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EFFECT OF PARAPROTEX AND THE MIXTURE OF GRAPE MARC, CINNAMON AND CLOVE ON THE CARP (*Cyprinus carpio*) GROWN IN RAS - RECIRCULATING AQUAPONIC SYSTEM

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ABSTRACT

Aquaponics, a complex cultivation method obtained by combining two different systems, hydroponics and aquaculture, operates on the principle that fish waste becomes a resource for plants. Recirculating aquaponic systems are mostly used in research, being a less polluting alternative and being more effective from the cost points of view. RAS used for the research was placed into an ICDIMPH-HORTING Bucharest greenhouse, using three plants callus tunnels for supporting two sections: the fish breeding section represented by *Cyprinus carpio*, and the plant growth represented by *L. sativa*. The biological material was placed into 8 tanks where phytobiotics represented by *Paraprotex* and *MCC* mixture were added into the daily fish feed to monitor the fish health and the effect that the two types of mixture have on the fish health The fish health status was monitored by performing blood tests in the first day of experiment, in the middle and at the end of the experiment. The values of hematological indicators showed variations during the experiment period, some of them being within the range values for *C. carpio*, while others fell within the normal limits. The aim of the study is to evaluate the fish health status and their development under the influence of natural phytocomplexes administrated in the feed.

1. Introduction

Climate change, which affects most natural ecosystems, can have several impacts on extensive and semi-controlled aquaculture systems by decreasing the water quality, leading to increased stress and diseases outbreaks (Handisyde *et al*., 2017). Aquaponia is a combined method of vegetable farming and fish farming that does not require any soil.

Aquaponics can be defined as a system that combines hydroponicagriculture with aquaculture, and it is an excellent way of reducing water usage and disposing of aquaculture effluents (Nuwansi *et al.,* 2017).

In this system, aquaculture wastewater is recycled and reused for the hydroponically grown plants instead of discharging to neighbouring water bodies (Shete *et al.,* 2015). These nutrients are absorbed by plants from wastewater before returning to the fish tank. Further, if remediated water is applied to an aquaponic system, the whole system becomes cost-effective, economically viable, and offer additional benefits like supplementation of extra nutrients, minimizing the establishment period of the hydroponic unit, and facilitates hosting decomposers (Nuwansi *et al*., 2019).

The Aquaponics is the symbiosis between hydroponics (growing plants without soil) and aquaculture (growing fish or other aquatic organisms), in which fish and plants grow together in a single integrated system (Rakocy *et al*., 2006).

Aquaponics can be useful, regardless of a country development degree which has limited resources of agricultural production, but also a large population (Mchunu *et al*., 2018). It can provide a variety of good quality food (animal protein and vegetal) for both rural and urban areas (Liang *et al*., 2013).

Aquaponic systems are a less polluting alternative to the environment compared to traditional food production techniques. This technological method has the potential to produce proteins with less labor, less soil, fewer chemicals and fewer water consumption (Specht *et al*., 2014).

The most recommended aquapocic systems are recirculating aquaponic systems because the nutrients in the water can be maintained at optimal concentrations for both fish and aquaponic cultures (Nair *et al*., 2005).

According to literature, this aquaponic technique consists in mounting floating supports directly on the surface of the water gloss related to the fish breeding units. In this case, polystyrene plates are used as supports for the plants whose roots are in permanent contact with the technological water loaded with nutrients (Jensen *et al*., 1985).

Medicinal plants, which harbour a myriad of bioactive metabolites, have arose in the last decade as an extremely attractive alternative in aquaculture (Reverter *et al*., 2014; Sutili *et al*., 2018; Zhu, 2020). The use of plant-enriched diets has shown to be able to improve growth, digestive enzymes, anti-oxidant activities and immune parameters of fish (Awad *et al*., 2013; Yousefi *et al*., 2020b; Zemheri-Navruz *et al.*, 2020). However, as shown before some plants also contain anti-nutritional compounds or toxic metabolites, and therefore careful examination of plant species and dosage are required before their broad use in aquaculture systems (Reverter *et al.*, 2020b).

Recent research showed that the introduction of relatively small concentrations of vitamins, probiotics, prebiotics and newest phytobiotics in animal diet can ensure some specific demands or influence in a positive way (directly or indirectly) the growth performances and the health status (Maurilio 2011; Kasiri *et al*., 2011; Antache *et al*., 2013).

Phytobiotics are natural compounds that lead to an optimization of animal productivity which, when incorporated into diets (Cristea *et al*., 2012). Increasing number of recent studies present the positive aspects of phytobiotics administration, in diets of different fish species: the immunostimulator (Khalil *et al*., 2009), bioproductive (El-Dakaret *et al*., 2008), antioxidant and antimicrobial effect.

In aquaculture, prophylactic administration of immunostimulants is one of the most effective method of strengthening the defense mechanism (Raa *et al*., 1992). In fish, the immunostimulants are known to increase immunity (Harikrishnan *et al*., 2010). Some nutrients are linked to the immunological status of fish. This has drawn the attention of fish nutritionists to the immunoprotection of fish, besides the growth. Sustainable aquaculture depends on the balance between health and growth condition of fish (Kumar *et al*., 2005).

The paper presents the monitoring and evaluation of the effect of the two types of mixtures of phytocomplex *ParaProteX* and *MCC* administered in different concentrations together with food, on the health of carp raised in the recirculating aquaponic system.

2. Materials and methods

2.1. Materials

The recirculating aquaponic system used for the research described in this paper, was placed into a ICDIMPH-HORTING Bucharest greenhouse, using three plants callus tunnels for supporting two sections: the fish breeding section represented by

Cyprinus carpio, Linnaeus 1758 and the plant growth represented by *L. sativa* L.

Two tunnels were reconditioned by modifying the supporting pedestals and the height, for the growth of the plants (*Lactuca sativa*). The 2 plant growth enclosures have a size of 7.4x1.8x0.3 m, and for carp growth a tunnel with dimensions 7.4x1.8x0.4 m was used, being divided into 8 pools of equal dimensions.The aquaponic system works on the principle of communicating vessels. The water from the aquaponic system circulates through the polypropylene pipe, from where it is taken with the help of a CPM self-priming pump with a flow rate of 7.2 m3 / h, placed at ground level and passed through a mechanical filter.

