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Ph.D. THESIS ABSTRACT

"THE GROWTH PERFORMANCE AND NUTRIENT RETENTION EFFICIENCY ON COMMON CARP FINGERLINGS (CYPRINUS CARPIO, LINNE, 1758) IN RECIRCULATING AQUACULTURE SYSTEMS"

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Anexa 2 –Copertă interioară/față

„Program for enhancing performance and innovation in excellent doctoral and postdoctoral research - PROINVENT”

IOSUD – Dunărea de Jos” University of Galaţi
The School for Doctoral Studies in Industrial Engineering

UNIVERSITAS GALATIENSIS

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CONTENT

INTRODUCTION - The opportunity of the approached topic

Aquaculture has been one of the fastest-growing agricultural sectors worldwide over the past two decades. Owing to the fact that aquaculture is a major source of high-quality food (fish and fish products, mollusks, crustaceans, algae, etc.) that complements the nutritional needs of human consumption.

Cultivating fish under controlled conditions in various culture systems significantly alters the ecological conditions for fish in natural basins. In Romania, in addition to traditional growth systems (systematic arrangements represented by ponds, semi-systematic arrangements represented by ponds, reservoirs, etc.) operated under extensive, semi-intensive, and intensive regimes, super-intensive fish farming systems have been successfully used lately. The most well-known of these systems are recirculating aquaculture systems (RAS).

The general objective of the doctoral thesis was to optimize the health status and growth performance of common carp fry in recirculating aquaculture systems (RAS) through the evaluation of various growth conditions (water quality, population density, feed quality, feed ration).

The specific objectives, established in accordance with the research program, aimed to conduct experiments in a laboratory-scale recirculating system and test technological elements under conditions that closely resemble the real production environment in a pilot-scale recirculating system. The purpose was to establish relevant technical management parameters for the growth of common carp fry. These specific objectives included:

- Establishing optimal stocking densities for the growth of common carp fry in semi-industrial/industrial complex recirculating systems.
- Selecting the best feeding management practices to enhance nutrient retention in the culture biomass.
- Validating the optimal technological model in terms of productive performance by evaluating the health status and condition of common carp fry at the two tested technological maturity levels (laboratory scale and pilot scale).
- Identifying technological differences when transitioning from the laboratory to the pilot system level.
- Practicing biosecurity measures throughout the experimental period.
- Evaluating nutrient retention efficiency from feeds in the flesh of common carp fry.

All the experiments were conducted in small-scale recirculating aquaculture systems (laboratory level) and medium-scale systems (pilot level). Through the addressed issues and the obtained results, numerous clarifications have been provided regarding the optimization of common carp fry growth technologies. The technological indicators obtained can serve as important benchmarks for improving the profitability of the fish farming sector in Romania.

PART I. STATE OF KNOWLEDGE IN THE FIELD
CHAPTER 1. STRATEGIC FRAMEWORK FOR AQUACULTURE DEVELOPMENT

1.1. Aquaculture - History, Current State

Aquaculture has become a crucial concern, fulfilling the growing global demand for aquatic products, becoming a significant source of income for millions of people worldwide. In addition to producing high-quality food for human consumption, aquaculture also helps reduce the anthropogenic pressure on wild fish stocks in both freshwater and marine environments.

Excluding algae, global production from fishing and aquaculture has shown a growth trend from 2000 to 2020, reaching 178 million tonnes in 2020, a slight increase of 0.2% compared to 2019 but a decrease of 0.7% from the record of 179 million tonnes in 2018 (FAO 2022). In Romania, in 2020, 20.2 thousand tonnes of fish and mollusks were recorded through fishing and aquaculture (Table 1.1).

Table 1.1 - Structure of capture and aquaculture production by species groups and geographical regions in 2020 (thousand tonnes) [FAO 2022].

Zone	Marine fish	Freshwater fish	Mollusks	Crustaceans.	Diadromous fish	Other aquatic animals	Total
Global	69 076,5	58 457,7	23 901,8	17 250,4	7 487,1	1 583,2	177 756,8
Africa	6 627,8	4 909,6	260,8	217,3	26,9	1,8	12 044,2
America	13 923,6	1 560,2	2 280,8	2 424,5	1 607,7	106,5	21 903,2
Asia	34 889,5	51 353,9	20 144,2	13 924,2	3 214,8	1 433,6	124 960,2
Europa	12 225,4	619,1	1 036,1	638,7	2 549,4	27,1	17 095,7
Oceania	1 409,2	14,9	180,0	45,7	88,3	14,3	1 752,5
Romania	0,2	12,4	4,2	0,0	3,3	0,0	20,2

In 2020, approximative 90.3 million tonnes of aquatic organisms were harvested through fishing, accounting for 51% of the total production, a decrease from the peak of 96.5 million tonnes in 2018. On the other hand, aquaculture accounted for 49% of the global total, amounting to 87.5 million tonnes.

The distribution of aquaculture production by continent varies, with Asia accounting for over 62%, Europe and Africa for 19%, and Oceania for 13%. In Romania, the fish caught through fishing in marine and inland waters increased from 2,700 tonnes to 8,000 tonnes during 2010-2020, while aquaculture production ranged from 9,700 to 12,200 tonnes during the same period (Table 1.2).

1.2. Growth of cyprinids internationally

The Cyprinidae family is one of the largest teleost fishes, comprising over 2000 species and over 200 genera (Hoole D. et al., 2001). Cyprinids are raised on almost all continents, including tropical, subtropical, and temperate regions. Among the species of cyprinids, carp from the Chinese complex are grown in Asia, Europe, and North America, mainly through semi-intensive systems but also extensive systems (in Asia) and intensive systems (in Europe).

Analyzing the dynamics of global cyprinid production from 2011 to 2019, as shown in Table 1.3, a consistent upward trend in production and earnings is observed. In 2011, a production of 59.8 million tonnes generated 154.8 billion USD, while in 2019, a production of 85.3 million tonnes yielded over 259.5 billion USD. Globally, in 2019, the sale of over 4.4 million tonnes of carp alone generated over 9 billion USD in revenue.

In 2019, Romanian aquaculture production was 12,848 tonnes, representing 0.94% of the EU production of over 1.36 million tonnes. The value of the commodity production was 31.8 million euros, accounting for 0.64% of EU revenue (Table 1.4).

Table 1.2- Dynamics of global cyprinid aquaculture production during 2011-2019 Q = t, V = USD 1000, [FAO, 2019].

Species		2011	2012	2013	2014	2015
Total global	Q	59 789 006	63 480 442	66 952 001	70 506 397	72 776 132
	V	154 793 562	169 771 629	191919 161	210 890 845	206 741 676
		2016	**2017**	**2018**	**2019**	
	Q	76 474 450	79 497 331	82 304 694	85 335 990	
	V	223 784 898	238 697 221	248 669 603	259 547 487	
Cyprinus carpio		**2011**	**2012**	**2013**	**2014**	**2015**
	Q	3 347 619	3 493 956	3 693 152	3 866 276	4 025 747
	V	6 010 570	6 690 898	7 470 252	8 269 983	8 358 118
		2016	**2017**	**2018**	**2019**	
	Q	4 054 802	3 859 635	4 222 839	4 411 900	
	V	8 538 248	8 167 128	8 792 843	9 046 263	

1.3. Growth of cyprinids in Romania

Extensive carp farming benefits the natural environment by improving its quality and retaining surface water, providing a habitat for protected fauna and flora species, and preserving biological diversity (Dobrowolski, 1995; Turkowski et al., 2007; Guziur, 2009).

The demand for cyprinids in Romania is relatively high. From 2010-2019, the total production increased from 8,981 tons to 12,848 tons, with a corresponding increase in value from 26.2 million USD to nearly 35.7 million USD. Carp remains the most important species for aquaculture in Romania. During the same period, carp production increased from 2,888 tons in 2010 to 4,191 tons in 2019 (Table 1.4).

*Table 1.4 - Total Aquaculture Production and Carp Production in Romania, 2010-2019 (Q=t, V=USD*1000), [FAO, 2019]*

Species		2010	2011	2012	2013	2014
Total production (fish, crustaceans, mollusks, etc.)	Q	8 981	8 353	10 004	10 146	10 680
	V	26 269	22 095	23 315	27 419	25 507
		2015	**2016**	**2017**	**2018**	**2019**
	Q	11 042	12 574	12 798	12 298	12 848
	V	24 303	30 637	37 011	36 142	35 680
Carp production (Cyprinus carpio)		**2010**	**2011**	**2012**	**2013**	**2014**
	Q	2 888	2 652	3 266	3 395	3 737
		2015	**2016**	**2017**	**2018**	**2019**
	Q	4 349	4 841	4 539	4 357	4 191

The production of cyprinids in Romania is regulated by specific legislation, and aquaculture activities require mandatory authorization. To obtain approval, operators must comply with quality and food safety standards, as well as conduct regular monitoring of water quality and fish health.

CHAPTER 2. ECOPHYSIOLOGICAL CHARACTERISTICS OF COMMON CARP

2.1. Taxonomy Elements

Common carp is a freshwater fish native to Eurasia, and it has been introduced to almost every corner of the world except for northern Asia and the polar regions. It is considered to be the oldest domesticated fish species.

Classification scientific:

Kingdom: Animalia
Phylum: *Chordata*
Subphylum: *Vertebrata*
Infraphylum: *Gnathostomata*
Nanophylum: *Pisces*
Superclass:*Osteichthyes*
Class: Actinopterygii
Subclass: *Neopterygii*
Infraclass: *Teleostei*
Superorder: *Ostariophysi*
Order: *Cypriniformes*
Suprafamilie: *Cyprinoidea*
Family: *Cyprinidae*
Subfamily: *Cyprininae*
Genus: *Cyprinus*
Species: *Cyprinus carpio*

2.1.1. Species, subspecies, varieties

The genus Cyprinus comprises five species: *Cyprinus carpio*, *Cyprinus micristius*, *Cyprinus rabaudi*, *Cyprinus*, and *Cyprinus mirrus*, of which the first one is found in Europe, while the latter is exclusively found in East Asia. Within the species *Cyprinus carpio*, three subspecies are specified: Cyprinus carpio haematopterus, Cyprinus carpio viridiviolaceus, and Cyprinus carpio carpio, with the last one also present in our waters (S. Stăncioiu, 1978).

2.1.2. Cultivated varieties

In addition to wild carp found in natural habitats, several cultivated forms of carp are raised in fish farms, belonging to two main races: the Lausitz race (with scales covering the body) and the Galicia race (with a body partially or completely devoid of scales). All cultivated carp forms differ, primarily in body shape, presence and arrangement of scales, and the value of the profile index.

2.2. Ecology and Biology

In the lower course of the Danube River, the common carp is a semi-migratory species that enters the ponds early in the spring (at 7-8ºC) and returns to the Danube when the water levels decrease. This retreat is determined by the decrease in water levels and the pronounced rise in temperature (24-26ºC).

Regarding the feeding habits of the common carp, initially, after the absorption of the yolk sac, the fry feed on zooplankton and phytoplankton, and from a length of 20 mm, they also start consuming benthic organisms. As they grow older, their benthophagous nature becomes more pronounced, and their nutritional spectrum widens. They do not feed during the winter.

CHAPTER 3. - RECIRCULATING AQUACULTURE SYSTEMS

3.1. General considerations

Recirculating aquaculture systems can be used at various intensity levels, depending on the amount of reused water and the stocking density of the culture material. In comparison, a traditional growth system, where water passes through the farm only once before being discharged, typically uses around 30 m3 of water per kilogram of produced fish, which is approximately 100 times more water compared to a typical RAS (Recirculating Aquaculture System) (FAO, 2022) (Figure 3.1, Figure 3.2).

Figure 3.1 - Recirculating Aquaculture System (RAS) in Indoor Facilities

Figure 3.2 - Recirculating Aquaculture System (RAS) in Outdoor Facilities

3.2. Components of a recirculating aquaculture system

- **Growth tanks**

Ensuring an appropriate environment in the growth tank is essential to meet the needs of the fish, both in terms of water quality and tank design. The correct design, including size, shape, water depth, and self-cleaning capacity, can significantly impact the growth performance of the fish.

- **The mechanical filter**

Mechanical filtration of water discharged from fish tanks has proven to be the practical and most efficient solution for removing organic waste. Almost all fish farms recirculate the water discharged from the tanks through a micro screen system equipped with a filter mesh typically ranging from 20 to 100 microns in size. This system effectively traps and removes solid particles and organic debris, improving water quality and reducing environmental impact.

- **The biological filter**

Not all organic matter is eliminated in mechanical filtration, as the finest particles and dissolved compounds, such as phosphorus and nitrogen, may pass through the filter. The breakdown of organic matter and ammonia is a biological process carried out by bacteria in the biofilter. Heterotrophic bacteria decompose organic matter by consuming oxygen and producing carbon dioxide, ammonia, and sludge. Nitrifying bacteria convert ammonia into nitrite (NO_2^-) and nitrate (NO_3^-).

