A COMPARATIVE ANALYSIS OF HEMATOLOGICAL PARAMETERS IN CARP INDIVIDUALS FROM DIFFERENT FISH FARMS

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Abstract

Air quality affects the terrestrial environment, while water quality is critical for the survival and well-being of aquatic animals. This is especially important when considering the potential use of underwater creatures for human consumption. However, it is unrealistic to expect water quality parameters to be constantly ideal for fish reproduction, growth, and development in fish farms. Thus, it is essential to study the impact of water quality on biological material, which is necessary for fish farm populations, and to understand how different environments affect fish growth and development. In this study, biological material from three fish farms located far apart was used to examine the impact of different water quality parameters on fish growth and development. The specimens used were clinically healthy and two years old. The differences in water quality parameters between the three environments had a significant impact on the specimens, causing significant changes in their growth and development when they were transferred to new environments. Some specimens even died during the experiment due to the differences in water quality. The study also found that the adaptability of fish to their environment is not transferable to new environments, which can lead to significant changes in hematological parameters. In some specimens, the studied parameters were halved after transfer, while in others, they doubled. The study concludes that heritability is influenced equally by genetic and environmental factors, and the environment plays a crucial role in determining the characteristics of fish.

Key words: *environment, heritability, quality, water*

INTRODUCTION

The environment modifies the genetic structures of the organisms that inhabit them by adding or canceling some characteristics they present at birth. As discovering the world around them, people lived in different environments and adapted not only psychologically to new temperatures, climates and views, but genetically too. The higher humans went above sea level, the lower the atmospheric pressure. This fact is described by fewer oxygen molecules in the air. Exposure to high altitude environments, for short periods, causes hypoxia that manifests itself in pulmonary and cerebral edema.

There are a lot of disorders that lower atmospheric pressure can induce: respiratory - dyspnea with wide breathing or regular breathing, circulatory - tachycardia, high blood pressure, palpitations, mental - euphoria, delirium, hallucinations, behavioral - apathy, memory - diminishing fixation and evocation, attention. The speed of orientation reactions and decisions decreases. Sensory disorders appear, manifested by tactile and painful hyperesthesia, accommodation disorders, etc. When exposing to these environments for a long time, it causes the appearance of pulmonary hypertension which increases the risk of pregnancy complications that can lead to its loss. Among other disorders, this

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is the most important when considering adaptation. People who migrated to high-altitude environments have adapted physiologically and genetically to live here but after very long periods, over several generations.

When moving, humans brought with them domestic animals that adapted just like them in the same long periods, over many generations [1].

This theory is the same when talking about fish and water as an environment. But, when we manage a fish farm the adaptation cannot take `many generations` because it means losing time and money. Further, this study refers to how the aquatic environment influences some hematological parameters of *Cyprinus carpio* and fundaments future studies on how reproduction should happen.

Cyprinus carpio got the attention of evolutionists, due to the large areas they are present on all continents and their presence in most fresh-water ecosystems. The common carp is one of the most important farmed freshwater fish species in the world [2].

Selective breeding programs for common carp are not frequently implemented. These carp breeding programs are based on the development of different genes used for crossbreeding, generally following growth-related traits [3; 4; 5]. When we refer to the genetics of fish populations we know that it is closely related to quantitative genetics. This is due to the frequency of the strain. The factors that modify this frequency, as well as the heredity of the quantitative characters, can be studied only at the population level. It has been observed that some pure strains appear to perform about as well as the best crosses [6]. One of the major problems of research in fish farming is represented by the selection and growth of fish populations to produce broods and breeders with superior qualities.

Another problem concerning the crossbreeding on common carp seems to be the consanguinity of the parental strains. This is a real problem related to costs because inbreeding counts reduced performance for traits like reproduction and survival but production traits too, which means expensive costs for maintaining the stains and not to improve the characters of economic interest.