The carp used in the experiments came from the Brateș Research and Development Base - Research and from Development Institute for Aquatic Ecology, Fisheries and Aquaculture Galati.

Figure 1. Recirculating aquaponic system (RAS).

For populating the aquaponic growth system with biological material, dimensions of the experimental tank and the volume of recirculated water were taken into consideration. Thus, the optimal population density was 5 kg of 2-year-old carp for each experimental tank. A total of 40 kg (172 fish) of carp evenly distributed in 8 experimental tanks, with an average weight of 232.5 g/fish were monitored (Figure 1).

In the last stage of the experiment, to ensure the health of reared fish species in aquaponic systems and to comply with their physiological requirements, feed additives were added to fish feed.

Juvenile carp (biological material) used for this experiments was represented of 40 kg fish biomass being equally distributed into the eight rearing units (M1 and M2) - control variant and three different concentrations of mixture natural phytocomplexes: B1.1. with Paraprotex 2g/100g feed dry and B2.1. with 2g/100g feed dry MCC; B1.2. with Paraprotex 4g/100g feed dry and B2.2. with 4g/100g feed dry MCC; B1.3. with Paraprotex 6g/100g feed dry and B2.3. with 6g/100g feed dry MC.

The natural phytocomplexes were incorporated directly in 100g of standard feed NC 60 II, the daily ration for each experimental tank, varying the concentrations.

The fish from the control tanks M1 and M2 were fed only with 100g of standard feed NC 60 II / day, with a crude protein concentration of 32-34%. The amount of daily food was 2% of the body weight of the fish in each experimental tank.

In order to monitor the health of fish reared in the aquaponic recirculating system (RAS), two experiments were performed by using for over 42 days *ParaProteX* phytobiotics and *MCC mixture* (grape marc, cinnamon and cloves) introduced classical as powder into the fish feed (Figure 2; Figure 3). The used phytocomplexes were purchased as powder, of trade.

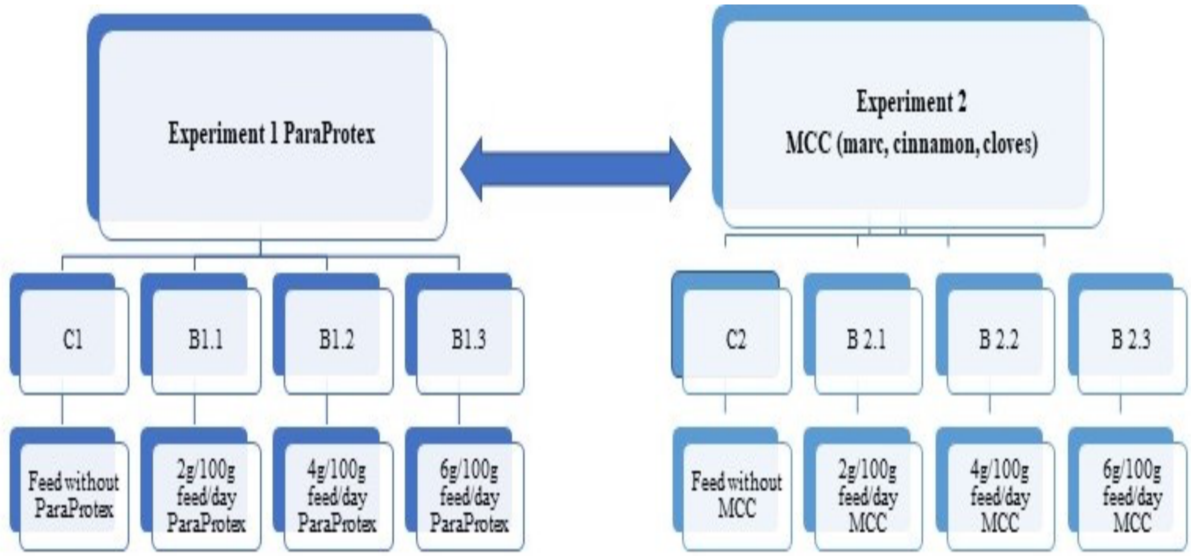

Figure 2. Experiment scheme.

Figure 3. MCC mixture (grape marc(a), cinnamon (b) and cloves (c)

2.2. Blood collection

Blood collection was performed using the cardiac puncture method, without the need to slaughter the fish.

Blood samples were collected at the beginning of the experiments (day 1), in the middle of the experiments (day 21) and at the end of the experiments (day 42), to determine the hematological and biochemical parameters.

Before collecting blood samples, the fish were anesthetized by soaking them in 0.5 ‰ 2-phenoxyethanol solution for 60 seconds.

From each experimental, 5 fish/experimental variants were analyzed, representing 25% of the total population. Disposable sterile syringes were used to collect blood. The needle penetration point is halfway between the base of the pectoral fins and the ventral line (Figure 4).

Two blood vacutainers were collected from each analyzed specimen: a vacutainer with EDTA anticoagulant for hematological examination and a vacutainer without additives for biochemical examination.

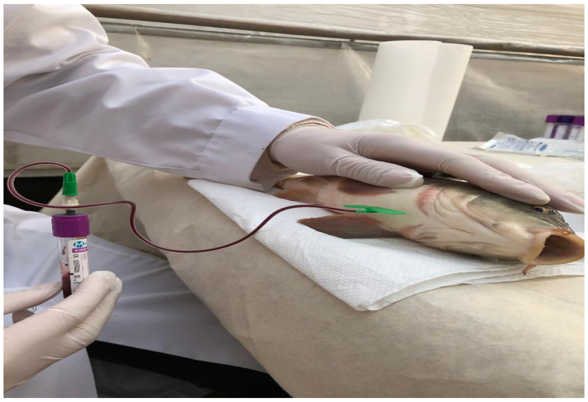

Figure 4. Blood collection by cardiac puncture method.

2.3. Methods of hematological and biochemical parameters investigation

After harvest, the blood samples were transported with a refrigerated box to the veterinary analysis laboratory.

Blood vacuum cleaners were introduced into the hematology analyzer for veterinary use Abacus Vet5, Figure 5. The following parameters were determined: no. of erythrocytes (E), hemoglobin (Hb), hematocrit (Ht) and erythrocyte indices MCV, MCH, MCHC.