- **The degassing unit**

Degassing reduces carbon dioxide and nitrogen to lower levels than can be achieved through simple aeration or overflow when complete gas removal to reach a saturation level below 100% is impossible. A vacuum degasser is used to extract gases from the water, thereby reducing the gas saturation to lower levels than can be achieved with traditional degassing methods.

- **UV treatment station**

Treatment with ultraviolet (UV) light aims to damage the DNA of biological organisms by applying a specific wavelength. In aquaculture, this treatment targets primarily pathogenic bacteria and unicellular organisms. The use of UV treatment for medical purposes has been widespread for several decades. It does not have a negative impact on fish, as the UV water treatment is performed in an enclosure protected from UV radiation outside the fish production area.

- **Oxygenation station**

Water aeration is a process in which oxygen is added to water through the exchange of gases between the water and the air in the environment. This process aims to maintain the oxygen balance in the water at approximately 100% saturation. In aquaculture facilities, the oxygen content decreases due to the respiration of the fish, typically reaching around 70-80%.

- **Ozone station**

The ozone treatment represents an efficient method of destroying unwanted organisms through the strong oxidation of organic matter and biological organisms. In this technology, microparticles are broken down into molecular structures that recombine and form larger particles, a process called coagulation. These larger particles are then captured in RAS filtration systems instead of passing through as microscopic particles. This technology, also known as water purification (disinfection), makes the water clearer and reduces the adherence of suspended solids and bacteria.

- **pH regulator**

The nitrification process in the biofilter produces acid, leading to a decrease in pH level. To maintain a stable pH in recirculating aquaculture systems, adding a base to the water is necessary. In most RAS systems, the pH ranges between 6.5 and 7.5, often stabilizing around 7.0. A higher pH within this range promotes nitrification in the biofilter, while a lower pH facilitates carbon dioxide removal through degassing. Sodium hydroxide (NaOH), or caustic soda or lye, is commonly used for pH adjustment. Alternatively, calcium hydroxide (Ca(OH)2), known as slaked lime, can be used.

- **Pumps**

To recirculate the water, various types of pumps are used. Pumping water typically requires a significant amount of electrical energy. Therefore, using efficient pumps and installing them correctly is important to keep costs at a minimum level.

- **Monitoring, control, and alarm systems**

Intensive fish farming requires careful monitoring and strict control of conditions to maintain optimal production and avoid significant losses. Technical failures can have serious consequences, which is why alarm systems are vital for ensuring operational safety.

3.3. Technical conditions regarding water quality in RAS

The quality of water in fish farming units has a significant impact on fish production. Fish have adapted to live in a relatively limited range of waters with different physicochemical properties (temperature, pH, dissolved oxygen, hardness, etc.). There are considerable variations among species in their tolerance range for these water parameters to the extent that certain water conditions may be optimal for one species but physiologically stressful for another. Adjusting the physical and chemical parameters ensures optimal conditions to achieve satisfactory fish growth rates.

Building and operating a recirculating aquaculture system is a costly investment. There is strong competition in the fish market, and to make a profit, production needs to be high. Selecting the right species and constructing an efficient system is crucial. The goal is to sell the fish at a high price while keeping production costs as low as possible.

PART II. EXPERIMENTAL ACTIVITY
CHAPTER 4. - MATERIALS AND RESEARCH METHODS

4.1. Research Infrastructure

The experimental research was conducted in two recirculating aquaculture systems represented by glass aquariums, namely the Laboratory Technological Level (LTL) and the Pilot Technological Level (PTL). This research infrastructure belongs to the Department of Food Science, Food Engineering, Biotechnology, and Aquaculture, Faculty of Food Science and Engineering at the "Dunărea de Jos" University in Galați.

The first growth system consists of 12 glass aquariums with a volume of 0.132 m^3 each (Figure 4.1). The water supply to the system is carried out by DAB A 80 180 XM pumps after sterilization and disinfection processes. Water sterilization is performed using the Tetra Quiet UV-C 35000 system. Oxygenation is achieved using a Fiap Air Active 1000 compressor, which introduces an air flow rate of 8400 l/h, evenly distributed in all the aquariums.

To ensure the removal of solid waste, the growth units are connected to two settling tanks, and through a pump, the water is sent to the mechanical filter composed of sponges, gravel, and sand with different granulations. After the mechanical filter retains the solid waste, the water is pumped to the biological filtration stage, which is achieved using bactoballs.

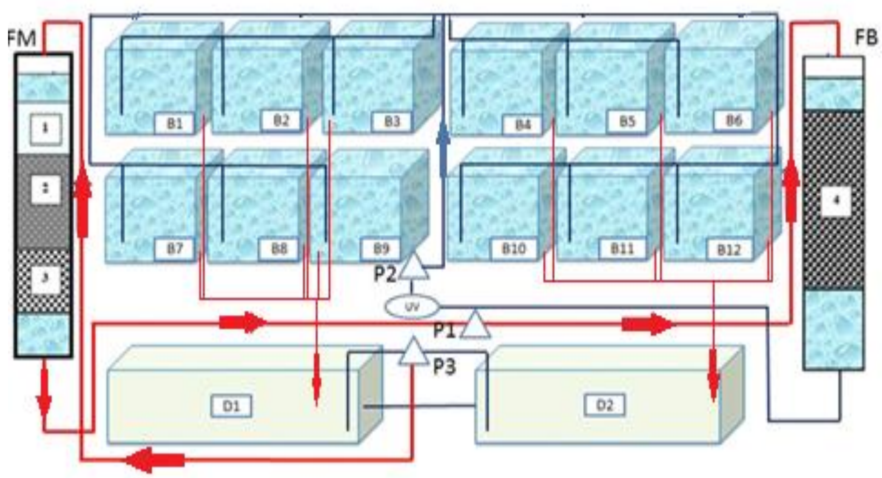

Figure 4.1- Experimental Recirculating Aquaculture System

B_1 - B_{12} – Fish growth units, D_{1-2}– Settling tanks, P_1, P_2, P_3 – pumps, UV - Sterilization lamp, FM- Mechanical filter, FB- Biological filter, 1 – Sponge, 2 – sad, 3 – gravel, 4 – bactoballs.

Water supply to the growth units

Water discharge from the growth units

The second experimental system relied on larger growth units, specifically tanks with a useful volume of 1 m^3 each. This more advanced pilot system was developed at the Romanian Center for Modeling of Recirculating Aquaculture Systems in Galați, Romania (abbreviated as MoRAS). The pilot recirculating system consists of three modules, each with a capacity of 8 m^3; each module contains eight growth units with an equal capacity of 1 m^3 (Figure 4.2).

The biomass monitoring system facilitates fish growth control, and feeding management is carried out using automated feeding equipment.

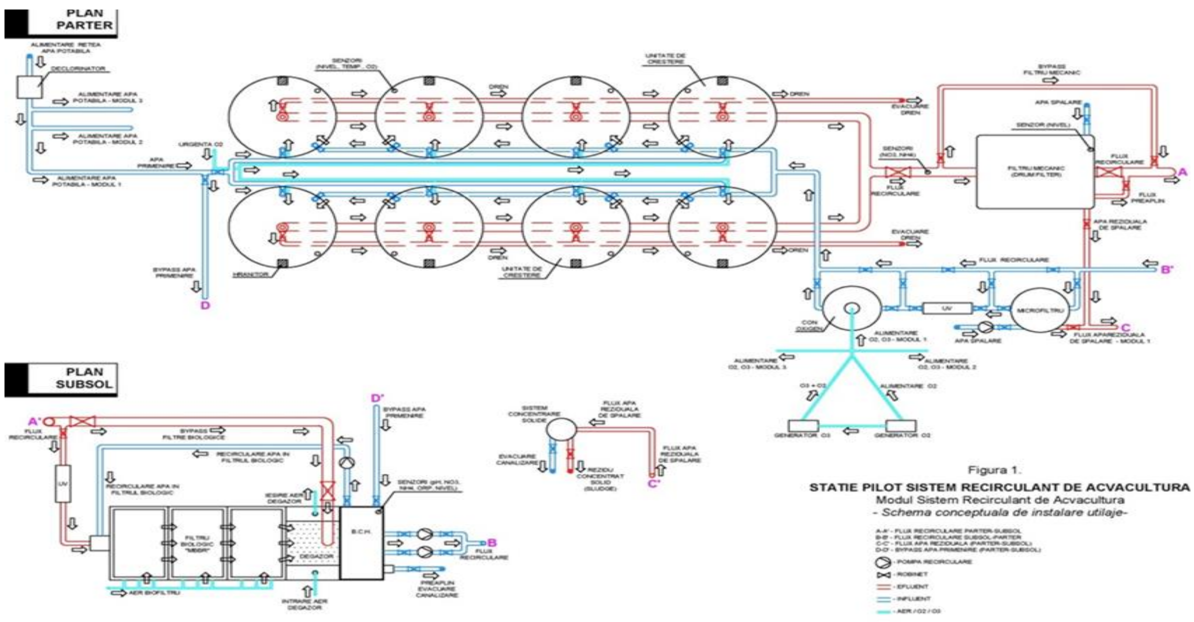

Figure 4.2 - Module of the Recirculating Aquaculture System (developed within the MoRAS project)

The pilot station is connected to the networks for water supply, electricity, and wastewater, and it is equipped with generators for oxygen, ozone, and electricity, as well as dechlorination filters and systems for concentrating solid waste from the water resulting from mechanical washing.

4.2. Biological Material

The chosen cultured species was the common carp. The initial experiments were conducted with carp fry obtained through natural reproduction in a pond during the summer of 2018. All individuals used had an approximate length of 4 cm and an average weight of 1.5 g.

To minimize stress and the possibility of injury, the fish were transported in open containers with the addition of oxygen. Prior to the start of the experiments, the fish were treated with malachite green (5 ml per 200 liters of water for 60 minutes) to eliminate ectoparasites from their skin. Subsequently, the fish were acclimatized for two weeks in a well-oxygenated quarantine tank. After acclimatization, the fish were transferred to the growth units to commence the experiments.

4.3. Water Quality Monitoring

Throughout the experimental period, special attention was given to monitoring the physicochemical parameters of water. For this purpose, daily monitoring of temperature, dissolved oxygen (DO), and pH was conducted. Concentrations of $N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$, and $P\text{-}PO_4$ were determined on a weekly basis. pH, dissolved oxygen, turbidity, and temperature were monitored using the YSI Pro O DO multiparameter instrument and the portable WTW Multi 3410 multiparameter instrument.

4.4. Technological indices for characterizing fish growth performance

At the end of each experiment, after weighing and measuring each individual fish, parameters were calculated to assess technological performance indicators.

- Actual growth rate (Sr) is defined as the difference between the final biomass (Bf) and the initial biomass (Bi). Sr = Bf - Bi (grams).

- Daily growth rate (GR) is determined by dividing the difference between the final biomass (Bf) and the initial biomass (Bi) by the number of days of growth (t): GR = (Bf - Bi) / t (g/day).

- Feed Conversion Ratio (FCR) is the ratio of the amount of feed (F) consumed to the growth increment of the fish (Sr). FCR = F / Sr (kg feed/kg growth).

- Specific Growth Rate (SGR) is expressed as a percentage and represents the daily biomass growth. It is determined using the formula: SGR = 100 × (ln Bf - ln Bi) / t (%/day).

- Protein Efficiency Ratio (PER) is calculated as the ratio of the growth increment (Sr) to the amount of feed consumed (F) multiplied by the crude protein of the feed (PB) (%). It is also known as the protein efficiency ratio. PER = (Wf - Wi) / (F * Pb) (g/g).

- Protein Utilization Efficiency (PUE) is calculated using the formula: PUE = 100 * (Wf * Pf - Wi * Pi) / (F * Pb) (%), where: Pi is the muscle tissue protein content at the beginning of the experimental period (%), Pf is the muscle tissue protein content at the end of the experimental period (%), Wi is the initial biomass (kg), Wf is the final biomass (kg), F is the total amount of feed consumed (kg), Pb is the protein concentration in the feed (%).

- The length-weight correlation was determined using the growth equation (Ricker, 1975): W = a * Lb, where "a" represents the intercept (initial growth coefficient) and "b" is the allometric coefficient, W is the body weight, L is the total length of the fish.

- In addition to the value of the allometric coefficient b, the value of R2 was also obtained, which indicates the homogeneity of the variable under consideration; the closer R2 is to 1, the more applicable the condition of b is for all individuals included in the calculation.

- The coefficient of variation (CV, %) was calculated as the ratio of the standard deviation to the mean weight to measure dispersion among the fish.

4.5. Evaluating physiological status

Hematology represents an important branch in fish medicine, helping researchers better understand and assess the health status of different fish species. Hematological indices, such as red blood cell count (RBC x 106 cells/µl of blood), hemoglobin (Hb - g/dl), hematocrit (Ht - %), and erythrocyte constants - mean corpuscular volume (MCV - μm^3), mean corpuscular hemoglobin (MCH - pg), and mean corpuscular hemoglobin concentration (MCHC - g/dl) - are important parameters for evaluating the overall physiological status of fish. Changes in these parameters can be a response to various environmental conditions (Bocioc E. et al., 2015).