In other words, crossbreeding does not bring a long-term plus, and it should be analyzed as an adjacency to the classic mating, looking for genetic effects that bring a consistent increase in production and fitness traits [7]. Selective breeding programs for the exploitation of additive genetic variation have not been widely implemented in common carp, however selective breeding programs for common carp include improvements for traits such as growth rate, external traits and viability and/or adaptation to breeding conditions, such as temperature variations [8].

Bowman [9] found large growth differences between half-sibling carp families, and Brody [10] estimated heritability for the growth parameter at 0.47 of the parent-offspring regression. In 2008, Vandeputte [11], obtained a heritability of 0.44 for the common carp weight parameter, analyzing the records of three generations. Despite all these studies and analyzes, the vast majority of world carp production was based on unimproved genetic stocks. This may be due to a long time of data collection and processing, which leads to pointless financial losses. The expenses with the improvement of some productive parameters are the same as those of a less perfect production.

It should not be neglected, however, that the development of genetically improved strains has a huge potential for more efficient production. What genetic improvement programs for common carp should consider is the improvement of productive traits and fitness traits (resistance to the herpes koi virus, which has become a problem in European carp production) [12; 13]. This adaptation of genetic improvement programs requires the

selection of additive genetic effects and strategies for capitalizing on non-additive effects if it is concluded that they are important. The introduction of these selection programs would guarantee an increase in productivity and production efficiency in the fishing industry. Large-scale experiments and selection programs in other fish species have shown that genetic changes can be obtained in each generation [14]. For the design of genetic improvement programs for common carp, it is important to obtain exact values of genetic parameters. The genetic parameters targeted are the heritability of the traits of interest and the genetic correlations between them. These parameters are necessary for a good understanding of the performances of the different strains and their crosses to obtain the features of productive importance. Heritability values for weight and length vary between 0 and 0.55, but many of these values are expected to tend to 1, due to environmental influences, inbreeding. Hematological parameters are very important in determining the health and physiological status of the fish [15]. In addition, these parameters reflect the changes in the organism correctly and play an important role in the detection of disease and metabolism of fish living in different ecological environments [16].

Fishes are poikilothermic creatures, in which changes are observed in hematological parameters due to environmental factors such as bacteria, parasites, water temperature, oxygen content, pH and so on. Hematological values in fish change with the effects of seasonal variations that are associated with changes in water temperature and climatic changes [16].

MATERIAL AND METHOD

The biological material was represented by eight carp individuals that were obtained from three fish farms which are at a great distance from each other. All samples were clinically healthy and were checked, measured and registered (Tab.1).

Table 1 Meristic measurements of the fish

Fish ID	Weight (kg)	Length (m)	Farm No.
L_1	1.95	0.35	1
L_2	1.90	0.34	1
L_3	1.95	0.35	1
C_1	2.00	0.36	2
C_2	2.10	0.37	2
C_3	2.20	0.38	2
B_1	2.00	0.35	3
B_2	2.10	0.35	3

When first came into the laboratory, 1 ml of blood was collected from each specimen then they were released in laboratory conditions (Tab.2).

Table 2 Distribution of fish in laboratory conditions

Fish ID	Weight (kg)	Length (m)	Environment
L_1	1.95	0.35	AL
L_2	1.90	0.34	AC
L_3	1.95	0.35	AP
C_1	2.00	0.36	AC
C_2	2.10	0.37	AL
C_3	2.20	0.38	AP
B_1	2.00	0.35	AL
B_2	2.10	0.35	AC

Every fish specimen was released in a tank in a volume of 50 l of water, at the same temperature, 12°C from 21st of April to 8th of May, when the last fish specimen was examined.

Blood samples were collected from the carp individuals using the following procedure: each fish was completely anesthetized by electronarcosis and then removed from its tank with the aid of a net. Once removed, the fish was placed on a clean towel on its side and carefully lifted to a comfortable handling position with both hands. The fish was positioned so that its head was on the left side, its tail was on the right side, and its abdomen was facing the operator. Using a wet towel, the fish was held in place with one hand while blood was collected from its caudal vein using a 23G needle inserted at a 90˚ angle. The blood was collected in green vacutainers containing 34

U.I. heparin. The entire process was carried out according to established protocols [17].

