 ha).

The hematological investigations continued after the stocking of polyodon in RP4 Nucet, making other two samples batches (the 3rd of August, 1995 and the 9th of October), extracted from population exemplaries of a weight under the mean body mass so that they could be killed. From the polyodon reared in RP5 Cazaci, the blood was sampled only at the end of the rearing season on the 24th of May, 1995.

The investigations for determination of the blood metabolic profile, on the same *Polyodon spathula$_2$* species, continued during the year on 1996 in May on the 15th after the wintering time, in full rearing season of the moth of July on the 23th day and in the autumn of the same year, on the 25th day of October.

Four year of age exemplaries of *Polyodon spathula$_2$* species were stocked at a density of 110 ex/ha in a 5 ha pond (RP4 Nucet) with *C. carpio, Aristhichthys nobilis, H. molitrix, Ct. idella*.

In 2007 year, after 12 years, the same hematological study was carried out on both ages samples: *P. spathula$_1$* and *P. spathula$_2$*.

Polyodon spathula$_1$ was stocked

The hematological investigations carried out in the spring of 2007, on the 29th of March, in the summer of the same year, 2007, on the 26th of July and in month of December, on the 19th, regarded the exemplaries species stocked on density of 250 ex/ha in BR2 Marata (0.4 ha) with cyprinids: *C. caripo*$_2$ and *Ct. idella*$_5$.

The same methods of investigation and technics of sampling were being used for all the ex[perimental days on *Polyodon spathula*$_2$ stocked with *C. carpio*$_1$, *H. molitrix*$_1$ and *Ct. idella*$_6$ in BR3 Cazaci (0.5 ha), on density of 80ex/ha.

For all experimental dais have been used the same methods and techniques of samplingand hematological investigation. Blood sampling was carried out by puncture of caudal artery, without anesthesia of fish (Svobodova, 1991). The EDTA anticoagulant was used, 5 mg/ 1 ml of blood was used to prevent blood coagulation (Blaxhall, 1973).

Erythrocyte count was made in Goreaev counting camera, on the microscope MC5 IOR (object glass 20 x 0.4 and eyeglass 7), using as a diluting solution the Cosma fluid (Parvu, 1984) for the samples dealt with in 1995, while for those dealt with in 2007, the Hendrik's solution was used. Dilution of sample was 1/200, the result being expressed as x 10^6/µl of bload.

Hemoglobin (Hb) was determined by Sahli method; the result being expressed as g/100 ml of blood. Haematocrit (Hct) was also determined by centrifugal action of 3.000 r.p.m. for 30 minutes, represented as percentage (%)

On the basis of the parameters values: Hb, Hct and RBC (Blaxhall, 1973) by calculation, the erythrocyte constants MCV, MCH and MCHC have been determined, providing connections on largeness, form and red blood cell hemoglobin loading.

- Mean corpuscular volume (μ^3) MCV = Hct x 10/RBCc;
- Mean corpuscular hemoglobin (pg Hb/erythrocyte) MCH = Hb x 10/RBCc;
- Mean corpuscular hemoglobin concentration (gHb/100 ml erythrocytes) MCHC = Hb x 100/Hct.

For determination of sanguine glycemia the method with *orto-toluidina* using total blood samples read inductively at 630 nm, was used.

The total serum proteins (PST) has been titrated by Biuret method, by serum centrifugation and read on inductively at 540 nm.

Samples reading has been made with/on a Specord 50 apparatus, after carrying out calibrating plot measurements for each method (PST and glycemia), registering the blood correspondent in g/dl and mg/ml respectively. For a leukocyte formula assessment colored blood smears have been made on May-Grunwald-Giemsa dyeing.

During all the rearing period, monitoring the main physico-chemical parameters of water (such as temperature, pH, dissolved oxygen organic meter, nitrate and phosphorus compounds etc) was being done. These facts were enframed within normal limits except for the temperature that, in 2007, generated (for a short time) a condition of discomfort for polyodon (water temperature during the day time exceeded 30 degrees C).

3. Results and Discussions

In 1995, the stocking of polyodon species was made tardy, on the 16th of May 1995 on a water temperature of 17 degrees Celsius, date whereat the first blood sample was made. At the same time the biometry and hematology data (as mean level and standard deviation) are shown for the *Polyodon spathula*$_1$ species in table 1 and table 2 and for *Polyodon spathula*$_2$ species in table 3 and table 4.