4.6. Determining the biochemical composition of fish meat and feed

To determine the biochemical composition of the carp meat, biological samples were collected at the beginning and at the end of the experiment. When collecting the samples, care was taken to ensure uniformity among the specimens to eliminate errors caused by differences in mass. The biochemical determinations were performed on whole fish samples with scales homogenized using a mortar, and representative aliquots were extracted from the obtained mixture. Additionally, the biochemical composition analysis was conducted on all types of feeds used throughout the experimental period to certify the declared ingredients in the feed recipe.

4.7. Methods of statistical data processing.

Excel 2010 for Windows and SPSS 21.0 for Windows were used for statistical analysis of the data. To test the statistical differences between variables, the t-test for comparing means with a significance level of $p<0.05$ and also the ANOVA test was used.

If significant differences were identified, in the data sets, posthoc testing was performed to determine subsets using the Duncan test. The Kolmogorov-Smirnov test was applied to assess the normality of the data used in the analysis.

CHAPTER 5 - THE INFLUENCE OF POPULATION DENSITY ON THE GROWTH PERFORMANCE OF CARP FRY

5.1. Experiment 1 - The influence of density on the growth performance of carp fry in a laboratory-scale recirculating aquaculture system.

5.1.1. Introduction

During the growth process, fish are exposed to various sources of stress caused by inadequate living conditions, repeated handling, fishing activities, etc. Combining a range of indicators to assess the effect of density on fish welfare and growth performance is the most reliable method to determine whether density negatively impacts on cultured fish.

5.1.2. Material and Methods

For this experiment, 2100 fish with an average initial individual body mass of 1.8 g/fish were distributed into the growth units of a recirculating aquaculture system (RAS) in four density treatments: V_1 - 70 fish with an initial stocking density of 0.9 kg/m^3, V_2 - 140 fish with an initial stocking density of 1.8 kg/m^3, V_3 - 210 fish with an initial stocking density of 2.6 kg/m^3, and V_4 - 280 fish with an initial stocking density of 3.5 kg/m^3. The experiment was conducted in triplicate growth units with 132 liters.

The fish were fed three times a day with an extruded granulated feed 50/14 (containing 50% crude protein and 14% fat) at a daily feeding rate of 5% of their body weight (5% * BW). The biochemical composition of the feed used is presented in Table 5.1.

Table 5.1- Feed Composition

Ingredients	U.M	Value
Protein	%	50
Fat	%	14
Cellulose	%	2
Lysine	%	2,5
Phosphorus	%	1
Copper	mg/kg	6
Vitamin A	IU/kg	20 000
Vitamin D3	IU/kg	2 000
Vitamin E	mg/kg	200
Vitamin C	mg/kg	200

Figure 5.1- *Cyprinus carpio* [original]

Water quality parameters such as dissolved oxygen, temperature, and pH were monitored daily using the multiparameter instrument described in Chapter 4 - Materials and Methods. Additionally, the concentration of nitrogen compounds was measured weekly using a spectrophotometer and Merck test kits. To maintain water quality, all growth units were cleaned twice a week, with approximately 75% of the water volume being replaced with fresh water from the city supply, previously dechlorinated. At the end of the trial, somatic measurements were performed on 50 fish from each experimental variant.

5.1.3. Results and discussions

- **Monitoring of water quality parameters**

The values of the physicochemical parameters of the water during the experiment are presented in Table 5.2. Analyzing the table, it can be observed that the importance of the physicochemical parameters were influenced by fish density and the amount of feed provided. However, statistically, there were no significant differences among the experimental variants.

The water temperature averaged 21.2±0.18˚C in V_1 and 21.6±0.14˚C in V_4. Throughout the 31 days of growth, the water temperature fluctuated within the range of 20.5-22.5˚C. Maintaining constant temperatures during growth is a major advantage of recirculating systems in enclosed facilities. The temperature range of 20-23˚C falls within the optimal growth conditions for the fish.

The water pH in the growth system was consistently maintained between 7-8 units, with the lowest values recorded in V4 (7.29±0.10). Throughout the 31 days of growth, the water pH fluctuated within 6.9-8.0 units. From a statistical point of view, no significant differences were observed among the four variants (verified through ANOVA, $p>0.05$). The concentration of hydrogen ions (water pH) is an important parameter influencing nutrient absorption capacity.

Dissolved oxygen in the water varied with average values of 7.05±0.08 mg/l in V_4 and 7.71±0.12 mg/l in V_1. Throughout the 31 days of growth, dissolved oxygen fluctuated within the range of 7.0-8.6 mg/l, which is optimal for the development of carp. From a statistical point of view, no significant differences were observed among the four variants (verified through ANOVA, $p>0.05$).

Nitrogen compounds are fundamental indicators of environmental quality. Deviations beyond the optimal limits can often lead to fish health problems and even mortality, mainly caused by nitrites or ammonia.

The average concentrations of ammonium ions ($N\text{-}NH_4+$) ranged from 0.17±0.02 mg/l in V_1 to 0.22±0.05 mg/l in V_4. Over the four weeks of growth, $N\text{-}NH_4+$ fluctuated within the range of 0.12-0.27 mg/l, within permissible concentrations for carp growth.

In an aquaculture system, the amount of nitrates produced is directly proportional to the fish population density (Endut et al., 2011). The average concentrations of nitrates ($N\text{-}NO_3-$) ranged from 17.9±0.65 mg/l in V_1 to 21.13±0.20 mg/l in V_4. Over the four weeks of growth, $N\text{-}NO_3-$ fluctuated within the range of 17.0-22 mg/l, which is permissible for carp growth.

Nitrites ($N\text{-}NO_2-$), more toxic than nitrates compounds, remained within permissible limits. The average concentrations for the four variants ranged from a minimum of 0.03±0.02 mg/l in V_1 to 0.05±0.02 in V_3. Over the four weeks of growth, $N\text{-}NO_2-$ fluctuated within the range of 0.02-0.06 mg/l, which is permissible for carp growth.

- **Analysis of the growth performance of carp fry**

Population density's influence on carp fry's growth performance in the RAS can be quantified by analyzing various technological indicators such as growth increment, daily growth rate, specific growth rate, feed conversion ratio, protein efficiency ratio, etc. (Table 5.3).

At the end of the experiment, the correlations between mean total length (TL) and mean total body weight (W) were analyzed to gain further insights into the growth pattern of the fish. The regression curves, obtained from a representative sample of each experimental variant, were plotted with the respective equations. The coefficient values (b) indicated a negative allometric growth, suggesting that the growth of the fish is more dependent on length rather than weight. In this experiment, a strong correlation was observed between length and weight, with all coefficient of determination values (R^2) greater than 0.80. The coefficient of determination (R^2) indicates the homogeneity of the variables considered; the closer R^2 is to 1, the more the condition of b applies to all the specimens considered, indicating a more homogeneous group.

Table 5.3- Technological performance indicators obtained at the end of the experimental period

Experimental Variant	V_1			V_2			V_3			V_4		
Calculated Parameters	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**	**A12**
Initial Biomass (g)	126	126	126	252	252	252	378	378	378	504	504	504
Initial Biomass (kg/m^3)	0,9	0,9	0,9	1,8	1,8	1,8	2,6	2,6	2,6	3,5	3,5	3,5
Final Biomass (g)	312	308	321	507	532	524	671	712	693	851	880	908
Final Biomass (kg/m^3)	2,2	2,2	2.2	3,5	3,7	3,7	4,7	5,0	4,9	6,0	6,2	6,4
Biomass Growth (g)	186	182	195	255	280	272	293	334	315	347	376	404
Biomass Growth (kg/m^3)	1,3	1,3	1,4	1,8	2,0	1,9	2,1	2,3	2,2	2,4	2,6	2,8
Initial Fish number	70	70	70	140	140	140	210	210	210	280	280	280
Final Fish number	68	67	69	119	122	125	172	178	165	216	221	228
Survival Rate (%)	97	96	99	85	87	89	82	85	79	77	79	81
Initial Average Weight (g/ex)	1,8	1,8	1,8	1,8	1,8	1,8	1,8	1,8	1,8	1,8	1,8	1,8
Final Average Weight (g/ex)	4,6	4,6	4,7	4,3	4,4	4,2	3,9	4,0	4,2	3,9	4,0	4,0
GR (Daily Growth Rate) (g/day)	5,3	5,2	5,6	7,3	8,0	7,8	8,4	9,5	9,0	9,9	10,7	11,5
SGR (%/day)	2,9	2,9	3,0	2,3	2,4	2,4	1,9	2,0	2,0	1,7	1,8	1,9
Individual Growth (g)	2,8	2,8	2,9	2,5	2,6	2,4	2,1	2,2	2,4	2,1	2,2	2,2
Total Feed Distributed (g)	345	345	345	691	691	691	1036	1036	1036	1382	1382	1382
FCR (g feed/g biomass growth)	1,86	1,90	1,77	2,71	2,47	2,54	3,54	3,10	3,29	3,98	3,68	3,42

The coefficient of variation (CV) showed a variation ranging from 20-40%, corresponding to a nearly homogeneous batch (V_1 - 24.47±3.11%) and moderately homogeneous batches (V_2 - 32.66±7.06%, V_3-32.53 ± 3.10%, V_4-36.35±3.88%). The variant with the lowest density was more homogeneous (Cordeli

A.N. et al., 2021). The results indicated that increasing population density leads to higher variation in individual growth. The increase in CV over time suggests the existence of inter-individual competition within the fish group (Azaza et al., 2013). In aquaculture, reducing variations in fish size and achieving uniform size is preferable to facilitate feeding, harvesting, marketing, and processing (Azaza et al., 2010, 2013).

Individual weight gain of the fish was significantly influenced by population density, with fish growing more when the stocking density is lower. Significant differences existed among the four experimental variants (ANOVA, $p<0.05$). Post-hoc Duncan tests revealed three distinct groups: the individual weight gain of fish in V1 was significantly different from V_2, while the individual weight gain of fish in V_3 and V_4 was similar. The final average body weight of the fish after 31 days of experimentation was: V_1-4.61±0.03 g, V_2-4.27±0.08 g, V_3-4.03±0.15 g, and V_4-3.97±0.02 g.

Regarding specific growth rate (SGR) and feed conversion ratio (FCR), the best values were obtained in the lowest stocking density (V_1). Post-hoc analysis showed that SGR values in V_1 (2.94±0.07%/day) were higher than those in V_2 (2.34±0.08%/day), while the values in V_3 (1.95±0.10%/day) and V_4 (1.80±0.10%/day) were similar ($p>0.05$).

Regarding FCR, Duncan's post hoc test divided the obtained values into four distinct groups, with the best values obtained in variant V_1. FCR varied from 1.84±0.06 in V_1 to 3.69±0.28 in V_4, increasing with higher fish stocking densities. Higher FCR values obtained at higher stocking densities indicate lower feed efficiency. Furthermore, an inversely proportional correlation was observed between population density and fish growth: at lower densities, fish grow more. To obtain relevant information about the effect of feed proteins on body proteins, the protein efficiency ratio and protein utilization efficiency were calculated.

ANOVA analysis revealed significant differences ($p<0.05$) between the experimental variants in PER and PUE. Duncan's multiple tests indicated that PER values in group V_1 were significantly different ($p<0.05$) from those obtained in group V_2, while no significant differences were observed between V_3 and V_4. Significant differences ($p<0.05$) were also found in PUE values. The evolution of PUE showed better protein utilization, indicating increased efficiency, inversely proportional to the increase in population density. Duncan's multiple tests identified four distinct groups corresponding to each tested stocking density.

Studies by Hayat et al. (2021) showed that population densities of 50, 75, 100, and 125 fish/m^3 led to similar weight gains and SGR as our experiments. Higher densities could be more profitable for common carp farms in less developed countries by reducing land and facility costs.

In a scientific article published by Nuwansi et al. in 2021, investigating the influence of stocking densities on carp growth, the same conclusion was reached: better fish growth at lower densities. Thus, in the variant with the lowest stocking density V_1 (1.4 kg/m^3), the lowest feed conversion ratio (2.64±0.05) was obtained, while for other variants, values of 2.1 kg/m^3 (V_2) and FCR 4.00±0.13, and 2.8 kg/m^3 (V_3) with an FCR of 5.68±0.15 were obtained (Cordeli A.N. et al., 2021).

Survival is an important indicator of fish health (Rey et al., 2019). In our experiments, survival was directly influenced by stocking density. Post-hoc analysis showed that the survival percentage of fish in group V_1 (97.14±1.43%) was significantly higher than that in V_2 (87.14±2.14%), while there were no significant differences between V_3 (81.75±3.1%) and V_4 (79.17±2.15%) ($p>0.05$).

- **The biochemical composition of fish flesh**

The biochemical composition of fish flesh is influenced by many factors, such as the cultured species, size, age, environmental conditions, and diet (Cho, 2001). The values obtained regarding the biochemical composition of carp bodies are similar to those reported by other authors.