The study determined two hematological parameters, namely hematocrit (Ht) and hemoglobin (Hb) (Tab.3). The hematocrit was determined by employing the microhematocrit method that utilizes heparin capillary tubes measuring 70 mm in length and 1 mm in diameter. Once the blood had permeated the capillary tube to a length of 60 mm, the open end of the tube was sealed using a gas flame. The centrifugation was performed using a Hettich Haematokrit 210 centrifuge at a speed of 13000 rpm for 120 seconds. The hematocrit value was directly obtained from the scale of the centrifuge device.

The hemoglobin concentration was measured using the Sahli colorimetric method with the hemoglobin-meter Sahli. This apparatus consists of a dial gauge and a capillary pipette with a volume capacity of 20 mm^3 (0.02 ml). The dial gauge includes a double blank, represented by two brown-yellow colored glass rods, and a special test tube with two scales, one in g/dl and the other in %. (16 % corresponds to 100 g/dl). Initially, HCl N/10 was introduced into the measuring tube up to division 10 of the percentage mark, using a Pasteur pipette. Subsequently, blood was aspirated to the capillary pipette indicator (0.02 ml), and the contents were released into the graduated tube at the bottom of the tube, where it was mixed with the HCl. After mixing, the mixture was allowed to stand for 5 minutes to enable the formation of hematine chloride, which results in a dark brown color. Then, distilled water was added drop by drop, and the contents were mixed with a glass rod until the color of the liquid in the test tube matched that of the test sample.

The hemoglobin reading was taken by identifying the corresponding graduation (in g/dl) at the lower meniscus. Blood cell morphology and appearance were observed through the creation of smears during each blood draw. The wedge or push technique is commonly used to prepare blood smears, which aims to produce a uniform and thin film of blood gradually becoming thinner towards the opposite end of the glass slide. The smear preparation technique involved placing a small blood drop at the midline of a glass slide, followed by applying another glass slide in front of the blood drop at a 30-45° angle and retracting it until it comes into contact with the blood.

To create a blood smear, a small drop of blood is placed at the end of a glass slide and another slide is applied at an angle to spread the blood across the width of the slide. The slide is then pushed forward with a continuous motion to create a uniform, thin film of blood on the glass slide. Ideally, the smear should cover about 2/3 of the slide's length.

For staining the smears, May-Grünwald's stain was used, which results in a red-to-violet coloration of the nuclei. This coloring is based on the molecular interaction between the dye and an Azure B-DNA complex. The intensity of the coloring depends on the content of the dyes as well as the ratio between them, and may vary depending on staining times and the pH of the solutions or buffers used.

Table 3 Initial blood parameters

Carp	Ht (%)	Hb (g/dl)
L_1	27.3	7.8
L_2	26.6	7.2
L_3	29.0	7.8
C_1	18.3	4.8
C_2	17.5	4.6
C_3	18.2	4.8
B_1	30	8.0
B_2	14	3.0

Also, the water was examined and some parameters were registered (Tab. 4).

Table 4 Initial water parameters

WATER				
VALUE	AP	AL	Ac	AB
KH	9	18	14	19
GH	9	22	15	12
PH	7.4	8	6.6	7.6
NH_4	<0.05	<0.05	>10	<0.05
NO_2	<0.01	0.025	<0.01	<0.01
NO_3	1	1	<0.5	<0.5
PO_4	<0.02	<0.02	<0.02	<0.02
SiO_2	<0.1	<0.1	3	<0.1
Fe	<0.02	1	0.05	0.8
Cu	<0.05	<0.05	<0.05	<0.05
O_2	10	6	10	10