Comparing the hematological results on *Polyodon spathula*$_1$ of 1995 with those of 2007, it can be observed the low level of hemoglobin for the imported sturgeon compared with that of its descendants. Also, the erythrocyte constant derivate MCHC has a very low level in spring, below 10 gHb/100 ml erythrocytes thing that suggest that the polyodon species imported (after one year of growing in the new habitat) present a sever hypochrom anemia. Note also that, on the microscopic

ichthyopathological examination it has not memorized the active presence of some specific external parasites on their skin level, fins or holes nose but only on the gill, the chronicle amount of parasite protozoa such as: *Trichodina sp.* has been met, in spring, and accidental *Trichodinella sp.,* in summer.

Table1. Biometrical and hematological indexes on *Polyodon spathula*$_1$ reared in ponds in poly culture

Specification	*Polyodon spathula*$_1$ (BI) 16.05.1995	HC4 Nucet (4 ha) 3.08.1995	HC4Nucet (4 ha) 9.10.1995	H5 Cazaci (1.8 ha)24.10.1995
TL (cm)	50.5 ± 1.63	53.6 ± 2.76	55.3 ± 2.16	67.0 ± 3.80
W(g)	310 ± 34.36	328.3±76.42	359.3 ± 39.63	772.9±144.42
Hb (g/100 ml)	3.8 ± 0.48	4.8 ± 0.85	6.7 ± 0.84	8.0 ± 0.86
Hct (%)	38.7 ±5.23	27.8 ± 6.49	24.5 ± 3.62	34.83 ± 4.39
RBC ($\times 10^6$/µl)	1.189 ±0.18	1.875 ± 0.200	1.156 ± 0.18	1.616 ± 0.42
MCV (cm^3/erythrocyte)	330.1 ±54.06	149.4 ± 35.02	861.2 ± 147.56	226.61 ± 54.08
MCH (pg)	32.4 ± 4.8	27.9 ± 5.33	58.6 ± 8.49	52.03 ± 12.48
MCHC (gHb/100 ml)	9.9 ± 1.199	19.4 ± 5.07	27.7 ± 4.49	23.26 ± 4.06
Proteins g/dl	-	1.24 ± 0.33	1.49 ± 0.86	2.26 ± 0.37
Glycemia g/dl	-	15.57 ± 4.85	19.96 ± 4.53	15.8 ± 4.45

Values as mean value ± SEM, Note: TL – total length; W – weight;

Table 2. Biometrical and hematological indexes on *Polyodon spathula*$_1$ reared in ponds in poly culture

Specification	*Polyodon spathula*$_1$ (BI) 29.03.2007	BR2 Marata (0.4 ha) 26.07.2007	BR2 Marata (0.4 ha)19.12.2007
TL (cm)	46.3 ± 3.17	74.1 ± 2.58	83.0 ± 1.69
W(g)	200.4 ± 40.28	1680.8±100.0	1613.8±128.86
Hb (g/100 ml)	6.2 ± 0.86	8.9 ± 1.0	9.0 ± 1.46
Hct (%)	29.9 ± 6.34	36.7 ± 6.8	37.1 ± 5.77
RBC ($\times 10^6$/µl)	1.046 ± 0.09	1.368 ± 0.1	1.501 ± 0.22
MCV (cm^3/erythrocyte)	284.29 ± 44.81	267.9 ± 45.0	248.78 ± 32.34
MCH (pg)	59.33 ± 6.86	64.5 ± 6.4	60.4 ± 9.00
MCHC (gHb/100 ml)	21.07 ± 2.41	24.3 ± 1.9	24.23 ± 0.89
Proteins g/dl	1.52 ± 0.56	2.21 ± 0.5	3.1 ± 0.81
Glycemia g/dl	14.0 ± 3.45	15.3 ± 3.3	15.8 ± 3.66

Values as mean value ± SEM, Note: TL – total length; W – weight;

Table 3. Biometrical and hematological indexes on *Polyodon spathula*$_2$ reared in ponds in poly culture