The biochemical parameters determined were: glucose, total proteins, calcium, phosphorus, cholesterol, urea, creatinine. Biochemistry analyzes were performed with the Skyla Vb1 Veterinary Chemistry Analyzer, Figure 6.

Biochemical analyzes were determined from serum. After harvesting, the blood is allowed to coagulate at room temperature. By retracting the clot followed by centrifugation for 10 minutes at 3000 rpm, the serum is obtained. For the statistical analysis the Program PRIMER 7 was used.

Figure 5. Hematology analyzer used to determine hematological parameters.

Figure 6. Skyla VB1 veterinary chemistry analyzer used to determine biochemical parameters.

3. Results and discusions

The use of plant supplements to boost immune parameters and disease resistance in fish has recently attracted much attention as a sustainable, low-cost and effective alternative in the prevention and treatment of aquaculture disease outbreaks (Van Hai, 2015; Reverter *et al.*, 2020a). Multiple studies have exposed the beneficial effects of using medicinal plant-based supplements in aquaculture, such as better growth, enhanced immune defenses and decreased pathogen and stress susceptibility (Hoseinifar *et al.*, 2020a; Reverter *et al.*, 2020b; Yousefi *et al.*, 2020a; Yousefi *et al.*, 2021).

In order to see the physiological response of fish to the stressful action of technological and physico-chemical factors in the recirculating aquaponic system, the dynamics of blood parameters were analyzed. There is a permanent dynamic balance between the fish environment, the physico-chemical parameters of the water, the application of breeding and feeding technology, so that any disturbance that occurs in this system can disrupt this balance and lead to changes in blood hematological and biochemical parameters. That is why it is very important to monitor these blood parameters in order to be able to draw a uniform conclusion about the health of the biological material.

3.1. Analysis of hematological results

The hematological parameters were used as fish health status indicators and for the detection of possible physiological changes occurred under conditions of stress caused by:

- administration of a feed with a modified composition by incorporating natural phytocomplexes of the *ParaProteX* type and *MCC mixture* was done by the direct method.
- the need for the fish organism to adapt to the water oxygenation conditions and in particular to the specific conditions of an aquaponic recirculation system.

The dynamics of hematological indicators determined in 2-year-old carp juveniles was analyzed in terms of the phytocomplexes action on fish health and in order to establish an effective feeding strategy.

3.1.1. Erythrocyte count (E)

In carp (*Cyprinus carpio*) the normal values of erythrocytes are between 1.1-2.2x10^6/µL (Ghittino, 1983; Misăilă, 2008). An increase in the number of erythrocytes is observed before reproduction and when the water has a high oxygen content. The massive decrease in the number of erythrocytes, leading to anemia is observed in various pathological conditions.

The average value of erythrocytes during experiment 1 (*ParaProteX*) decreased in the first 21 days, at the end of the experiment, the the number of erythrocytes recording a value of 1.12 µg/L in B1.3, falling within the normal values for carp (Figure 7).

Following the analysis of the average number of erythrocytes during the experiment with the MCC mixure, a slight increase of the obtained values was observed, the most significant in B2.3 (Figure 7).

There were no significant differences between the values of erythrocytes in the control tank and the values obtained in the *ParaProteX* tanks (Figure 7).

3.1.2. Hemoglobin

To assess the physiological state of fish, hemoglobin dosing is considered a rapid test to verify hematological homeostasis (Misăilă, 2008).

Physical or environmental stress causes a rapid increase in blood hemoglobin concentration, due to the recruitment of erythrocytes from the spleen and hemoconcentration installed as a result of plasma water loss.

The normal values for carp (*Cyprinus carpio*) are between 6.5-10.6 g/dL. Mean hemoglobin values ranged from one tank to another and from one concentration of administered phytocomplex to another. In Figure 7 it can be seen that in tanks B1.1 and B1.3 at the analyzes performed on the first day and on day 21, a decrease is observed, showing that the change of living environment related to water quality, and that the administration of *ParaProteX* in different concentrations, caused discomfort to the carp fish population (Figure 8).

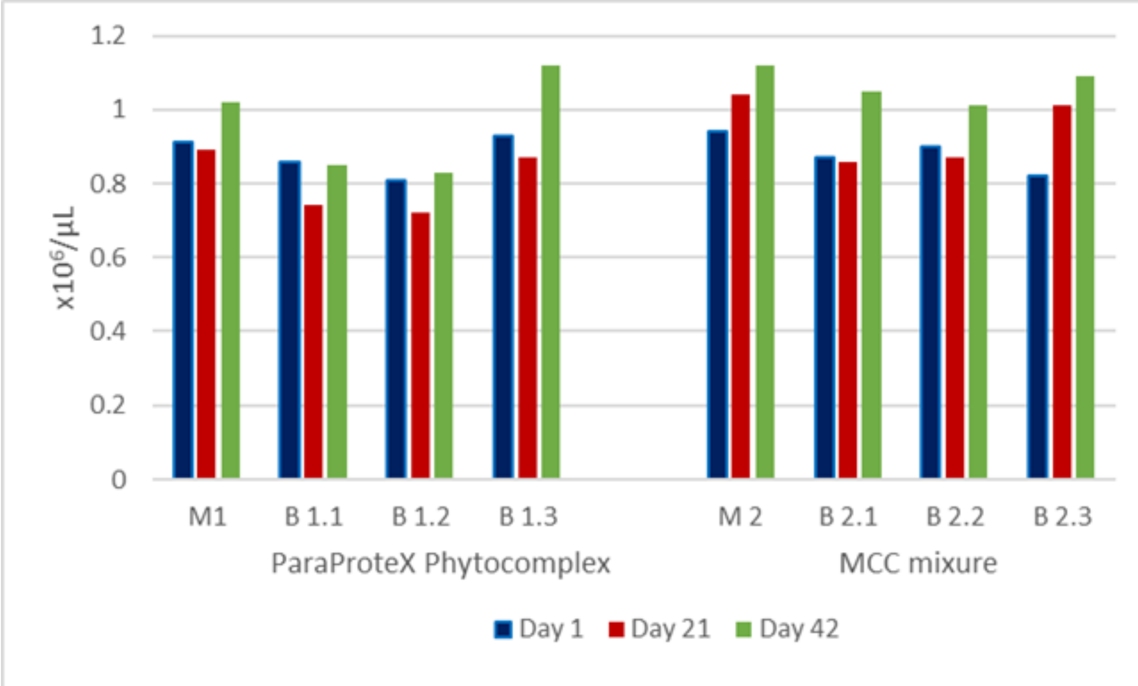

Figure 7. Variation in erythrocyte values in fish blood from the two experiments.
(M1, M2- control variant; B1.1., B1.2., B1.3.with Paraprotex; B2.1., B2.2., B2.3. with MCC)