Table 5.4 Composition of carp fry meat at different population densities

Parameter	Experimental Variants				
	Initial	**V_1 final**	**V_2 final**	**V_3 final**	**V_4 final**
Water (%)	76,11±0,18	76,48±0,07	76,33±0,22	75,20±0,22	75,32±0,08
Proteins (%)	12,29±0,18	13,15±0,02	13,26±0,02	13,15±0,16	13,53±0,47
Lipids (%)	7,78±0,07	8,80±0,08	8,71±0,2	8,81±0,21	8,63±0,21
Ash (%)	2,19±0,10	1,42±0,02	1,39±0,07	1,46±0,03	1,44±0,03

Note: Data is presented as the mean of triplicates ± standard deviation (SD)

The obtained results revealed significant differences in the water content of the fish among the four experimental variants. The water content in V_1 and V_2 was higher, significantly different from those in V_3 and V_4. Compared to the initial time point (initial concentrations), a slight increase in meat moisture was observed in V_1 and V_2, while V_3 and V_4 showed lower moisture content.

Regarding the protein, lipid, and ash content, no significant differences (ANOVA, $p>0.05$) were recorded among the four stocking densities. Still, significant differences were observed compared to the initial time point (ANOVA, $p<0.05$). These differences included higher protein and lipid and lower ash content (mineral substances). Surprisingly, a high concentration of body lipids was observed in all final variants, ranging from 8-9%. This phenomenon can be explained by the fact that the fish were fed a lipid-rich diet (14%). Therefore, to avoid excessive fat accumulation in the meat, as the fish grow, the protein and lipid concentrations in the feed should be lower, around 35-40% protein and 7-10% lipids.

5.1.4. Conclusions

The stocking density of the biological material in the growth units significantly influences the growth performance of fish. Lower densities in a small-scale Recirculating Aquaculture System (RAS) result in better performance, including improved feed utilization. Excessive stocking densities lead to overcrowding and competition for food and space, resulting in slower fish growth and reduced feeding efficiency. Density also considerably affects fish behavior, with high stocking densities shown to increase aggressive behavior. Additionally, higher densities have led to increased feeding time compared to lower densities.

Regarding nutrient retention from the feed in fish meat, research has resulted in the biochemical profile, reflecting how the overall chemical composition changes over time. The chemical composition of the feed directly influences the composition of the meat. As fish are fed, the percentage of body proteins and lipids increases, accompanied by a decrease in the water content of the meat, which is a positive aspect.

Careful attention must be given to the choice of culture species, environmental conditions, and feeding practices to achieve positive technological results. The selection of the culture species should consider the ecophysiological characteristics of the species, especially its growth potential. Carp is a species with exceptional ecotechnological plasticity, suitable for cultivation in various systems.

5.2. Experiment 2 - Influence of Density on the Growth Performance of Carp in a Recirculating Aquaculture System (Pilot-scale Tanks)

5.2.1. Introduction

Stocking density is an important technological indicator in intensive fish farming, as it can be a potential source of chronic stress with adverse effects on the health and behavior of fish (Ashley, 2007). Numerous scientific studies have demonstrated that stocking density causes changes in the immune system and leads to a reduced ability to fight against pathogens, potentially resulting in illnesses or mortalities (Maule et al., 1989, Mazur and Iwama, 1993, Rotllant et al., 1997).

5.2.2. Materials and Methods

At the beginning of the experiment, 2800 fish with an average initial weight of 1.5 g were allocated to eight rearing units in four density variants in duplicate. The first variant (V_1) had a density of 200 individuals/tank (0.6 kg/m^3), V_2 had 300 individuals/tank (0.9 kg/m^3), while V_3 and V_4 had densities of 400 (1.2 kg/m^3) and 500 individuals/tank (1.5 kg/m^3), respectively. Feeding was carried out automatically three times a day (at 08:00, 13:00, and 18:00) with a ratio of 3% of the body weight. The duration of this experiment was also 31 days, identical to the time of the first experiment. The rearing units were plastic tanks with 1000 liters of water capacity.

5.2.3. Results and Discussions

- **Monitoring of water quality parameters**

The water temperature during the experiment ranged from 19.5˚C to 20.6˚C. This temperature range falls within the optimum range for the species, but it is at a lower limit. For carp, the optimal growth temperatures are in the range of 20-27˚C. The water temperature dynamics were similar in all experimental variants because the water in the recirculating system is a simple, reusable physical substrate. Furthermore, the statistical analysis performed on the four experimental variants confirmed no significant differences ($p>0.05$).

Dissolved oxygen in the water varied between 7.5-9 mg/l in all variants. The oxygen concentration varies inversely with temperature, meaning higher temperatures result in lower oxygen levels. However, in our experiment, with constant water temperatures, the oxygen concentrations remained constant and similar across the four variants.

The pH value fluctuated between 6.8-8.1 units throughout the experimental period, falling within the optimal range for the studied species.

Ammonium nitrogen ($N\text{-}NH_4+$), represented by the ammonium ion, is a product resulting from the decomposition of organic matter residues, such as unconsumed feed and excreta, by heterotrophic bacteria. Ammonium is an unstable compound formed during this process, which can be converted into ammonia. During the research, ammonium levels were monitored weekly, ranging from 0.09-0.2 mg/l.

Nitrate levels were generally low (7-12.5 mg/l), except for the experimental variant with the highest density, where values ranged from 16-22.5 mg/l.

- **Analysis of fingerling carp growth performance**

The highest weight gain for fingerling carp was observed at the initial stocking densities ranging from 0.6 kg/m^3 (V_1) to 0.9 kg/m^3 (V_2).

Regarding the feed conversion ratio (FCR), a significant decrease was observed as the stocking densities decreased. The average FCR values ranged from 4.22±0.23 (V4) to 1.54±0.11 (V_1) (Figure 5.22). The best results in terms of FCR were obtained in V_1 (200 initial fish) and V2 (300 initial fish), followed by V_3 and V_4 (400 and 500 initial fish), with no statistically significant differences between these two experimental variants ($p>0.05$). The specific growth rate (SGR) significantly increased as the stocking density decreased. Thus, SGR was 2.21±0.12%/day (V_1), followed by 1.71±0.06%/day (V_2), 1.3±0.07%/day (V_3), and 1±0.06%/day (V_4).

The calculated technological performance indicators based on the initial and final data obtained are summarized in Table 5.5.

Table 5.5 - Growth performance of fingerling carp at different stocking densities

Experimental variants.	V1		V2		V3		V4	
Calculations/Unit of growth	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**
Initial biomass (g)	300	300	450	450	600	600	750	750
Initial biomass (kg/m^3)	0,60	0,60	0,90	0,90	1,20	1,20	1,50	1,50
Final biomass (g)	611	580	759	771	893	905	1030	1009
Final biomass (kg/m^3)	1,22	1,16	1,52	1,54	1,79	1,81	2,06	2,02
Biomass growth (g)	311	280	309	321	293	305	280	259
Biomass growth (kg/m^3)	0,62	0,56	0,62	0,64	0,59	0,61	0,56	0,52
Initial fish number	200	200	300	300	400	400	500	500
Final fish number	185	187	271	257	319	335	367	381
Survival rate (%)	93	94	90	86	80	84	73	76
Average initial weight (g/ex)	1,5	1,5	1,5	1,5	1,5	1,5	1,5	1,5
Average final weight (g/ex)	3,3	3,1	2,8	3,0	2,8	2,7	2,8	2,6
Growth days	31	31	31	31	31	31	31	31
GR (Daily growth rate) (g/day)	10,0	9,0	10,0	10,4	9,5	9,8	9,0	8,4
SGR (%/day)	2,3	2,1	1,7	1,7	1,3	1,3	1,0	1,0
Individual growth (g)	1,8	1,6	1,3	1,5	1,3	1,2	1,3	1,1
Total feed distributed (g)	454	454	681	681	908	908	1135	1135
FCR (g feed/g biomass growth)	1,46	1,62	2,20	2,12	3,10	2,98	4,05	4,38

Regarding the daily growth rate of fish biomass, no significant differences were observed among the four variants ($p>0.05$). The best results were obtained in variant V1 (9.5 g/day), while the lowest results were recorded in variant V_4 (8.7 g/day).

After 31 days of research, the following average values of individual body masses were obtained: V_1: 3.2 ± 0.14 g, V_2: 2.9 ± 0.14 g, V_3: 2.75 ± 0.07 g, and V_4: 2.73 ± 0.11 g (Cordeli A.N. et al., 2019). Post-hoc Duncan analysis indicated that the final weight of fish in variants V1 and V2 was significantly higher than in variants V3 and V4. This confirms the hypothesis that lower stocking densities favor fish growth.

Regression curve equations were derived to obtain additional information about fish growth patterns using length and weight data. The allometric coefficient (b) values obtained for all experimental variants indicated a negative allometric growth, suggesting that fish grew more in length than in weight.

The protein efficiency ratio (PER) and protein utilization efficiency (PUE) further supports the conclusion that lower stocking densities result in better individual growth. The proteins from the distributed feed led to a higher individual growth increment in V_1 (0.75 g biomass growth/1 g ingested protein) compared to V_2 (0.41 g), V_3 (0.28 g), and V_4 (0.22 g). The same trend is observed for PUE.

➢ The biochemical composition of common carp meat

Regarding the protein, lipid, and ash content, no significant differences were observed (ANOVA, $p>0.05$) among the four stocking densities, but significant differences were found compared to the initial measurements (ANOVA, $p<0.05$).

At the beginning of the experiment, the water-to-protein ratio was 5.71±0.04. At the end of the experiment, this ratio decreased in the first two variants and slightly increased in V3 and V4. The final values were on average, 5.52±0.15 in V_1, 5.65±0.16 in V_2, 5.76±0.12 in V_3, and 5.89±0.16 in V_4 (Cordeli A.N. et al., 2019).

The biochemical composition of common carp meat in the four different stocking density variants is presented in Table 5.6 (at the beginning and the end of the experiments).

Table 5.6 - Biochemical composition of common carp meat grown at different stocking densities.

Parameter	Experimental Variants				
	Initial	V_1	V_2	V_3	V_4
Water (%)	75,83±0,17	75,80±0,03	75,90±0,05	75,66±0,12	76,29±0,22
Protein (%)	13,27±0,45	13,72±0,02	13,43±0,03	13,12±0,01	12,94±0,14
Lipids (%)	8,48±0,17	8,95±0,06	8,54±0,07	9,34±0,2	8,88±0,11
Ash (%)	1,62±0,05	2,05±0,01	1,70±0,01	1,82±0,06	1,61±0,02

Note: Data is presented as the mean of triplicates±SD.;

5.2.4. Conclusions

The research conducted in the pilot recirculating system yielded similar results to laboratory studies: stocking density influences the individual growth performance of fish, with lower densities leading to larger body masses. On the other hand, total biomass (final production) varies directly with stocking densities, as higher densities result in greater final production. These findings align with research conducted in other countries with the same species.

It is crucial to consider the negative effects that can occur when stocking densities are too high. Increased competition for food, aggression among fish, and subsequent stress can have detrimental effects on their well-being. Additionally, high stocking densities facilitate the spread of pathogens.

To ensure profitability in fish farming, farmers must properly manage environmental conditions, have a solid understanding of fish nutrition and feeding practices, be knowledgeable about preventive measures and disease treatment, choose the most cost-effective cultured species, and implement innovative growth technologies suitable for the selected farming system.

CHAPTER 6. THE INFLUENCE OF FEED QUALITY ON THE GROWTH PERFORMANCE OF COMMON CARP FRY

6.1. Experiment 3 - The influence of feed quality on the growth performance of carp in a recirculating aquaculture system, laboratory level

6.1.1. Introduction

Numerous physiological, endogenous, and exogenous factors influence the chemical composition of fish meat. Endogenous factors include metabolic processes, genetic parameters, and sexual maturity, while exogenous factors include microclimate, water quality, feed quality, and quantity (Shearer, 1994; Huss, 1995; Tkaczewska et al., 2014). Feed quality is one of the most important exogenous factors influencing fish meat's biochemical composition and overall growth performance. Feeds with high-energy content promote rapid growth and reduce production time. However, it should be noted that feeds rich in fats and proteins promote fat accumulation, while the protein content in the meat remains constant (Fauconneau et al., 1995; Kaushik, 1995). The fatty acid composition of fish fat is essential for meat quality.

6.1.2. Materials and Methods

At the beginning of the experiment, the average body weight of the fry was 2.98 g ± 0.05 g, and the total length was 5.42 ± 1.17 mm. The fish were distributed into four experimental groups, each with three replicates. Four types of extruded granulated feeds were tested, with the following protein and lipid contents: V_1 - 50% protein and 14% lipid, V_2 - 30% protein and 7% lipid, V_3 - diet with 45% protein and 16% lipid, and V_4 - diet with 44% protein and 22% lipid (shown in Table 6.1). The duration of the experiment was the same as the previous one, i.e., 31 days. Throughout the research period, the physicochemical parameters of the water were constantly monitored: temperature, pH, dissolved oxygen (determined daily using portable multiparameter instruments), and weekly measurement of nitrogen compounds (N-NO_2^-, N-NO_3^-, N-NH_4^+) using a portable Spectroquant NOVA 60 spectrophotometer.