The still water used in the experiment was commercially purchased and selected for its pH value of 7.4. The initial water parameters were obtained by filling the tanks, each with a capacity of 50 liters. Specifically, two tanks were filled with 50 liters of still water (AP), three tanks were filled with 50 liters of water from fish farm no. 1 (AL), and three tanks were filled with 50 liters of water from fish farm no. 2 (Ac). To determine the chemical parameters, a Jbl Pro Aquatest Lab, Koi, Marine, Proscape kit was utilized, which employs reagents to measure values such as Kh, Gh, pH, NH_4, NO_2, NO_3, PO_4, SiO_2, Fe, Cu, and O_2. Water samples were collected from each fish pond in 1-liter glass receptacles and the parameters were determined immediately after collection for initial determination.

RESULTS

The findings were analyzed by presenting the changes in the blood and water parameters throughout the experiment using graphical representations. Furthermore, the initial and final smears were compared to interpret the results. The observed changes in blood parameters indicated that the environmental conditions have a significant impact on the health of fish. Specifically, in sample L_1, there was a notable increase in the number of degenerated lymphocytes and reactive neutrophils, as well as a decrease in the N/C ratio, compared to the initial moment (Fig. 1 and Fig. 2).

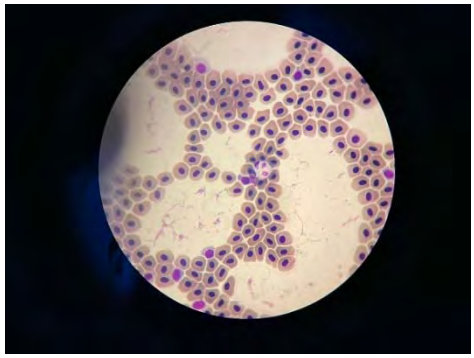

Fig. 1 L_1- degenerate-looking lymphocytes

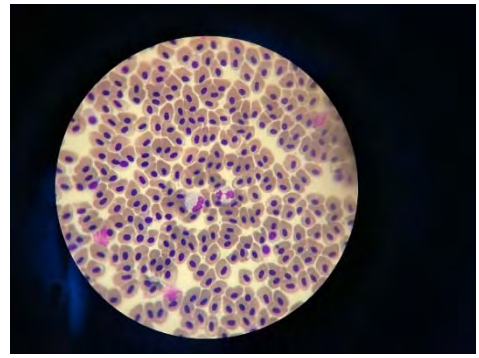

Fig. 2 L_1- intensely reactive neutrophils, increased quantitatively

In sample L_2, in addition to numerous degenerate-looking lymphocytes and a low N/C ratio, numerous segmented neutrophils were also observed (Fig. 3).

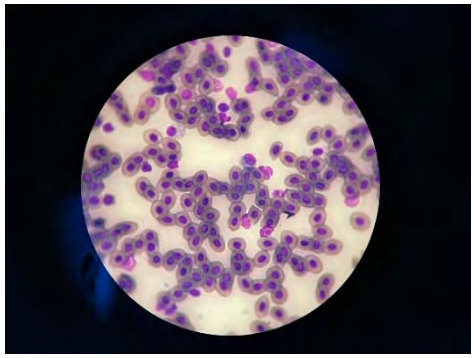

Fig. 3 L_2- segmented neutrophils

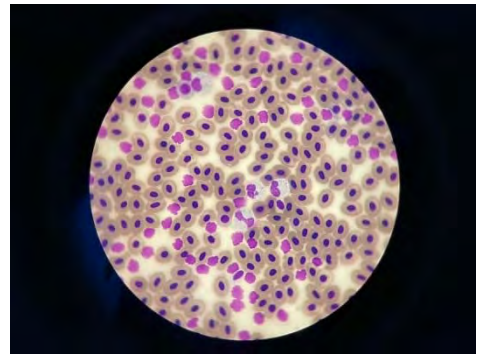

Fig. 4 C_1- intensely reactive neutrophils, increased quantitatively and numerous segmented neutrophils

Sample L_3 exhibited only numerous degenerate-looking lymphocytes and segmented neutrophils at the end of the experiment. All three samples, L_1, L_2, and L_3, showed increased cell fragility at the initial moment. Sample C_1, on the other hand, registered intensely reactive neutrophils that increased quantitatively, as well as numerous segmented neutrophils (Fig. 4).