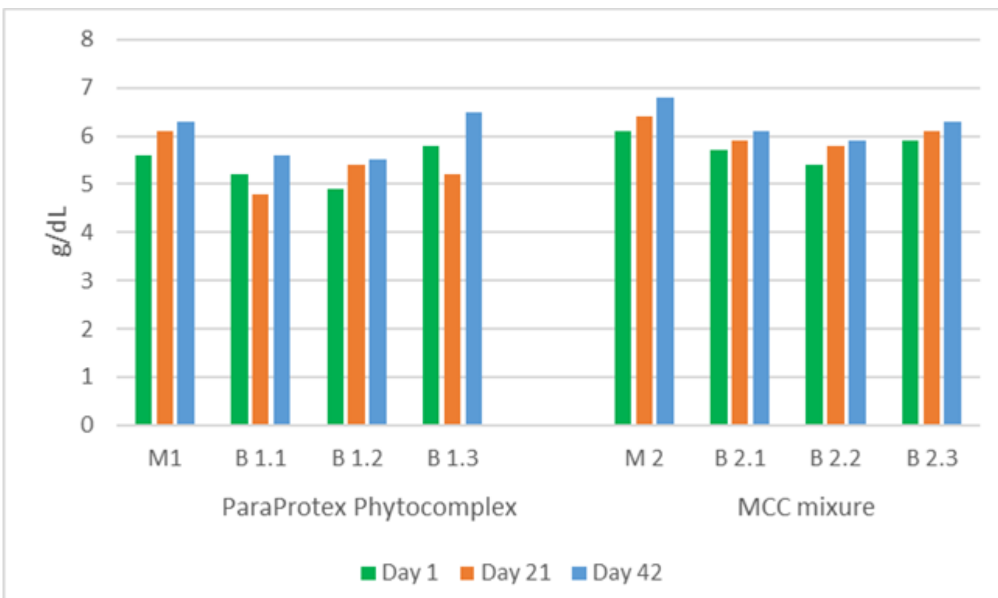

Figure 8. Variation in hemoglobin values in fish blood from the two experiments.
(M1, M2- control variant; B1.1., B1.2., B1.3.with Paraprotex; B2.1., B2.2., B2.3. with MCC)

3.1.3. Hematocrit

The hematocrit value, expressed as percentage, shows the volume of erythrocytes in relation to the total blood volume. In carp (*Cyprinus carpio*) the normal values of hematocrit are between 32-43.9% (Ghittino, 1983; Misăilă, 2008).

Pathological decreases in hemoglobin and hematocrit indicate anemia. During the experiments it is found that in the tanks where the food mixed with *ParaProtex* was administered, the fish population is more difficult to accommodate, towards the end of the experiment the discomfort process showing a decrease (Figure 9).

All hematological parameters determined at the end of the experiments with *ParaProteX* mixture, were within the normal values reported in the literature for the carp species, *Cyprinus carpio*.

Compared to *PraraProtex,* the *MCC mixture* had a beneficial effect on the fish, shortening their period of adaptation to the new living environment in the recirculating aquaponic system type RAS (Figure 9).

All hematological parameters determined at the end of the experiment with *MCC mixture*, were within the normal values of the species. Increases of WBC, which are cells with immune functions, is also considered a sign of enhanced immune response and has been previously reported after plant supplementation in fish (Mbokane and Moyo, 2018; Nhu *et al*., 2019).

There were no significant differences between the values of the hematological parameters in the control tank and the values obtained in *the ParaProteX* and *MCC mixture* tanks.

3.2. Analysis of the results of biochemical parameters

The biochemical parameters determined in the *ParaProteX* and *MCC* experiments were used to establish the blood metabolic profile and to draw a unitary conclusion on the general physiological condition of the fish population used in the experiments.

The dynamics of the biochemical indicators determined in the 2-year-old carp juvenile was analyzed in the light of the action of *ParaProteX* and *MCC* mixture on the health of the fish and the establishment of an efficient feeding strategy.