Table 6.1 - Chemical composition of the tested feeds

Parameters/Feeds	U.M.	V_1 (50/14)	V_2 (30/7)	V_3 (45/16)	V_4 (44/22)
Moisture	%	10	10	10	10
Protein	%	50	30	45	44
Lipids	%	14	7	16	22
Ash	%	-	7	7	7,2
Cellulose	%	2	5	2	1,8
Lysine	%	2,5	-	-	-
Phosphorus	%	1	0,8	1	1,02
Copper	mg/kg	6	30	5	5
Calcium	mg/kg	-	1,2	1,3	1,7
Sodium	mg/kg	-	0,2	0,3	0,3
Vitamin A	mg/kg	-	10 000	10 000	10 000
Vitamin D3	mg/kg	20 000	1 800	-	1 463
Vitamin E	mg/kg	200	60	200	200
Vitamin C	mg/kg	200	-	150	250
Digestible Energy	Mj/kg	17,65	15,36	18,5	19,1

6.1.3. Results and discussions

➢ **Monitoring water quality parameters**

The water temperature remained constant throughout the research, ranging from 21°C to 24.3°C, with an average of 23.71±0.85°C. These values fall within the optimal growth conditions for carp fry. No significant differences were observed among the four variants since the same total volume of water was recirculated, undergoing prior mechanical and biological filtration.

The water pH varied between 6.9 and 8.5 units, with an average of 8.13±0.09. This parameter experienced some fluctuations during the experiment, especially in variants V_3 and V_4. However, it is worth noting that all values in all four variants fell within the permissible limits for proper carp growth. The dissolved oxygen in the water fluctuated but remained within the optimal range for carp fry. The average values were 7.46±0.31 mg/l in V_1 and 8.04±0.06 mg/l in V_4.

Nitrogen compounds play an important role in assessing water quality. Various studies have highlighted the negative effects of exposure to high concentrations of nitrogen compounds on fish in aquaculture systems. In recirculating systems, it is recommended to maintain nitrite levels below 0.2 ppm to ensure healthy carp growth, while nitrate levels, which are less toxic, can reach up to 50 ppm.

Throughout the experiment, the nitrate level in the water was generally low, ranging from 16.41±6.08 mg/l. These values can be attributed in part to the proper functioning of the mechanical filter and, especially, the biological filter. By adopting appropriate measures to monitor and maintain nitrite and nitrate concentrations within recommended limits, good technological water quality can be ensured, thereby protecting the health and well-being of carp in recirculating aquaculture systems.

During the analyzed period, the concentrations of ammonium (NH_4+) at the outlet of the biological filter varied between 0.01-0.02 mg/l, while in the growth units, they had an average value of 0.18-0.20 mg/l. These values are below the maximum allowable limit for fish, which is 2 mg/l.

The concentrations of nitrites (N-NO_2-) in the water remained within permissible limits throughout the experiment. The average values recorded for the four experimental variants were 0.20±0.24 mg/l, with a slightly higher average value observed in the experimental variant (V_1), where the fish were fed a higher protein content feed.

Monitoring nitrite levels in the aquatic environment is important as they can have harmful effects on fish health. The fluctuations within the permissible limits indicate proper feeding and water quality management during the experimental research. The higher values recorded in the experimental variant, where the fish were fed a feed with a higher protein content, may indicate an influence of the feed on nitrite levels in the water.

Phosphorus is an essential nutrient for aquatic organisms and plays a crucial role in fish and aquatic flora's metabolism, growth, and development.

In recirculating systems, the phosphorus concentration can vary depending on the distributed feed, as it only comes from the distributed feeds. During the research period, the average phosphorus concentration in the technological water (expressed as P_2O_5) was 5.7±0.23 mg/l. Higher values were obtained in variant V_1 with a 50/14 feed (5.6-5.8 mg/l), while the lowest concentrations were in V_4 with a 44/22 feed (5-5.5 mg/l).

➢ **Analysis of carp fry growth performance.**

The technological performance indicators obtained throughout the experiment are presented in Table 6.2.

Table 6.2 - Technological performance indicators obtained during the experimental period.

Experimental Variant	**V1**			**V2**			**V3**			**V4**		
Elemente de calcul	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**	**A12**
Initial Biomass (g)	280	280	280	280	280	280	280	280	280	280	280	280
Initial Biomass (kg/m^3)	1,96	1,96	1,96	1,96	1,96	1,96	1,96	1,96	1,96	1,96	1,96	1,96
Final Biomass (g)	739	770	782	707	722	784	752	750	778	894	926	992
Final Biomass (kg/m^3)	5,17	5,39	5,47	4,95	5,05	5,49	5,26	5,25	5,45	6,26	6,48	6,94
Biomass Growth (g)	459	490	502	427	442	504	472	470	498	614	646	712
Biomass Growth (kg/m^3)	3,21	3,43	3,51	2,99	3,09	3,83	3,30	3,29	3,49	4,30	4,52	4,98
Initial Fish number	70	70	70	70	70	70	70	70	70	70	70	70
Final Fish number	68	68	68	67	70	70	67	67	67	67	68	69
Survival Rate (%)	97	97	97	96	100	100	96	96	96	96	97	99
Initial Average Weight (g/ex)	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0
Final Average Weight (g/ex)	10,87	11,32	11,50	10,55	10,31	11,20	11,22	11,19	11,61	13,34	13,62	14,38
GR (Daily Growth Rate) (g/day)	14,81	15,81	16,19	13,77	14,26	16,26	15,23	15,16	16,06	19,81	20,84	22,97
SGR (%/day)	3,13	3,26	3,31	2,99	3,06	3,32	3,19	3,18	3,30	3,74	3,86	4,08
Individual Growth (g)	6,9	7,3	7,5	6,6	6,3	7,2	7,2	7,2	7,6	9,3	9,6	10,4
Total Feed distributed (g)	810	810	810	810	810	810	810	810	810	810	810	810
FCR (g feed/g biomass growth)	1,77	1,65	1,61	1,90	1,83	1,61	1,72	1,72	1,63	1,32	1,25	1,14
Crude protein/ crude fat feed	50/14	50/14	50/14	30/7	30/7	30/7	45/16	45/16	45/16	44/22	44/22	44/22

Among the most important parameters determined were daily growth rate, total biomass gain, individual growth gain, specific growth rate, and feed conversion ratio. An optimal diet for carp fry should contain nutrients (especially proteins, lipids, and carbohydrates) in quantities that fully meet the species' nutritional requirements (including vitamins, trace elements, and macroelements) (Satpathy et al., 2003). Maintaining an optimal balance of nutrients is important, as doses that are too low or too high can negatively affect fish growth performance.

The results indicate better growth in the experimental group, where fish were fed diets containing 44% protein and 22% fat (V_4). In contrast, no significant differences in fish growth were observed in the other three groups. This suggests that a higher content of proteins and lipids has a beneficial effect on growth gain and final body mass in carp fry.

Furthermore, at the end of the experiment, a higher specific growth rate (SGR) was observed in the growth units where carp were fed a diet containing 44% crude protein and 22% fat (V_4 with an SGR of 3.93 %/day). Lower values were obtained in the other variants: 3.23 in V_1, 3.12 in V_2, and 3.22 in V_3. The same trend was maintained for the daily growth rate (GR) parameter with the following values: 15-16 grams/day in V_1, V_2, and V_3, and nearly 22 grams/day in V_4. The higher SGR value, combined with the best daily growth rate (GR), highlights the effectiveness of the 44/22 feed.

- **The biochemical composition of carp fry meat**

The biochemical composition of carp fry meat, fed with different commercial feeds containing varying protein contents, is detailed in Table 6.3. Environmental factors, but especially technological factors (feed quality, daily ration, feeding intensity, etc.), are often associated with good meat quality in fish. Additionally, genetic factors can influence the body's protein and lipid content, compensating for feeding's effects on growth (Fauconneau et al., 1991). As a general rule, farmed carp meat will have a higher fat content compared to wild carp.

Table 6.3 - Composition of carp meat for the four feed variants

Parameter	Inițial	V_1 final	V_2 final	V_3 final	V_4 final
Water (%)	75,91±0,19	77,67±0,95	72,59±1,20	75,71±2,19	75,32±0,08
Protein (%)	13,30±0,2	10,38±0,96	12,65±0,20	10,24±1,71	13,53±0,47
Lipids (%)	8,93±0,05	8,98±0,07	13,06±1,46	10,10±0,54	8,63±0,21
Ash (%)	1,8±0,09	1,97±0,02	1,74±0,01	1,82±0,006	1,44±0,03

Note: Data presented as the mean of triplicates ± standard deviation (SD)

In our experiment, the values of the biochemical composition of carp meat are consistent with those reported by other authors. The average water content varied between the limits of 72.59% in V_2 and 77.67% in V_1, crude protein content ranged from 10.24% (V_3) to 13.53% (V_4), lipid content ranged from 8.63% (V_4) to 13.06% (V_2), and ash content ranged from 1.44% (V_4) to 1.97% (V_1).

6.1.4. Conclusions

Optimizing feeding technology is a major challenge for achieving high and profitable yields. In recent years, more and more farmers have started to rear carp at high densities and use combined granulated, extruded/expanded feeds from different manufacturers.

Too low or too high protein levels in feeds can negatively influence fish growth performance, regardless of the chosen culture species (slow growth, decreased immunity, reproductive disorders, and overall health impairment). It is essential to understand the nutritional preferences of the species and then choose the appropriate farming system, suitable feed, and optimal daily feeding ration.

Protein concentration is not the only factor that influences carp growth. The overall composition of the diet, consisting of all essential nutrients (proteins, lipids, carbohydrates, vitamins, minerals), is essential. In addition to feed, environmental conditions (temperature, dissolved oxygen, pH, nitrogen compounds, etc.) also significantly impact the achieved production levels.

6.2. The Influence of Feed Quality on Carp Growth Performance in a Pilot-Scale Recirculating Aquaculture System

6.2.1. Introduction

The most important feed ingredients are of animal origin, primarily fishmeal, which has a 50-75% protein content, depending on its quality. In recent years, fish nutrition research has also focused on finding substitutes for fishmeal, some of which are of plant origin.

6.2.2. Materials and methods

In this experiment, 1600 juvenile carp with approximately equal body weight and size, averaging 2.98 ± 0.05 g, were selected. The fish were randomly distributed into four experimental groups, with two replicates each, resulting in 200 fish per tank. The experimental groups were designed to evaluate fish growth performance using four different commercial feed formulations: V_1 (30/7) - 30% crude protein and 7% lipids, V_2 (44/22) - 44% crude protein and 22% lipids, V_3 (45/16) - 45% crude protein and 16% lipids, and V_4 (50/14) - 50% crude protein and 14% lipids. The duration of the experiment was the same as in previous experiments, 31 days.

6.2.3. Results and discussions

- **Water Quality Parameter Monitoring**

The main physicochemical parameters of the water were measured daily using integrated sensors and a monitoring system. Additionally, nitrogen compounds ($N\text{-}NO_2$-, $N\text{-}NO_3$-, $N\text{-}NH_4$+) were measured once a week using a spectrophotometer compatible with Merck kits. A water sample was collected from each tank in the morning before feeding for these analyses. Water temperature significantly impacts fish growth, directly influencing physiological processes such as respiration, feeding efficiency, growth rate, behavior, and reproduction. In our experiment, the water temperature was maintained within the optimal range for common carp growth, ranging from 20.8°C to 22.5°C, with an average value of 21.70±0.82 °C. According to ANOVA ($p>0.05$), no significant differences among the four variants indicated that water temperature is not influenced by feed quality.

Significant variations in pH can negatively affect fish, causing stress in their bodies. During the experimental period, pH fluctuated between 6.9 to 7.9, with an average value of 7.93±0.09 pH units. This value falls within the optimal range for fish growth, typically between 6.5 and 8.5 pH units. There were no significant differences among the four variants, according to ANOVA ($p>0.05$), indicating that water pH is not decisively influenced by feed quality. The small differences between the four variants could be attributed to the quantities of unconsumed feed remaining in the rearing units. Dissolved oxygen level in water is crucial for fish growth. In this experiment, dissolved oxygen concentration varied between 7.01 mg/l and 7.99 mg/l, with an average of 7.93±0.27 mg/l. These results indicate that the oxygen level was maintained within the adequate range to support healthy fish growth during the experiment. There were no significant differences among the four variants, according to ANOVA ($p > 0.05$), suggesting that dissolved oxygen in water is not significantly influenced by feed quality. However, when the daily ratio is excessive, and not all feed is consumed, oxygen is consumed to mineralize the organic matter undergoing decomposition, resulting in a decrease in water oxygen concentration. This phenomenon is less common in recirculating systems and more frequent in ponds and lakes.