The samples C_2 and C_3 exhibited only a low N/C ratio compared to the initial moment. Samples B_1 and B_2, on the other hand, did not show significant changes during the experiment, but had numerous degenerate-looking lymphocytes and a low N/C ratio both at the initial and final moments (Fig. 5 and Fig. 6). These specimens exhibited greater adaptability compared to the other specimens, which is consistent with the environmental parameters from which they originated.

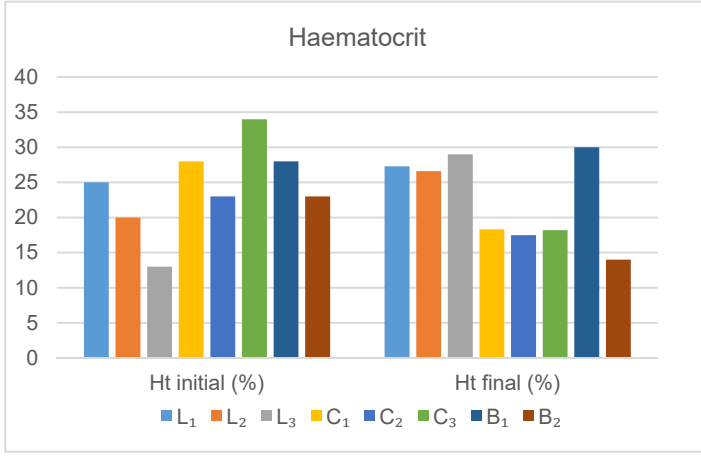

Fig. 5 Hematocrit values

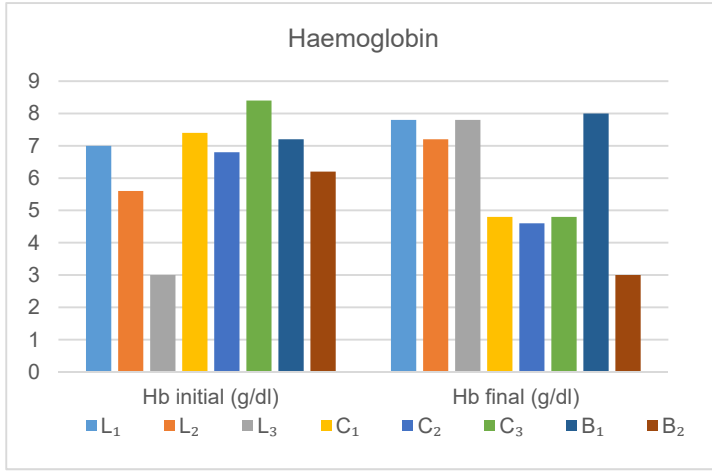

Fig. 6 Hemoglobin values

Through an integration of all data, it became evident that the specimens and the environment mutually influenced each other, resulting in notable changes in blood parameters as an adaptation to the new conditions.

DISCUSSIONS

Due to their position in the food chain and genotypic traits, fish are of significant interest in the study of adaptation processes. The rapid changes that occur at the biochemical level make them even more intriguing. Fish genotypic characteristics are highly influential in establishing favorable or unfavorable environments, and some species or individuals have the ability to adapt to different aquatic environment parameters. Fish have a large number of multiple hemoglobins, and extensive research has reported their biochemical adaptations, demonstrating that fish genetic material can be influenced by environmental parameters [18; 19].