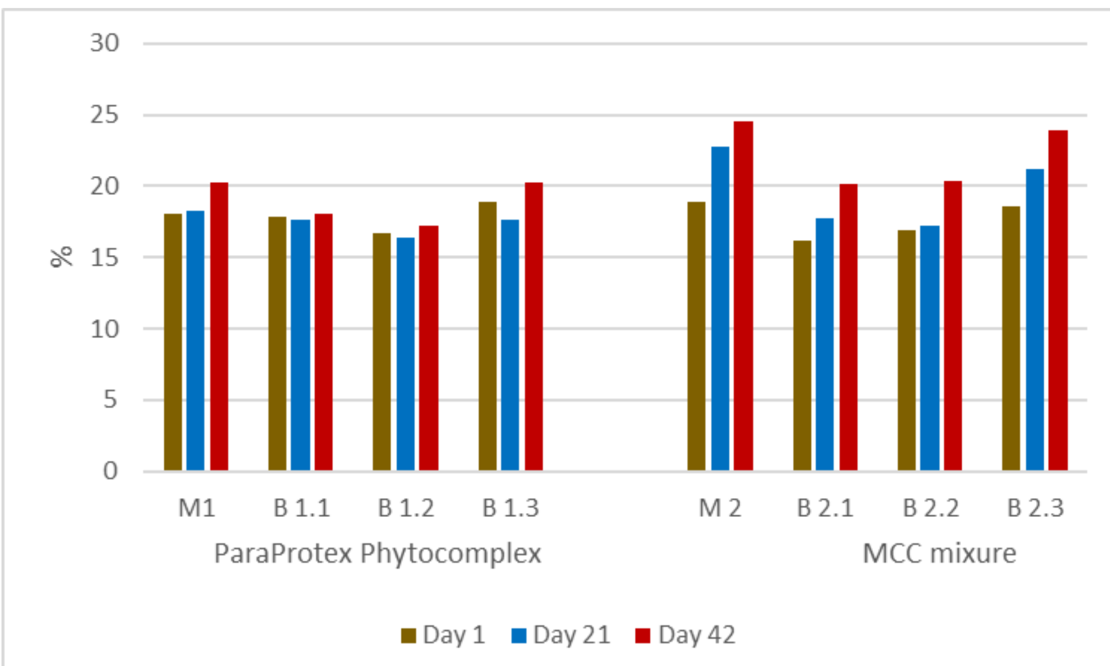

Figure 9. Variation of hematocrit values in fish blood from the two experiments.
(M1, M2- control variant; B1.1., B1.2., B1.3.with Paraprotex; B2.1., B2.2., B2.3. with MCC)

3.2.1. Total protein level

Knowing the total protein level is the most important biochemical indicator of the nutritional status and fish health.

Plasma protein levels in fish are influenced primarily by the protein value of the used feed, as well as by species, age, sex, physiological status, stage of sexual maturity, water temperature.

Total protein is often considered an indicator of the abundance of immune-related proteins and as such total protein increases in fish serum have been often suggested as signs of enhanced immune responses (Ghelichpour *et al*., 2017; Dehghani *et al*., 2020).

Normal values of total serum protein in 2-year-old carp juveniles are between 3.4-4.2 g/dL (Patriche T *et al*., 2010), and in adult carp range between 3.5-5.5 g/dL (Siwieki *et al*., 1993).

Total serum proteins recorded high values at the end of the experiment in all experimental tanks with *ParaProteX,* compared to the values recorded at the beginning of the experiment. It results that the food improved with the *ParaProteX* phytocomplex was well consumed by fish, high values being registered in the experimental tank B 1.3, where the fish was fed with the highest concentration of *ParaProteX*, 6g/100g feed (Figure 10).

Total serum proteins at the end of the experiment are higher in all experimental tanks where *MCC* was used, compared to the values recorded at the beginning of the experiment.

It turns out that the improved food with the *MCC* mixture was consumed by the fish, the best values being recorded in experimental tank B 2.2 (4g/dL), where the fish were fed with feed enriched with *MCC* mixture (4g grape marc + 4g cinnamon + 4g cloves per 100g feed / day / tank) (Figure 10).

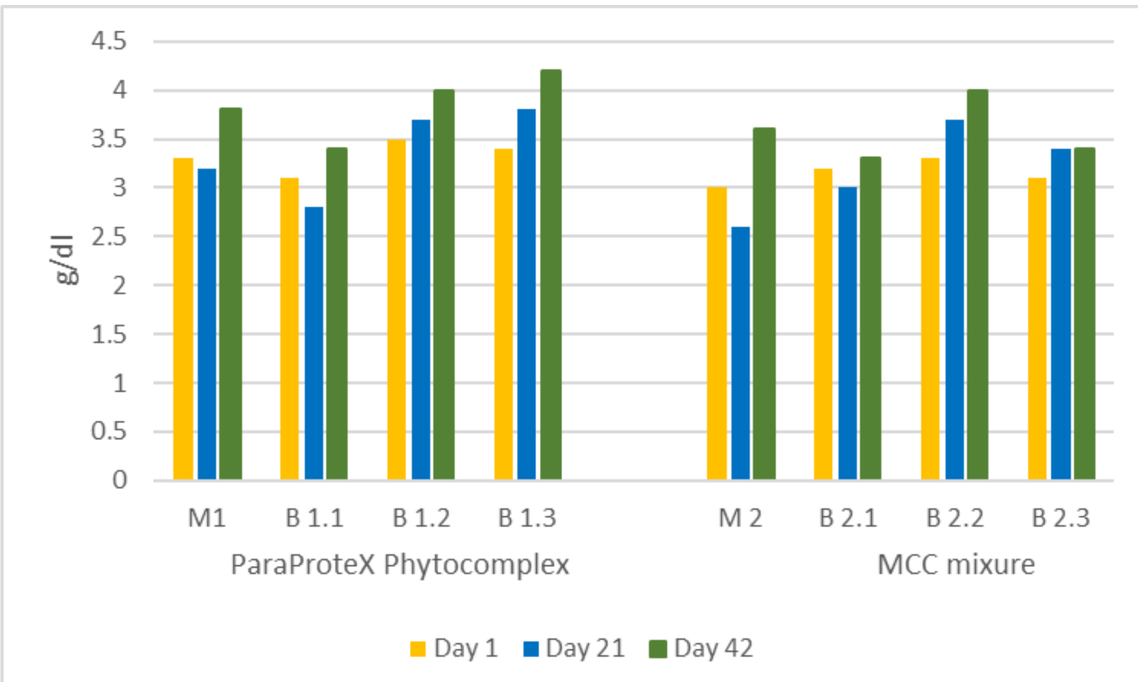

Figure 10. The evolution of total proteins in the blood of fish from the two experiments.
(M1, M2- control variant; B1.1., B1.2., B1.3.with Paraprotex; B2.1., B2.2., B2.3. with MCC)

3.2.2. Serum glucose

Serum glucose is the fastest method of assessing all acute or chronic stress states that occur in fish population.

In experiment 1 (*ParaProteX*) the serum glucose values recorded in the carp species, *Cyprinus carpio*, 2-year-old brood, at the beginning and end of the experiment were within normal values (V.N. = 40-90 mg/dL).

Serum glucose determined in experiment 2 (*MCC*) at the beginning and end of the experiment, recorded values that fell within the normal range indicated in the literature for the carp species, *Cyprinus carpio*, 2-year-old brood (VN = 40-90 mg/dL).

The values determined in the middle of the experiment show a slight increase, due to the stress of handling and overcrowding, as well as the adjustment period necessary to change the diet by incorporating *MCC* mixture (grape marc, cinnamon, cloves) in the feed, indicated by the value of serum glucose, within the range at the end of the experiment (Figure 11).