Significant differences were observed among the experimental variants regarding the ammonia ion level. The lowest value was recorded in variant V_2, with an ammonia concentration of 0.13 mg/l, while variant V_4 recorded an ammonia concentration of up to 0.22 mg/l. These results indicate that ammonia levels in water increased with increased dietary protein concentration. Nitrates ($N\text{-}NO_3$-) represent the end product of the nitrification process. Although they are generally not considered toxic to fish, their concentration in the water used in technological systems must fall within acceptable limits for the species being reared. According to the specialized literature, maintaining nitrate concentrations between 20 and 40 mg/l is recommended for fish growth in water recirculation systems. In this experiment, the recorded values ranged from 17.9 in V_3 to 19.1 mg/l in V_4, with an average value of 18.11±3.59 mg/l.

Nitrite levels recorded during the experiment ranged from 0.04 to 0.07 mg/L, with an average value of 0.06±0.05 mg/l. The highest nitrite levels were recorded in variants V_2 and V_3 at approximately 0.07 mg/l, while the lowest values were recorded in variants V_1 and V_4 at about 0.04 mg/l. Differences between the variants may be attributed to the quantities of unconsumed feed accumulated in the rearing units.

The recorded values for phosphate concentration in the process water during the experiment varied between a minimum of 5 mg/l in variant V_1 and 5.6 mg/l in variants V_2, V_3, and V_4, indicating a moderate concentration. It is important to monitor and maintain these values within acceptable limits to ensure healthy growth and an appropriate environment for the fish.

➢ Growth Performance Analysis of Carp Juveniles

In juvenile carp, lipids, and carbohydrates are equally efficient in providing the energy required for vital processes. Proteins should be used for muscle mass accumulation as the primary plastic nutrients and secondary energy sources. Previous studies have shown that using milk casein as the sole protein source led to an optimal digestible energy-to-protein ratio between 13,000 and 15,000 kJ/kg, with a protein content of 31%-32% (Takeuchi et al., 1979b). Data regarding growth performance and feed efficiency are presented in Table 6.4.

Table 6.4 - Influence of Feed Quality on Growth Performance.

Parameter/Variant	V_1 (30/7)	V_2 (44/22)	V_3 (45/16)	V_4 (50/14)
Initial biomass (g)	597,50±7,95	595,00±4,24	593,00±0.01	595,50±3,54
Final biomass (g)	914,50±7,78	1110,00±31,11	1142,50±27,58	1227,50±45,96
Initial fish number	200	200	200	200
Total growth gain (g)	317,00±12,73	515,00±35,36	549,50±27,58	632,00±49,50
Survival (%)	95,25±0,35	98,75±1,77	96,25±1,77	95,75±3,18
Initial average weight (g/ex)	2,99±0,02	2,98±0,02	2,97±0,00	2,98±0,02
Final average weight (g/ex)	4,80±0,06	5,62±0,06	5,93±0,03	6,41±0,03
Daily growth rate (g/day)	10,23±0,41	16,61±1,14	17,73±0,89	20,39±1,60
Individual growth gain (g/ex)	1,81±0,08	2,64±0,08	2,97±0,03	3,43±0,04
FCR	2,74±0,11	1,69±0,12	1,58±0,08	1,38±0,11
SGR (%/day)	1,37±0,05	2,01±0,11	2,11±0,08	2,33±0,14
PER	1,220±0,05	1,35±0,09	1,41±0,07	1,46±0,11

Note: Average values of duplicates ± SD

No significant differences were observed regarding the initial average body mass of the carp fry in each group ($p>0.05$). The survival rate recorded in each variant was above 95%, with no significant differences ($p>0.05$).

At the end of the experiment, significant differences were observed ($p<0.05$) in terms of the final body mass of the fish. The lowest body mass was recorded in the diet with 30% crude protein (V_1), while the highest body mass was recorded in the diet with 50% crude protein (V_4).

Statistical analysis (Duncan's test) revealed four distinct groups of fish based on their weight at the end of the experiment. Thus, in variant V_1, the average individual final mass was 4.80±0.06 g/fish; in V_2, it was 5.62±0.06 g/fish; in V_3, it was 5.93±0.03 g/fish; and in V_4, it was 6.41±0.03 g/fish. The 50/14 feed, with a protein content of 50% and lipid content of 14%, led to the highest weight gain, significantly different ($p<0.05$) from the growth recorded in fish fed diets containing 45%, 44%, and 30% protein.

The coefficient of determination (R^2) values, generally above 0.9 (90%), demonstrate the homogeneity of the results. The best dispersion of points corresponding to the (W, L) values were observed in variant V_3, with an (R^2) of 0.934. In this experiment, the growth coefficient (b) values indicated a negative allometry (b<3) in V_1 and V_4, meaning that the growth of the fish, proportionally, relied more on length than on weight. This indicates that in some tanks, the growth conditions were not optimal. Positive allometry (b>3) was observed in variants V_2 and V_3, indicating that the fish grew more in weight than in length, which is preferred.

Among all variants, the weakest results were obtained in variant V_1, where a feed with 30% protein and only 7% lipids was used. This result suggests that a low content of protein and lipids leads to poor growth performance.

Increasing the protein level in the diets had a significant impact ($p<0.05$) on the final body mass of the fish and the biomass gain, specific growth rate (SGR), and feed conversion ratio (FCR). Only the protein efficiency ratio (PER) did not show a significant difference ($p>0.05$) among the four experimental variants.

A significant reduction in the feed conversion ratio (FCR) was observed as the protein level in the diet increased, ranging from 2.74 to 1.27.

The best conversion rate was recorded in V4 (50% crude protein), followed by V_3 and V_2 (45% and 44% protein), with no significant differences between V_3 and V_2 ($p>0.05$).

Fish growth performance and feed utilization efficiency progressively improved with increasing protein levels in the diet from 30% to 50%. The best growth results were achieved when the fish were fed a diet with 50% protein, and the growth rate was significantly different from the groups fed diets with 30%, 44%, and 45% protein. The specific growth rate (SGR) showed a significant increasing trend with the increase in protein level in the diet ($p<0.05$). The SGR values were recorded as 1.37±0.05%, 2.01±0.11%, 2.11±0.08%, and 2.33±0.11% per day for V_1, V_2, V_3, and V_4, respectively.

According to the research conducted by Ebrahimi A. and colleagues in 2020, it was found that protein content had a significant impact on weight gain, growth increment, feed conversion ratio (FCR), protein utilization efficiency (PER), and specific growth rate (SGR) in common carp fry with an initial weight of 30.5±3.1 g.

These findings suggest that optimal dietary protein levels may vary depending on the developmental stage of common carp, and adapting diets to meet the specific nutritional needs of each growth stage can improve fish growth performance. Continued research in this field is important to develop efficient nutritional strategies for optimal common carp growth.

- **Fish Meat Biochemical Composition**

Fish is an important source of animal protein for human consumption. Since good meat quality is associated with a healthy animal, meat analysis can improve the marketing of aquaculture products. Various authors have studied the biochemical composition of carp meat. Changes in chemical composition related to fish size/age and the effect of growth conditions are crucial for evaluating fish meat quality. The biochemical composition of carp fry meat, fed with different protein content commercial feeds, is detailed in Table 6.6.

Influence of feed quality on the biochemical composition of common carp meat.

Parameter	**Experimental Variants**				
	Initial	**V_1(30/7)**	**V_2(44/22)**	**V_3(45/16)**	**V_4(50/14)**
Water (%)	75,85±0,07	76,22±0,83	72,84±2,78	73,90±2,00	71,71±1,08
Protein (%)	13,57±0,20	12,16±0,11	12,30±0,80	11,36±1,73	12,76±1,23
Lipids (%)	8,75±0,28	9,32±0,85	11,52±0,16	12,31±1,77	12,69±1,35
Ash(%)	1,87±0,15	1,89±0,10	1,86±0,06	1,57±0,014	1,89±0,10

Note: Data is presented as the mean of triplicates ± SD at the end of the experiment.

The best results were obtained in ascending order V_1, V_2, V_3, and V_4, proportional to the percentage of protein in the feeds (30%, 44%, 45%, and 50%). The concentrations of lipids also contributed to these results, but not in an increasing order like proteins. As a non-predatory species, Carp does not require high concentrations of lipids above 15% in its diet, as trout does. The 50/14 feed is the most balanced in terms of energy, so it yielded the best results.

6.2.4. Conclusions

Proteins play an essential role in body mass growth and in the production of enzymes, hormones, and antibodies, being involved in vital processes of organisms. A protein deficiency in the fish diet leads to growth slowdown and weight loss. On the other hand, an excess of protein in the diet is usually converted into energy and can lead to an increase in nitrogen levels in the water, affecting the quality of the aquatic environment.

The protein level in carp feeds significantly impacts growth enhancement, feed conversion ratio, and specific growth rate. Identifying the optimal protein levels for each stage of carp development is a prerequisite for achieving technological performance.

To achieve optimal growth at the lowest cost, estimating the optimal level of nutrients in the fish diet is necessary. This involves finding an appropriate balance between proteins, lipids, and carbohydrates in the feed to support growth and vital functions without exceeding the necessary level, which can lead to environmental issues and additional costs.

CHAPTER 7 - THE INFLUENCE OF DAILY FEEDING RATE ON THE GROWTH PERFORMANCE OF CARP FRY

7.1. The Influence of Daily Feeding Rate on the Growth Performance of Carp Fry, Laboratory Level

7.1.1. Introduction

The costs associated with fish feed can represent 40-50% of the total operating costs (El-Sayed, 2006). To reduce these costs, feed producers provide feeding tables/guides that indicate the daily feeding rate, i.e., the amount of feed required based on fish size, body weight, and water temperature. Among various measures to optimize feed management to maximize their benefits, feeding rate is an important factor, which has been reported for several cultured species (Hung et al., 2001).

7.1.2. Materials and methods

The experiment aimed to determine the optimal feeding rate for carp fry with an initial body weight of approximately 10-12 g/fish in a high-performance recirculating system. Before the start of the experiment, the fish were acclimated under laboratory conditions for one week in a 500-liter tank. After this acclimation period, 720 carp specimens were distributed into twelve 132-liter tanks, with four experimental groups, each in triplicate: V_1 - daily feeding rate of 3% of body weight (% BW/day), V_2 - 3.9% BW/day, V_3 - 4.9% BW/day, and V_4 - 6% BW/day. The daily feedings were divided into three meals, scheduled at 08:00, 13:00, and 18:00. The fish were fed with a commercial feed containing 45% crude protein and 16% lipids (Table 7.2).

Table 7.2 - Composition of the feed used during the experimental period.

Ingredients	U.M	Quantity	Ingredients	U.M	Quantity
Protein	%	45	Vitamin C	mg/kg	150
Lipids	%	16	Fe	mg/kg	60
Cellulose	%	2	Cu	mg/kg	6
Ash	%	7	Zn	mg/kg	100
Calcium	%	1,3	Mn	mg/kg	25
Sodium	%	0,30	Ca	mg/kg	2,5
Phosphorus	%	1	BHA (E320)	mg/kg	30
Vitamin A	IU/kg	10 000	BHT (E321)	mg/kg	29
Vitamin E	mg/kg	200			

Ingredients: feather meal, wheat, fish meal, sunflower concentrate, wheat flour, blood meal, fish oil, rapeseed cake, rapeseed oil, hemoglobin powder, sodium chloride, calcium carbonate.

The physicochemical parameters of water were recorded daily using the portable multiparameter described in previous chapters. The concentration of nitrogen compounds was measured twice a week. At the end of the experiment, seven fish from each growth unit were sacrificed for the analysis of flesh composition (whole fish, including scales). Conventional, standardized methods determined moisture, crude protein, lipids, and ash. The analyses were performed in triplicate, and the results were calculated based on the wet weight of the samples.

7.1.3. Results and Discussions

- **Monitoring water quality parameters**

Maintaining good water quality is essential for fish species' growth, survival, and production. Water quality parameters, except for ammonia concentration, did not show significant differences ($p > 0.05$) (Table 7.3). Ammonia concentrations significantly increased with the higher feeding level ($p < 0.05$).

The water temperature fluctuated within the range of 23.28-23.4°C, falling within the optimal limits for the growth of carp fry. It is evident that the thermal oscillations of the water are directly related to variations in the air temperature in the surrounding environment. Water temperature dynamics cannot be associated with technological elements such as feeding rate.

Table 7.3 - Mean values (± SD) of the main physicochemical parameters of water

Parameter	V_1	V_2	V_2	V_4
T°C	23,30±0,48	23,40±0,68	23,30±0,5	23,28±0,46
pH (pH unit)	8,09±0,13	8,15±0,12	8,15±0,14	8,15±0,14
OD (mg/ l)	7,59±0,36	7,87±0,42	7,95±0,32	7,84±0,35
$N\text{-}NO_2^-$ (mg/ l)	0,25 ±0,09	0,26±0,13	0,26±0,10	0,27±0,13
$N\text{-}NO_3^-$ (mg/ l)	18,56±5,30	18,80±4,76	18,87±6,47	19,78±4,76
$N\text{-}NH_4^+$ (mg/ l)	0,21±0,11	0,32±0,09	0,38±0,16	0,45±0,18
$P\text{-}PO_4^+$(mg/ l)	0,62±0,48	0,64±0,23	0,67±0,23	0,68±0,30

Dissolved oxygen is undoubtedly the most important parameter of aquaculture water. Smaller fish require a higher amount of oxygen compared to larger fish due to their higher metabolic rate. For the carp species, which thrives optimally between 22-28°C, the optimal oxygen concentration is around 5-7 mg/l. In this context, maintaining the oxygen concentration within acceptable technological limits was pursued. Figure 7.2 shows that throughout the experiment, the oxygen concentration in all variants fluctuated within the range of 7-8 mg/l.