Specification	*Polyodon spathula*$_2$ (BI) 15.15.1996	HC4 Nucet (4 ha) 23.07.1996	HC4 Nucet (4 ha) 25.10.1996
TL (cm)	68.5 ± 2.35	67.5 ± 2.92	75.3 ± 4.03
W(g)	777.0 ± 83.9	863.3 ± 116.8	1.002 ± 269.0
Hb (g/100 ml)	8.0 ± 0.95	8.1 ± 2.05	7.9 ± 2.02
Hct (%)	40.5 ± 5.53	41.6 ± 4.06	41.3 ± 4.08
RBC ($\times 10^6$/µl)	2.252 ± 0.25	1.103 ± 0.19	1.364 ± 0.35
MCV (cm^3/erythrocyte)	180.85 ± 26.12	383.44 ± 47.06	314.54 ± 49.34
MCH (pg)	35.88 ± 4.66	74.38 ± 19.53	59.1 ± 6.46
MCHC(gHb/100 ml)	20.18 ± 3.74	19.34 ± 4.01	19.1 ± 3.17
Proteins g/dl	1.5 ± 0.23	1.85 ± 0.68	1.4 ± 0.55
Glycemia g/dl	7.8 ± 2.45	7.5 ± 2.03	7.6 ± 2.08

Values as mean value ± SEM, Note: TL – total length; W – weight;

Analyzing all the aspects that bear significance upon the biological and material life environment of sturgeon population brought from abroad, we believe that the adaptation condition of polyodon species offered by the new habitat have not influenced the low hematological rate level. Assuming that the absence of specific food from the pond in which the polyodon species had to stay until mid-May meant a limiting factor as the plankton biomass from this pond did not exceeded the level of 0.7

g/m^3, in April and in May was 1.2 g/m^3. As a resource of feeding, the plankton biomass represented by algae and rotifers (lowest energy forms) was totally insufficient. *Polyodon spathula* recorded the best growth rate when plankton biomass was more then 5 g/m^3 (Melcenkov, 1991).

Table 4. Biometrical and hematological indexes on *Polyodon spathula$_2$* reared in ponds in poly culture

Specification	*Polyodon spathula$_2$ (BI)* 28.03.2007	*BR3 Cazaci (0.5 ha)* 26.07.2007	*BR3 Cazaci (0.5 ha)* 22.11.2007
TL (cm)	78.8 ±1.84	95.4 ± 2.35	98.5 ± 7.57
W(g)	1193.7 ± 57.56	3767.5± 334.92	3299.7± 680.92
Hb (g/100 ml)	9.9 ± 1.7	9.5 ± 0.95	9.5 ± 0.32
Hct (%)	39.3 ± 8.0	38.2 ± 9.80	35.1 ± 5.71
RBC (×10^6/µl)	1.299 ± 0.2	1.239 ± 0.11	1.250 ± 0.16
MCV (cm^3/erythrocyte)	300.8 ± 24.0	249.7 ± 73.34	270.3 ± 53.77
MCH (pg)	76.9 ± 7.2	64.2 ± 9.12	76.6 ± 7.16
MCHC (gHb/100 ml)	25.7 ± 3.7	22.2 ± 4.78	28.9 ± 3.83
Proteins g/dl	2.9 ± 0.7	3.4 ± 0.62	3.7 ± 0.62
Glycemia g/dl	13.3 ± 2.4	15.1 ± 2.59	14.3 ± 2.85

Values as mean value ± SEM, Note: TL – total length; W – weight;

Among the leading factors of the appearance and maintenance of the polyodon sp. hyperchrom anemia during the spring-summer time, from the rearing experiment of the second summer (of the 1995 year), mention must be made of: the agglomeration stress, the "extended starvation" and the stocking formulas.

The hematological results acquired during the researches in the growing season (summer-autumn 1995), recorded amazing positive changes, suggesting that ensuring the North American sturgeon *Polyodon spathula$_1$* the appropriate life conditions (i.e., an adequate feeding and hydrochemical regime), a maxim of three months was necessary to restore the metabolic blood profile disturbed (Figure 1). The image in Figure 1 justified the above mentioned above, showing suggestively how the evolution of erythrocyte constant HCHC of *Polyodon spathula$_1$* in the year 1995, knows an ascendent course tending to reach the level registered for *Polyodon spathula$_1$* in the year 2007.