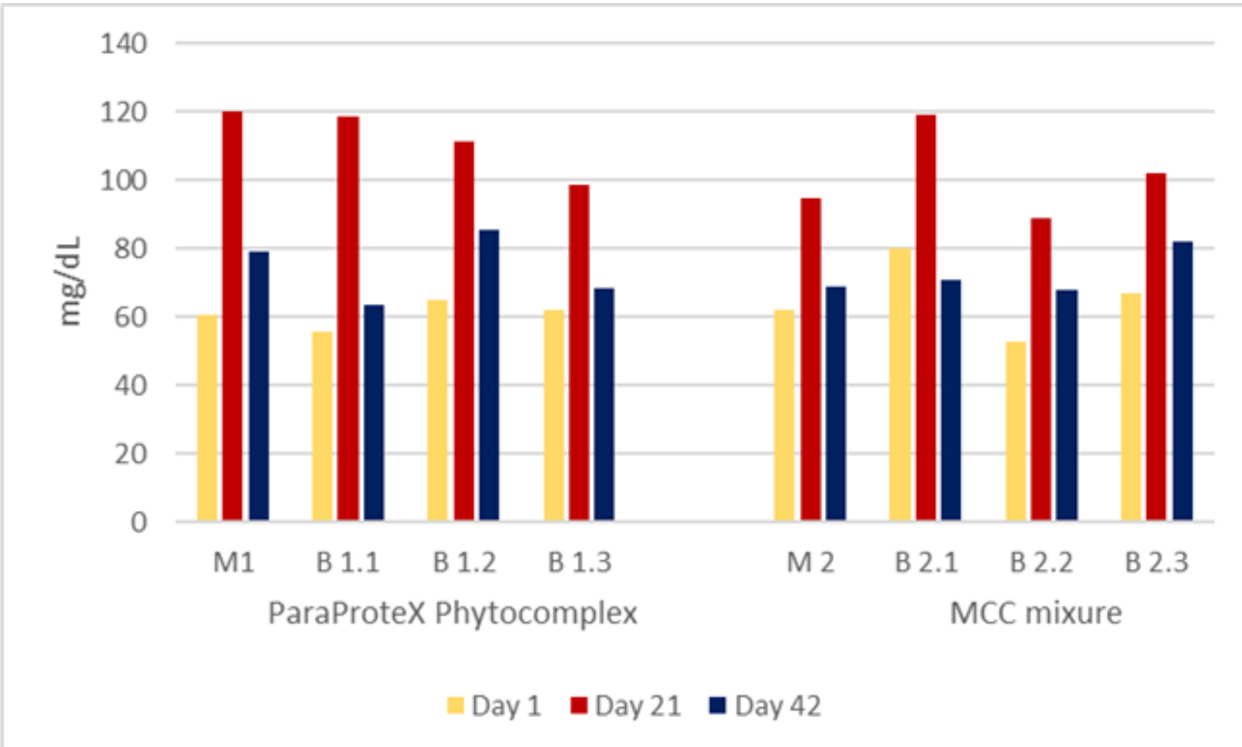

Figure 11. The evolution of serum glucose in the blood of fish from the two experiments.
(M1, M2- control variant; B1.1., B1.2., B1.3.with Paraprotex; B2.1., B2.2., B2.3. with MCC)

3.2.3. Calcium and serum phosphorus

Calcium (Ca) is the most important mineral component with major implications for bone metabolism. Phosphorus (P) is after calcium, the most abundant mineral element in the body. Phosphorus is found in most tissues, but the highest amount is found in bones, so the Ca/P ratio is important in ossification (Patriche *et al*., 2010).

At the beginning of the experiment with ParaProteX mixture, the fish recorded values below the lower limit given by the specialized literature for the 2-year-old carp brood (VN Ca = 12.1-13.3 mg/dL; P = 11.0-17.4 mg/dL (Patriche *et al*., 2010), especially phosphorus. Then, the values increased constantly, at the end of the experiment recording values close to the normal limit. This indicates an intense bone metabolism, specific to the growth period of the carp juvenile and which was stimulated by *ParaProteX* (Table 1).

Calcium and serum phosphorus recorded at the beginning of experiment 2 (*MCC*) values below the lower limit given by the literature, especially for phosphorus, afterwards the values increased constantly, being at the end of the experiment close to the normal limit. This indicates an intense bone metabolism, specific to the carp juveniles growth period, which was stimulated by the *MCC* mixture (grape marc, cinnamon, cloves) (Table 2).

3.2.4. Serum cholesterol

Cholesterol is an important component of cell membranes and the outer layer of plasma lipoproteins (Patriche *et al*., 2010).

At the beginning of experiment 1 with *ParaProteX*, it was within the normal limits indicated by the literature for the 2-year-old carp brood (VN = 116.5-132.1 mg/dL (Patriche *et al*., 2010), then it increased during the experiment, with values above the upper limit at the end of the experiment.

This indicates that the state of stress caused by recirculating aquaponic fish farming technology (handling and overpopulation stress) was maintained throughout the experiment (Table 1).

Serum cholesterol at the beginning of experiment 2 (*MCC*) was within normal limits, then it increased during the experiment, with values above the upper limit at the end of the experiment. This indicates that the state of stress caused by intensive fish farming technology (handling and overcrowding stress) was maintained throughout the experiment (Table 2).

3.2.5. Urea and serum creatinine

Urea is the end product of protein catabolism. Serum urea concentration is an important indicator of liver and kidney function (Patriche *et al.*, 2010).Creatinine comes only from muscle metabolism. The amount of creatinine in the blood depends only on the total muscle mass, therefore it is the least variable blood parameter (Patriche *et al.*, 2010). In the experiment values that were within the normal limits given by the literature for carp

carp for 2 years were recorded (VN Urea = 8.4-11.2 mg/dL; Creatinine = 0.34-0.68 mg/dL (Patriche *et al.*, 2010)), showing that the fish were properly fed (Table 1).

Urea and serum creatinine recorded values that were within normal limits, which tells us that the fish were fed with quality feed, and the addition of natural phytocomplex mixture *MCC* (grape marc, cinnamon, cloves) did not affect metabolic processes of fish and did not cause liver and kidney dysfunction (Table 2).