The water pH varied within the optimal limits imposed by the species-specific technological requirements (7-8.5 units). The minimum recorded value was 8.09 in variant V_1, while the maximum was 8.15 in variants V_2, V_3, and V_4.

- **Analysis of fish growth performance**

At the beginning of the experiment, the fish had almost equal body weights, and no significant differences were recorded between the variants. The Levene test verified and confirmed the homogeneity of values ($p>0.05$). After 31 days of growth, it was found that the mean final body weight was significantly higher in V_1 (20.29±1.21 g) compared to the other three variants ($p<0.05$). However, no significant differences were recorded between variants V_2 (18.90±0.53 g), V_3 (18.86±0.40 g), and V_4 (18.74±0.37 g) ($p>0.05$).

The coefficient of determination (R^2) values above 0.8 (80%) demonstrate the homogeneity of the results. The best dispersion of points corresponding to the (W, L) value pairs was recorded in variant V_3, with an R^2 of 0.911. In this experiment, the growth coefficient (b) values indicated negative allometry ($b<3$) in V_1, V_2, and V_4, meaning that the fish's growth was more proportionate to length rather than weight. However, the obtained values are very close to isometry situations. In variant V_3, isometry ($b=3$) was recorded, indicating that the fish grew in weight and length, a preferred phenomenon.

Although fish survival was high, ranging from 94.67% to 98.33%, ANOVA analysis showed that the survival rate was not significantly influenced by the daily feeding ratio ($p>0.05$). The highest survival rate was achieved in variant V_1, where a 3% BW ratio was used.

The following technological indicators were obtained at the end of the growth experiment (Table 7.4).

Table 7.4 - Technological indicators for carp growth performance in NTL

Variant Experiment	V1			V2			V3			V4		
Parameters	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
Initial Biomass (g)	663	666	665	665	663	664	667	665	665	667	660	668
Initial Biomass (kg/m³)	4,64	4,66	4,66	4,66	4,64	4,65	4,67	4,65	4,66	4,67	4,62	4,68
Final Biomass (g)	1193	1188	1190	1101	1105	1100	1102	1100	1098	1091	1095	1093
Final Biomass (kg/m³)	8,35	8,32	8,33	7,71	7,74	7,70	7,71	7,70	7,69	7,64	7,67	7,65
Biomass Growth Rate (g)	530	522	525	436	442	436	435	435	433	424	435	425
Biomass Growth Rate (kg/m³)	3,71	3,65	3,68	3,05	3,09	3,05	3,04	3,05	3,03	2,97	3,05	2,98
Initial Fish number	60	60	60	60	60	60	60	60	60	60	60	60
Final Fish number	60	57	59	58	57	60	57	59	59	69	59	57
Count Survival Rate (%)	100	97	98	91	93	100	95	98	98	98	98	95
Average Initial Weight (g/ex)	11,06	11,1	11,1	11,1	11,1	11,1	11,1	11,1	11,1	11,1	11,0	11,1
Average Final Weight (g/ex)	19,88	20,84	20,17	18,98	19,39	18,33	19,33	18,64	18,61	18,49	18,56	19,18
GR (Daily Growth Rate) (g/day)	17,08	16,84	16,94	14,05	14,24	14,07	14,02	14,05	13,97	13,68	14,03	13,71
SGR (%/zi)	1,89	1,87	1,88	1,62	1,65	1,63	1,62	1,63	1,62	1,59	1,63	1,69
Individual Growth Rate (g)	8,8	9,7	9,1	7,9	8,3	7,3	8,2	7,6	7,5	7,4	7,6	8,0
Total Feed Distributed (g)	617	619	618	804	802	803	1014	1009	1010	1220	1207	1222
FCR (g feed/g biomass gain)	1,16	1,19	1,18	1,85	1,82	1,84	2,33	2,32	2,33	2,88	2,78	2,84

Regarding individual and biomass growth rates, the best results were obtained in variant V_1, with a daily feed ratio of 3% BW (9.2 g/fish, 525.6 g biomass). In the other variants, the following average values were obtained: V_2 (7.8 g/fish, 437.6 g biomass), V_3 (7.7 g/fish, 434.3 g biomass), V_4 (7.6 g/fish, 428.0 g biomass). The differences between V_1 and the other three variants were significant.

Feed management is an essential condition for achieving profitable and cost-effective production. The best feed conversion ratio (FCR) was obtained in variant V_1 (1.18 kg feed per 1 kg biomass gain). The differences were significant compared to the other variants (FCR of 1.84, 2.33, and 2.84).

Significant differences (p>0.05) were observed in the growth rate (GR) and specific growth rate (SGR) values obtained in the four experimental variants, with the best deals recorded in variant V_1 (16.95 grams/day for GR, 1.88%/day for SGR). In comparison, lower average values were obtained in V_2 (14.12 g/day for GR, 1.63%/day for SGR), V_3 (14.01 g/day for GR, 1.62%/day for SGR), V_4 (13.81 g/day for GR, 1.58%/day for SGR).

Our research's final growth parameters of the carp fry are close to the results obtained in other studies. Markovic et al. (2012) observed a maximum specific growth rate (SGR) of 1.09%/day and a feed conversion ratio (FCR) ranging from 1.49 to 2.82 over a 90-day growth period.

By critically analyzing all the technological indicators in this experiment, it can be concluded that the performance of carp fry growth in a recirculating system depends on the amount of feed distributed, including the daily ratio, but fish growth is not directly proportional. Unfortunately, it is possible for a feed ratio to be miscalculated, either too low or too high, with negative influences on the fish's growth rate.

- **The biochemical composition of fish meat**

Regarding the biochemical composition of fish meat, the results regarding the effects of feeding levels on the biochemical composition are presented in Table 7.5. The research has shown that the daily feed size significantly affects the fish's body's water, protein, lipid, and ash content (p<0.05). Regarding protein content, significantly higher values were obtained in variant V_4 (14.56%) compared to V_1 (13.78%), V_2 (12.31%), and V_3 (14.32%) (Cordeli A.N. et al., 2021).

Table 7.5 - Influence of daily feed ratio on the biochemical composition of carp fry meat

Parametru	Experimental variant			
	V_1	V_2	V_3	V_4
Water (%)	72,68±0,17	72,98±0,16	70,43±0,17	70,43±0,13
Proteins (%)	13,78±0,08	12,31±0,16	14,32±0,76	14,56±0,31
Lipids (%)	11,58±0,42	12,37±0,14	12,29±0,32	12,85±0,63
Ash (%)	1,47±0,03	1,67±0,02	1,72±0,07	1,78±0,05

Note: The data is presented as the mean of triplicates ± standard deviation.

A significant lipid and ash content increase was observed with increasing feeding levels (p<0.05). The lowest values were recorded in variant V_1, with the lowest daily ratio of 3% BW. Although surprising, these results can be explained by the usual increase in moisture content at the expense of lipid concentration, while protein levels remain relatively stable. In our case, protein content was close to 14%, except in variant V_2, which was 12.31%.

7.1.4. Conclusions

Feeding management is essential in aquaculture to minimize production costs and maximize profitability. The optimal daily feeding ratio should be based on the capacity and performance of the culture system, species, and food quality. Increasing the feeding level beyond certain limits does not significantly improve fish growth performance. Based on the results obtained in this experiment, it has been established that for common carp fingerlings (*Cyprinus carpio*) with a body weight of 11-21 g per individual, the best daily feeding ratio is approximately 3% of body weight per day. Higher values have led to unsatisfactory technological results and pollution of the growth system.

7.2. Experiment 6 - Influence of Daily Feeding Ratio on the Growth Performance of Carp Fingerlings, Pilot Level

7.2.1. Introduction

Consumer demand for fish and other aquatic organisms has led farmers to develop production infrastructure and apply new, innovative technologies that allow for high stocking densities and quality feed to achieve high yields that cover all production costs and generate profits.

The experiment aimed to determine the influence of the daily feeding ratio on the performance of carp fingerlings grown in a recirculating system using plastic tanks with a capacity of 1 m^3 as growth units. The choice of the optimal ratio significantly affects production levels and production costs.

7.2.2. Material and methods

In this experiment, the biological material consisted of carp fingerlings with an initial average body weight (W) of 5.49 ± 0.06 g and a total length (L) of 60 ± 0.5 mm. The fish were divided into four experimental variants, each variant in duplicate: V_1 - with a daily ratio of 3.0% BW, V_2 - with a daily ratio of 3.4% BW, V_3 - with a daily ratio of 4.0% BW, and V_4 - with a daily ratio of 4.4% BW. The daily ratios were administered to the fish in three meals scheduled at 08:00, 13:00, and 18:00. The fish were fed with a commercial feed 45/16 (45% crude protein and 16% lipids).

The recirculating system consisted of eight growth units of 1 m^3 each, water recirculation pumps, mechanical and biological filters, water sterilization equipment, and equipment for measuring the physicochemical parameters of the water. The physicochemical parameters of the water were monitored daily using sensors integrated into the RAS system.

At the end of the experiment, seven fish from each growth unit were sacrificed to determine the biochemical composition of the fish body. Moisture, crude protein, lipids, and ash were determined using standard methods. The analyses were performed in triplicate, and the results were calculated based on the wet weight of the samples.

7.2.3. Results and discussions

- **Monitoring of Water Quality Parameters**

Feeding intensity, composition, metabolic activity, and unconsumed feed affect the water quality in the culture tanks.

The water temperature remained relatively constant throughout the experiment, considering that the water was recirculated. The average temperature recorded in the four variants (eight growth units) fluctuated between 20ºC and 23.8ºC.

Dissolved oxygen in the water also did not show significant differences between the four experimental variants, usually 7-8 mg/l.

Ammonia is an unstable compound that can transform into ammonia, which is extremely toxic to cultured biomass. The concentration of nitrates, the final product of ammonia oxidation by the Nitrosomonas-Nitrobacter bacterial complex, represents another important parameter of water quality that was continuously monitored during the experiment. The dynamics of nitrate concentration in the water of the growth units indicated values ranging from 11.5 mg/l (minimum value) to 18.6 mg/l (maximum value), which were below the technologically acceptable threshold.

- **Analysis of Fish Growth Performance**

The results of the main technological performance indicators obtained from the analysis of the initial and final data are presented in Table 7.6.

Table 7.6 - Technological indicators at the end of the experimental period

Parameter	V_1		V_2		V_3		V_4	
	V_1R_1	V_1R_2	V_2R_1	V_2R_2	V_3R1	V_3R_2	V_4R_1	V_4R_2
Initial biomass (g)	1097,84	1095,73	1079,48	1095,18	1112,24	1084,47	1108,6	1111,67
Initial biomass (kg/m^3)	2,20	2,19	2,16	2,19	2,22	2,17	2,22	2,22
Final biomass (g)	2537	2189	1934	1827	2018	2060	2075	1994
Final biomass (kg/m^3)	5,07	4,38	3,87	3,65	4,04	4,12	4,15	3,99
Biomass growth increment (g)	1439	1093	855	732	906	976	966	882
Biomass growth increment (kg/m^3)	2,88	2,19	1,71	1,46	1,81	1,95	1,93	1,76
Number of initial fish	200	200	200	200	200	200	200	200
Number of final fish	192	195	195	197	199	195	194	196
Survival rate (%)	96	98	98	99	100	98	97	98
Initial average weight (g/ex)	5,49	5,48	5,40	5,48	5,56	5,42	5,54	5,56
Final average weight (g/ex)	13	11	10	9	10	11	11	10
GR (Specific growth rate)(g/day)	46,42	35,27	27,57	23,61	29,22	31,47	31,17	28,46
SGR (%/day)	2,70	2,23	1,88	1,65	1,92	2,07	2,02	1,88
Individual growth increment (g)	8	6	5	4	5	5	5	5
Total feed distributed (g)	1439	1437	1707	1725	2172	2133	2518	2523
FCR (g feed/g biomass increment)	1,00	1,31	2,00	2,36	2,40	2,19	2,61	2,86
Daily feeding ratio (g/kg body weight)	10,5	10,5	12	12	14	14	15,5	15,5
Daily feeding ratio (% biomass)	3,0	3,0	3,4	3,4	4,0	4,0	4,4	4,4

Regression curve equations were obtained to establish the degree of correlation between total length and body weight, and the coefficient of determination (R^2) was calculated for each of the four experimental variants. Excellent values were obtained for the coefficient of determination, exceeding 0.90 in variants V_1, V_2, and V_4 and 0.86 in V_3. All values indicate a good dispersion of points relative to the regression curve (Cordeli A.N. et al., 2019).