Hemoglobins play an important role in fish adaptation as they act as a mediator between the organism and its environment [20]. Fish, unlike terrestrial animals, face a disadvantageous condition of a highly variable environment with spatiotemporal fluctuations in oxygen levels. Nevertheless, physiological modifications have been observed in fish exposed to hypoxic conditions, to enhance oxygen transfer. In certain Amazonian fish species, anatomical adjustments have been noted as an adaptation to their air-breathing needs [21; 22].

Regarding the hemoglobin profile, *Cyprinus carpio* blood exhibits four bands of hemoglobins on isoelectric focusing and three on starch gel [23]. In fish species, the hemoglobin forms are referred to as isohemoglobins [24; 25; 26].

The common carp's hemoglobin polymorphism is believed to have originated primarily from the polymorphism of globin constituents. Two theories have been proposed to explain this phenomenon: the "selectionist" theory and the "neutralist" theory, which accounts for the diversity of hemoglobin through neutral mutations [27]. According to Pérez et al. [28], the latter theory is responsible for most sequence variations, as evidenced by the analysis of hemoglobin data collected from various fish species. In a selectionist framework, it is suggested that multiple hemoglobins, rather than a single hemoglobin, working together with functional diversity, may enhance gas transport during environmental changes.

In unstable environments, hemoglobin heterogeneity is believed to have a selective advantage, as it can facilitate the transport of gases during environmental variations. This hypothesis is supported by studies that demonstrate a variation in the hemoglobin pattern of Carassius auratus in response to acclimation to different temperatures [29], which highlights the role of polymorphisms as an adaptive mechanism.

Based on the results of our study, it is apparent that each individual organism displays a unique pace of adaptation, influenced by the environmental conditions in which it has developed. Notably, specimens C_1, C_2, and C_3, originating from fish farm 2 with environmental parameters of pH 6.6, NH_4 over 10 mg/l, and SiO_2 of 3 mg/l exhibited initial Ht and Hb values that were approximately half of those observed in the other fish (Tab. 3). Upon being transferred to new environments, these values underwent a substantial increase, nearly doubling. These findings highlight the importance of environmental factors in shaping the adaptive abilities of organisms.

Specimen C_3 was introduced into a still water environment with a pH of -7.4, NH_4 levels lower than 0.05 mg/l, and SiO_2 lower than 0.1 (Tab. 4) and experienced mortality after 24 hours.

It has been well established by aquatic toxicologists that embryos and larvae represent the most vulnerable life stages of aquatic organisms [29]. As the samples under study were only 2 years old, it should be noted that younger individuals are more susceptible to drastic environmental changes, which may lead to significant adverse effects including mortality.

CONCLUSIONS

This paper offers a comprehensive examination of the consequences and insights obtained from research conducted on *Cyprinus carpio* and their ability to adapt to diverse surroundings.

The study underscores the challenges associated with selective breeding programs in common carp. Issues like consanguinity among parental strains and the high costs involved in maintaining strains without significant improvements in economic traits are significant considerations.

Despite the challenges, the research highlights the significant potential for genetic improvement programs in common carp farming. Focusing on traits related to productivity and fitness, as well as considering additive and non-additive genetic effects, could lead to more efficient and profitable carp production.

The study emphasizes the importance of hematological parameters, such as hematocrit and hemoglobin, in assessing the health and physiological status of fish. These parameters serve as valuable indicators of the fish's adaptation to its environment.

The observed changes in hematological parameters among the fish samples indicate that environmental conditions have a significant impact on the health of fish. This underscores the need for careful consideration of environmental factors in fish farming and conservation efforts.

The presence of multiple hemoglobin forms in common carp suggests a level of genetic variation that can contribute to their adaptation to fluctuating oxygen levels. This genetic diversity may be an evolutionary advantage in dealing with environmental changes.

The study highlights that younger fish are more susceptible to drastic environmental alterations. Understanding this age-related sensitivity is important for managing fish populations, particularly in aquaculture settings.

The findings have broader implications for the conservation of aquatic ecosystems. Understanding how fish adapt to changing environmental conditions can inform conservation strategies to protect vulnerable species and habitats.

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