Table 1. The average values recorded for biochemical indicators - calcium, phosphorus, cholesterol, urea, creatinine in Experiment 1 – *ParaProteX.*

Parameter	Experiment 1 ParaProteX	Experiment 1 Day 1	Experiment 1 Day 21	Experiment 1 Day 42	Standard deviantion
Calcium [mg/dL] /SD	Control Tank 1	9.1 ±0.05	9.3 ±0.1	9.3 ±0.1	± 0.1
	Tank 1.1	9.7 ±0.1	9.6 ±0.1	9.8 ±0.2	± 0.1
	Tank 1.2	9.9 ±0.2	10.1 ±0.2	10.5 ±0.5	± 0.3
	Tank 1.3	**9.6 ± 0.7**	**10.5 ±0.9**	**11.4 ± 1.0**	**±0.9**
Phosphorus [mg/dL] /SD	Control Tank 1	5.1 ± 0.4	4.8 ±0.2	5.5 ± 0.5	±0.4
	Tank 1.1	5.9 ± 0.9	5.5 ± 0.8	7.3 ± 1.0	±0.9
	Tank 1.2	5.2 ± 0.9	6.9 ± 1.0	7.1 ± 1.2	±1.0
	Tank 1.3	**6 ± 0.9**	**6.7 ± 0.9**	**8.1± .1.0**	**±1.0**
Cholesterol [mg/dL] /SD	Control Tank 1	123 ± 24.0	136 ± 25.6	172 ± 26.0	±25.4
	Tank 1.1	116 ± 23.4	145 ± 24.0	165 ± 26.7	±24.6
	Tank 1.2	118 ± 23.9	154 ± 25.8	169 ± 28.6	±26.2
	Tank 1.3	**117 ± 12.1**	**124 ± 12**	**141 ± 13.3**	**±12.3**
Urea [mg/dL] /SD	Control Tank 1	8.0 ± 0.3	7.9 ± 0.3	7.5 ± 0.2	±0.3
	Tank 1.1	7.9 ± 0.3	8.5 ± 0.5	7.7 ± 0.3	±0.4
	Tank 1.2	7.9 ± 1.0	7.9 ± 1.0	6.2 ± 1.0	±1.0
	Tank 1.3	**8.3 ± 0.5**	**9.2 ±0.5**	**8.9±0.5**	**±0.5**
Creatinine [mg/dL] /SD	Control Tank 1	0.30 ± 0.02	0.29 ± 0.02	0.33 ± 0.03	±0.02
	Tank 1.1	0.32 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	±0.01
	Tank 1.2	0.29 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	±0.01
	Tank 1.3	**0.30 ± 0.01**	**0.28 ± 0.01**	**0.30± 0.01**	**±0.01**

Table 2. Average values recorded for biochemical indicators - calcium, phosphorus, cholesterol, urea, creatinine in Experiment 2 - phytocomplex mixture *MCC* (grape marc, cinnamon, cloves).

Parameter	Experiment 2 MCC	Experiment 2 Day 1	Experiment 2 Day 21	Experiment 2 Day 42	Standard deviantion
Calcium [mg/dL] /SD	Control Tank 2	9.5 ±0.2	9.7 ±0.3	10.1 ±0.4	±0.3
	Tank 2.1	9.3 ±0.1	9.5 ±0.1	9.5 ±0.1	±0.1
	Tank 2.2	**9.2 ±1.0**	**9.8 ±2.0**	**11.1 ±3.0**	**±2.0**
	Tank 2.3	9.4 ±0.4	10.3 ±0.2	9.75 ±0.1	±0.5
Phosphorus [mg/dL] /SD	Control Tank 2	5.0 ±0.1	4.9 ±0.1	5.4 ±0.1	±0.3
	Tank 2.1	5.7 ±0.02	5.8 ±0.03	5.6 ±0.02	±0.1
	Tank 2.2	**6.0 ±0.1**	**6.9 ±0.2**	**8.0 ±0.6**	**±1.0**
	Tank 2.3	5.5±0.2	5.7 ±0.2	5.9 ±0.2	±0.2
Cholesterol [mg/dL] /SD	Control Tank 2	119 ±14.2	131 ±15.5	149 ±16.0	±15.1
	Tank 2.1	120 ±26.4	177 ±29.3	157 ±30.0	±29.0
	Tank 2.2	**114 ±5.0**	**113 ±5**	**125 ±8.0**	**±6.7**
	Tank 2.3	112 ±18.4	143 ±21.5	156 ±23.4	±22.6
Urea [mg/dL] /SD	Control Tank 2	8.5 ±0.8	8.3 ±0.8	7.2 ±0.6	±0.7
	Tank 2.1	8.4 ±0.2	8.8±0.2	8.7 ±0.2	±0.2
	Tank 2.2	**8.1 ±0.4**	**8.5 ±0.5**	**7.8 ±0.4**	**±0.4**
	Tank 2.3	8.4 ±0.2	8.7 ±0.2	9.0 ±0.5	±0.3
Creatinine [mg/dL] /SD	Control Tank 2	0.31 ±0.01	0.34 ±0.01	0.32 ±0.01	±0.01
	Tank 2.1	0.35 ±0.01	0.36 ±0.01	0.33±0.01	±0.01
	Tank 2.2	**0.33 ±0.02**	**0.32 ±0.02**	**0.36 ±0.02**	**±0.02**
	Tank 2.3	0.40 ±0.03	0.37 ±0.02	0.34 ±0.03	±0.03