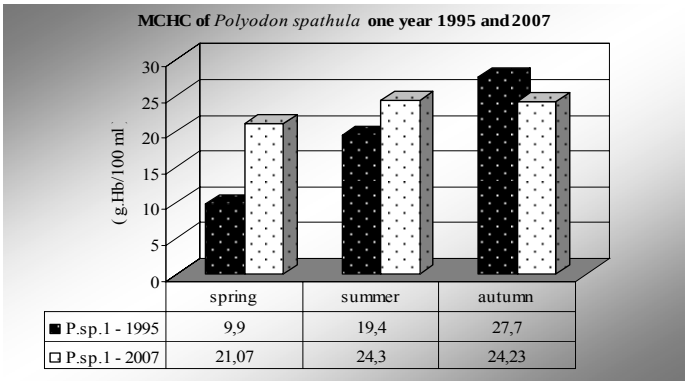

Figure1. Mean corpuscular hemoglobin concentration MCHC of *Polyodon spathula$_1$* both 1995 and 2007

In 1996, *Polyodon spathula$_2$* blood samples, examined clinically (integument macroscopic exam) from as far as 20 exemplars, evidenced the existence of three external parasites respectively, the crustacean *Lernea sp.* and up to 5 – 7 traces left by the parasite (Figure 2).

On account of the growth space, stocking of polyodon in pond took place in May, on the 15th 1996, after having suffered two successive manipulations (by fishing) in a fairly short period (of 35 days). Before the final stocking, blood samples were taken and carried out the biometry.

On the next bath of determinations (1996, the 20th of July), it was found that the polyodon grown up on the third summer, carried traces of external parasite, *Lernea sp.* as well as a number of 12 – 39 external parasites (*Lernea sp.*) fixed under tegument. On the fishing in autumn (1996, the 25th of

October), could be seen on the fish teguments only traces of external parasite *Lernea sp.* (small elevations as cone form having a few mm).

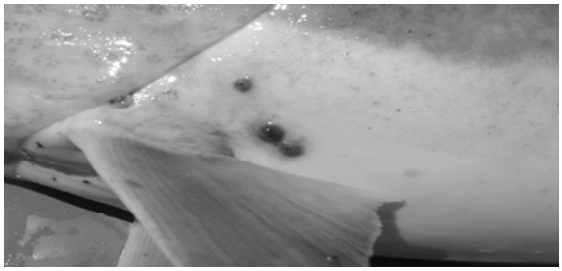

Figure 2. Skin changes caused by ectoparasites Lernaea sp. sturgeon species North - American paddlefish

Results of the hematological determinations carried out on two years old *P. spathula,* in spring of 1996, have shown that the influence of the wintering period and of their being repeateded handled in that spring feet more seriously in the biochemical balance of blood (PST and glycemia), parameters that decreased almost to the half of the determinations value as compared to the autumn of 1995 (Table 3). Against this background, to the mucus secretive cells balance standing as a natural barrier in defending the body was disturbed, making the polyodon more vulnerable to external parasite such as the crustacean *Lernea sp.*

In 2007, a superiority of the *Polyodon spathula*$_1$ and *Polyodon spathula*$_2$ (both ages) body mass was noticed as compared with the 1995 and 1996 exemplaries brought from abroad. Both in 1995 and 2007 *Polyodon spathula*$_2$ species have maintained a value around the initial one during the rearing time, the MCHC level changing and registering a slight decrease on parasitized fish with *Lernea sp.* as compared to the exemplaries having a good physiological condition (Fig. 3).

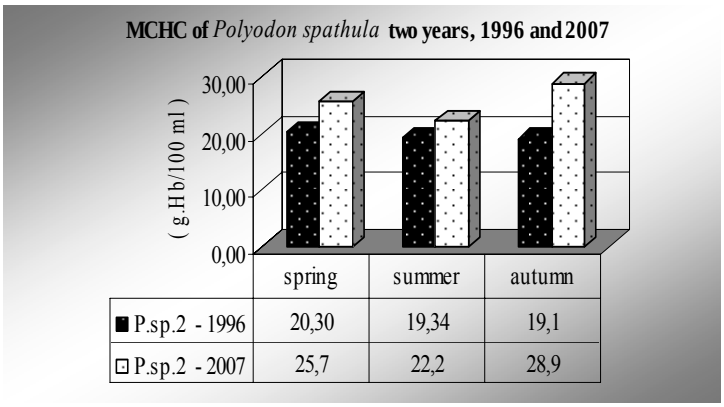

Figure 3. Mean corpuscular hemoglobin concentration MCHC of *Polyodon spathula*$_2$ both 1996 and 2007

The leukocyte formula of North American sturgeon *Polyodon spathula*$_1$ at the age of one year, clinically healthy, is somewhat similar to that of sturgeons (sterlet), (Ivanova, 1983) and is represented by following board analysis: lymphocytes 45 %, neutrophil granulocytes 53 % (promyelocyte 29 %, myelocyte 22 %, metamyelocyte 2 %), eosinophils 1 % and monocyts 1 %. Defining the board of leukocyte formula through the prism of nuclear index proposed by Arneth (Manolescu, 1978) one can say that it is recorded a strong "deviation to the left", deviation determined by the presence of "young" cells in the granulocyte batch, fact indicating a "regenerative" reaction bearing favourable consequences.

After the examination of smears, we mention that on *Polyodon spathula* at the age of 2 years (fish with a low amount of parasitizing), in the red batch of ellipsoid form appear erythrocytes as well as young erythrocytes with a tendency towards roundness and white cytoplasm. The white batch is

represented by lymphocytes with the average of 56 %, varying between 43 – 75 % for the smears from of 1996, May, 15th and 51.3 % with limits between 22 – 68 % in summer and autumn. Granulocytes are represented exclusively by young cells (promyelocyte), variable as greatness, with white low aciduric cytoplasm, dotted with specific purple granulations. This alternation of tint is explained by some authors as a super functional element and by other as toxic granulations. Level of neutrophil granulations in the summer time was of 41.5 % and respectively 43.8 %. In spring, the eosinophil granulocytes represented 0.8 % in the formula, in the summer (when fish was hermed by external parasite *Lernea sp.*) and in the autumn 1.9 %. Monocyts have not exceeded 1.6 %.

Our hematological research on the North American sturgeon *Polyodon spathula* remain an open domain for other researches and studies because this species have a special way to react to external stimulations as stress factors, proving thatit is a valuable fishy resource that will also be of a great ecologic importance.

4. Conclusions

The information provided by the biochemical and morphological examination of blood, correlated with the results of ichthyopathological analysis are of a grate importance from the pathological standpoint anticipating the presence of some asymmetries of an internal environment before any clinical behavior. The immunity system responds to the internal or external stimulation indicating differend kinds humoral reactions. Thus, deflation of PST and glycemia level, assort with increasing of the lymphocyte number 56 % (varying from 43 to 75 %) and the transmutation of the morphologic aspect of the neutrophil granulocytes blood samples of the latest spring clinical tegument exam indicate the presence of some dents of external parasite *Lernaea sp.* that can be accepted as a humoral answer of the immunity system. The granulocytes, exclusively young cells (promielocites) of different sizes and having a low aciduric cytoplasm level, with distinctive granulations colored grate basophile are also interpreted as a hyper functional element. Increasing of RBC from 1.103 to 1.346 × 10^6/µl and diminution in autumn, of eosinophile granulocytes number to 1.9 % (comparatively with 3.2 % when fish was infected with parasite with *Lernaea sp.* type) is a sign that humoral response recorded a recovery process to normality, even if PST and glycemia levels were still low.

In the case of lerneosis – morbidity occurrence brought abont by an external parasite *Lernea sp* – with all that does not produce essential changes peril or alter the sanguine metabolic profile of the fish or of the meet quality and is not a public health food threat, being disagreeable in integument appearance amendments, it is recomanded to be postponed as it takes marketing fish, avoiding the negative impact of the way food image acts upon the consumer.

Results acquired at the end of hematological study on *Polyodon spathula* species reared in ponds, on different stocking formula, on the second and the third summer, indicated that this sturgeon has an amazing capacity for adaptation to specific conditions imposed by the technology used in controlled system for rearing and recovering after having suffered serious influence of the different natural factors (such as agglomerating stress, fishing and multiple handiness, long periods of starvation and even the presence of some external parasites).

Assurance of a specific qualitative and quantitative food level is the main condition for a good health and a special kind of growth.

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