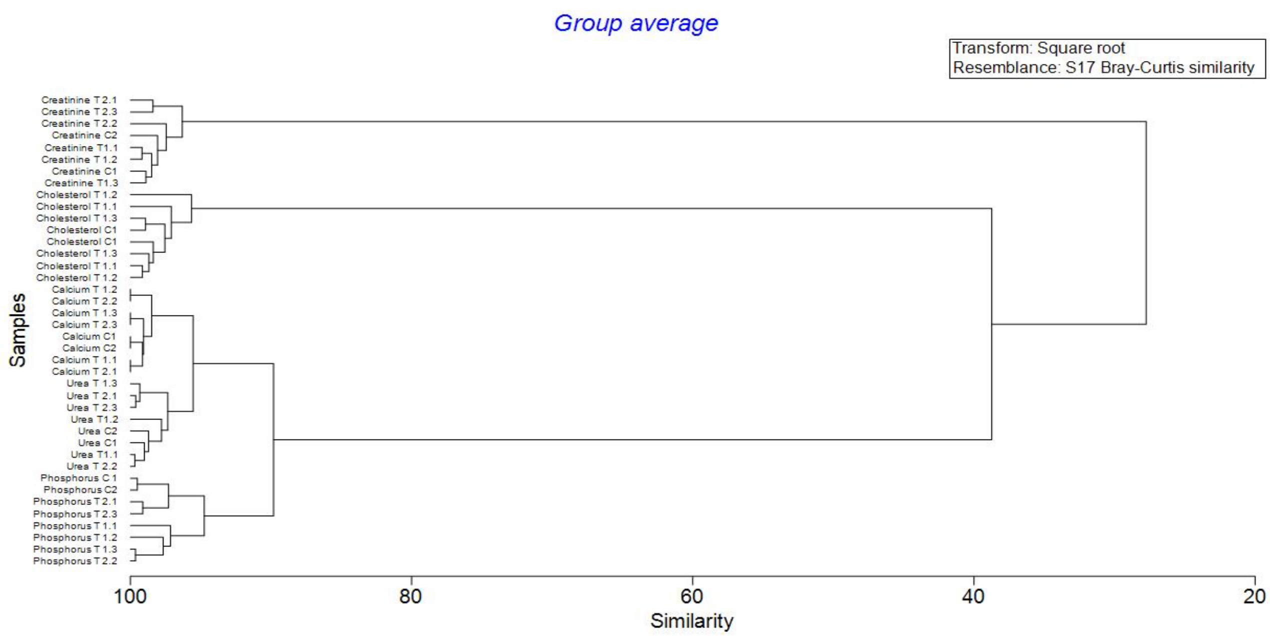

Figure 11. Bray- Curtis matrix – data obtained from the two experiments (square root transformation).

Bray-Curtis matrix showed high similarities (98-100%) between the two experiments in which the two natural phytocomplexes ParaProteX and MCC (grape marc, cinnamon, and cloves) were used (Figure 11).

At the end of the two experiments we can say that the use of the two natural phytocomplexes *ParaProteX* and *MCC* (grape marc, cinnamon and cloves) embedded in fish feed raised in an experimental recirculating aquaponic system type RAS, helped to: grow and develop a healthy biological material, as demonstrated by the values recorded by the hematological and biochemical parameters determined and the improvement of the technological comfort state by reducing the stress and the period of accommodation of the fish to the aquaponic recirculating system. Despite the rich biochemical composition of phytocomplexes used and its multiple bioactivities, the effects synergistic on fish health status are still unknown. The common carp is one of the most popular freshwater fish species cultured throughout the world. It is the fourth most cultured fish with production of more than 4.1 million tons in 2018 contributing 7.7% of the world's total fish aquaculture production (FAO, 2020).

There were no significant differences between the values of the biochemical parameters in the control tank and the values

obtained in the *ParaProteX* and *MCC mixture* tanks.

3.3. Discussions

Aquaponics is a complex cultivation method that has been obtained by combining two different systems, hydroponics and aquaculture. Aquaponic systems operates on the principle that fish waste becomes a resource for plants.

The aim of the experiments was to evaluate the synergistic influence of the combination of standard feed NC 60 II with a content of 32-34% crude protein and a natural plant phytocomplexes, *ParaProteX* mixture, respectively *MCC* mixture (grape marc, cinnamon and cloves) on the health of the 2-year-old carp juvenile for a period of over 42 days. The two mixtures of *ParaProteX* and *MCC* phytocomplexes were chosen for their special antiparasitic, antibacterial, antifungal and antiviral properties.

The dynamics of hematological and biochemical indicators determined in carp juveniles was analyzed in terms of phytocomplexes used in experiments to assess the physiological response of fish to stressful action of technological, physico-chemical factors and to establish a correct and efficient feeding strategy.

A slight decrease in the values recorded for haematological indicators on the 21st day from the beginning of the experiments compared to the values recorded at the beginning of the experiments was observed. Compared to the initial time (day 1) the biological material has undergone a period of adaptation to the new environmental conditions in the RAS type aquaponic recirculation system. Also, the incorporation of *ParaProteX* and *MCC* mixtures in different concentration caused discomfort to the fish population. All hematological parameters recorded at the end of the experiments were within the normal values reported in the literature for the carp species, *C. carpio*.

Total serum proteins recorded values that fall within the normal range of the carp species *C. carpio*, 2-year-old brood. The values recorded for serum proteins at the end of the experiments are increased in all tanks compared to the values recorded at the beginning of the experiments. It turns out that the food improved with the phytocomplexes of *ParaProteX* and *MCC* was well consumed by fish.

Calcium and serum phosphorus recorded at the beginning of the experiments, values below the lower limit given by the literature, especially phosphorus, after which the values increased constantly, registering values close to the normal limit, at the end of the experiments This indicates an intense bone metabolism, specific to the 2-year-old carp hatchery growth period.

Serum cholesterol at the beginning of the experiment was within the normal limits given by the literature, then it increased during the experiment, registering values above the upper limit at the end of the experiment. This indicates that the stress caused by intensive fish farming technology was maintained throughout the experiments.

Urea and serum creatinine recorded values that were within the normal limits given by the literature, which show that the fish were fed with proper feed, the incorporation of the natural phytocomplexes *ParaProteX* and *MCC* in the feed did not cause digestive discomfort to the fish.

4. Conclusions

In conclusion, these variations in the blood parameters underlined the role of the hematology and biochemical parameters in assessing the homeostatic response of fish to different technological conditions. From the data analysis regarding the hematological and biochemical indicators which emphasize the changes on the physiological status assessment of carp reared in recirculating aquaponic system, it appears that natural phytocomplexes mixture can be used for health improvement and remediation, thus improving the resistance to disease of biological material. The natural phytocomplexes used in feeding the carp have contributed to its adaptation and growth in optimal conditions, specific to the recirculating aquaponic system, through the synergistic action of its components with antibacterial, antiviral, antifungal and antiparasitic effect.

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