From the body weight-total length regression analysis, very close values of the allometric factor "b" can be observed for all four experimental variants. The highest value of the allometric factor was calculated for experimental variant V_1 (b=3.21), followed by experimental variants V_3 (b=3.18) and V_2 (b=3.04). In these cases, allometry was positive (b>3), indicating that fish growth was mainly driven by weight rather than length, a positive phenomenon indicating good living conditions for fish. Lower values of the allometric factor were obtained for the 4% biomass ratio, V_4 (b=2.98), which is still close to positive allometry.

For a more accurate assessment of the influence of feeding on the growth performance of common carp fingerlings, the biomass increment was analyzed for the four experimental variants. The highest fish biomass growth increment (1266 g) was recorded in variant V_1, with a daily feeding ratio of 3.0% BW, while the minimum value of 732 grams was obtained in experimental variant V_2. The average individual body weights at the end of the experiment were 12 g/fish in V_1, 9.5 g/fish in V_2, 10.5 g/fish in V_3, and 11 g/fish in V_4. These results confirm that V_1, with the lowest daily feeding ratio of 3.0% BW was the most effective variant.

Regarding the feed conversion ratio (FCR) values, the statistical analysis highlighted significant differences ($p<0.05$) between variants V_1 (1.16), V_2 (2.18), V_3 (2.30), and V_4 (2.74) g feed/g biomass increment, indicating better nutrient utilization and higher economic efficiency in the case of the lowest feeding ratio.

As for the specific growth rate (SGR), a decrease was observed with an increasing feeding ratio, and the differences between experimental variants were significant ($p<0.05$). SGR recorded average values of 2.47% BW/day in V_1, 1.77% BW/day in V_2, 2.00% BW/day in V_3, and 1.95% BW/day in V_4. The daily growth rate (GR) had the following average values: 40.85 g in V_1, 25.59 g in V_2, 30.35 g in V_3, and 29.82 g in V_4. These results confirm that the variant with a daily feeding ratio of 3% BW was the most efficient.

- **The biochemical composition of fish meat**

The influence of daily feed ration on the biochemical composition of fish meat (whole fish) is presented in Table 7.7. Research has demonstrated that the size of the daily ration significantly affects ($p<0.05$) the water, protein, lipid, and ash content in the fish body, similar to the previous experiment conducted at NTL. Regarding protein content, significantly higher values were obtained in variant V_3 (15.26%) compared to V_1 (13.66%), V_4 (13.05%), and V_2 (12.19%) (Cordeli A.N. et al., 2021).

Table 7.7. - Influence of feed ration on the biochemical composition of fish body

Parameter	Experimental variants				
	Initial	V_1 final	V_2 final	V_3 final	V_4 final
Water (%)	71,50±0,11	73,08±0,13	73,15±0,13	73,40±2,00	73,62±0,06
Proteins (%)	14,51±0,38	13,66±0,26	12,19±0,01	15,26±0,04	13,05±0,09
Lipids (%)	12,69±0,35	12,04±0,13	12,69±0,30	11,76±0,24	11,48±0,038
Ash (%)	1,58±0,04	1,47±0,069	1,59±0,02	1,51±0,018	1,59±0,097

Note: Data is presented as the mean of triplicates ± standard deviation.

The lipid content had higher values in V_1 (12.04%) and V_2 (12.69%). Paradoxically, the lowest value was obtained in variant V_3 (11.76%), with a higher daily ration (4% BW). The inverse proportional variation between water and lipid content is confirmed, meaning that a decrease in water content leads to an increase in body lipids. Regarding ash content, no significant statistical differences were recorded between the experimental variants or compared to the values obtained from the initial samples.

7.2.4. Conclusions

The growth performance of fish in a recirculating aquaculture system is significantly influenced by a series of ecotechnological and biological factors such as system carrying capacity, water quality, fish species, developmental stage or age, and nutritional requirements, among others.

The obtained biotechnological results demonstrated that, in terms of growth performance and feeding efficiency, the variant with a daily ration of 3% of body weight is most suitable for the growth of carp fry in small-scale farming units, such as NTL or NTP systems. Notably, the daily ration cannot be arbitrarily increased as it can lead to unjustified consumption of significant amounts of feed. Based on the obtained results, it can be concluded that excessively high daily feed rations can lead to decreased production. Additionally, choosing an optimal feeding ratio must consider the efficiency of feed utilization and its impact on water quality. Administering excessive amounts of feed can also deteriorate water quality.

The selection of the daily feed ration and, implicitly, the calculation of the feed requirement should be done in correlation with the existing fish biomass in the growth system. An important technological aspect is also the frequency of daily feedings. Dividing the feed into a minimum of three meals per day is recommended.

CHAPTER 8 - GROWTH PERFORMANCE, PHYSIOLOGICAL STATUS, AND NUTRIENT RETENTION EFFICIENCY IN CARP FRY

8.1. Introduction

Nutrient retention efficiency from feeds in fish meat can vary depending on several factors, such as the growth environment, species, feed quality, and health status. A balanced and species-specific diet and the application of sustainable and responsible aquaculture practices can contribute to achieving high production yields.

The research aimed to determine nutrient retention efficiency from feeds in fish meat within a recirculating aquaculture system based on the experience gained from the first six experiments.

8.2. Material and methods

The biological material consisted of carp fry with an initial average body weight of 18 ± 0.56 g/individual. 1200 fish were distributed into four density variants, each with two replicates: V_1 - 40 fish/growing unit; V_2 - 80 fish/producing unit; V_3 - 160 fish/growing unit; V_4 - 320 fish/growing unit. To assess the physiological status, hematological parameters were determined, including red blood cell count, hematocrit (Ht), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Table 8.1 - Chemical composition of the feed used during the experimental period.

Ingredients	U.M	Value	Ingredients	U.M	Value
Proteins	%	45	Vitamin C	mg/kg	150
Lipids	%	16	Fe	mg/kg	60
Cellulose	%	2	Cu	mg/kg	6
Ash	%	7	Zn	mg/kg	100
Calcium	%	1,3	Mn	mg/kg	25
Sodium	%	0,30	Ca	mg/kg	2,5
Phosphorus	%	1	BHA (E320)	mg/kg	30
Vitamin A	IU/kg	10 000	BHT (E321)	mg/kg	29
Vitamin E	mg/kg	200			

Ingredients: poultry meal, wheat, fish meal, sunflower concentrate, wheat flour, blood meal, fish oil, rapeseed meal, rapeseed oil, hemoglobin powder, sodium chloride, and calcium carbonate.

At the end of the experiment, ten fish were sacrificed from each growth unit to determine the biochemical composition of fish bodies. Moisture, crude protein, lipids, and ash were determined using standard methods. The analyses were performed in triplicate, and the results were calculated based on the wet weight of the samples.

8.3. Results and Discussions

➢ Monitoring water quality parameters

In super-intensive fish farming systems, the technological water serves as a physical substrate without influencing productivity. The living environment should provide an optimal level of technological comfort for the cultured biomass (Stepanowska K. et al., 2006).

The physicochemical parameters of the water (temperature, dissolved oxygen level, and pH) were monitored daily using sensors incorporated into the system. Nitrogen compounds (nitrites $N\text{-}NO_2^-$, nitrates $N\text{-}NO_3^-$, ammonium $N\text{-}NH_4^+$) were measured weekly.

Throughout the experiment, all physicochemical parameters of the water fluctuated within permissible limits, close to the technological optimum (Table 8.2). There were no significant differences between the experimental variants (confirmed by ANOVA analysis, p > 0.05).

Table 8.2 - Mean values of the main physicochemical parameters of water (±SD)

Parameter	V_1	V_2	V_3	V_4
Temperature˚C	20,5±1,34	20,7±1,36	20,3±1,38	20,1±1,52
Dissolved oxygen (mg/l)	14±2	13±2	12±1	12
pH (pH unit)	7	7	7	7
$N\text{-}NO_3^-$(mg/l)	24,56±4,26	24,84±4,54	25,28±4,86	26,84±2,17
$N\text{-}NO_2^-$(mg/ l)	0,07±0,02	0,08±0,023	0,08±0,04	0,084±0,03
$N\text{-}NH_4^+$(mg/ l)	0,06±0,03	0.07±0,04	0,12±0,049	0,12±0,06

The water temperature varied within the range of 20.1±1.52˚C in V_4 and 20.7±1.36˚C in V_3.

The dissolved oxygen content showed variations between 12 mg/l in V_4 and 14±2 mg/l in V_1.

The water pH remained constant as well. Due to the proper functioning of the recirculating system and the fact that the filtered water passed through all the growth units, no differences were observed between the experimental variants.

The nitrogen compounds remained within the optimal range for carp growth. The variation in nitrate concentration is presented in Figure 8.2. Once the bacterial population stabilized, the biofilter functioned at optimal parameters, promoting effective water filtration. Throughout the experiment, nitrate levels ranged from 24.4 to 26.8 mg/l, averaging 25.38 mg/l. The average nitrite concentration values analysis for the entire experimental period indicated no significant differences between the four evaluated stocking densities. The average concentrations of $N\text{-}NO_2$- for each growth unit were 0.08±0.023 mg/l (V_1), 0.08±0.04 mg/l (V_2), 0.084±0.03 mg/l (V_3), and 0.07±0.02 mg/l for V_4.

The ammonium ion ($N\text{-}NH_4$+) values did not show significant differences between the experimental variants. Its values fluctuated within the range of 0.01 to 0.20 mg/l, with an average of 0.05±0.12 mg/l. Maintaining the ammonia level within acceptable technological limits indicates that the first stage of the biological nitrification process proceeded normally.

- **Analysis of the growth performance of carp fry**

At the end of the experiment, significant differences (ANOVA, p>0.05) were observed between the four density variants for the following technological indicators: body weight, feed conversion ratio, specific growth rate, and protein efficiency ratio (Table 8.3). Although fish survival rates were high, ranging from 94% to 100%, ANOVA analysis showed that survival rate was not significantly influenced by stocking density (p>0.05). The highest survival rate was observed at the lowest density (V_1).

The correlation between total length and body weight (Lt-M) was established based on measurements taken at the end of the experiment. The data obtained from a sample of 50 specimens from each experimental variant were analyzed to develop a growth model and determine a growth equation for carp fry. Growth estimation was performed using linear regression and logarithmic equations.

The regression analysis of the length-body weight relationship for the four experimental variants showed high determination coefficients (R^2=0.9352 in V_2 with an initial density of 80 fish/growth unit, corresponding to 2.85 kg/m^3, R^2=0.9495 in V_1 with an initial density of 40 fish/growth unit, corresponding to 1.52 kg/m^3, R^2=0.9567 in V_4 with an initial density of 320 fish/growth unit, corresponding to 11.18 kg/m^3, R^2=0.9601 in V_3 with an initial density of 160 fish/growth unit, corresponding to 5.68 kg/m^3).

Table 8.3 - Technological indicators obtained for carp growth

Experimental Variant	V_1		V_2		V_3		V_4	
	V_1R_1	V_1R_2	V_2R_1	V_2R_2	V_3R_1	V_3R_2	V_4R_1	V_4R_2
Initial biomass (g)	759	749	1426	1429	2840	2838	5594	5585
Average individual weight (g)	19	18,7	17,8	17,9	17,8	17,7	17,5	17,5
Initial biomass (kg/m^3)	1,52	1,50	2,85	2,86	5,68	5,68	11,19	11,17
Final biomass (g)	1800	1846	3440	3326	5255	5515	9242	9359
Final biomass (kg/m^3)	3,60	3,69	6,88	6,65	10,51	11,03	18,48	18,72
Biomass growth increment (g)	1041	1097	2014	1897	2415	2677	3648	3774
Biomass growth increment (kg/m^3)	2,08	2,19	4,03	3,79	4,83	5,35	7,30	7,55
Initial fish number	40	40	80	80	160	160	320	320
Final fish number	39	38	80	79	157	150	303	303
Survival rate (%)	98	95	100	99	98	94	95	95
Average initial weight (g/ex)	19,0	18,7	17,8	17,9	17,8	17,7	17,5	17,5
Average final weight (g/ex)	46,2	48,6	43,0	42,1	33,5	36,8	30,5	30,9
Growth days	61	61	61	61	61	61	61	61
GR (Daily growth rate) (g/day)	0,45	0,49	0,41	0,40	0,26	0,31	0,21	0,22
SGR (%/day)	1,4	1,5	1,4	1,4	1,0	1,1	0,8	0,8
Individual growth increment (g)	27,2	29,8	25,2	24,2	15,7	19,0	13,0	13,4
Total feed distributed (g)	1374	1362	2627	2632	5239	5236	10362	10351
FCR (g feed/g biomass increment)	1,32	1,24	1,30	1,39	2,17	1,96	2,84	2,74
Daily feed ratio (g/kg body weight)	9	9	9	9	9	9	9	9
Daily feed ratio (% biomass)	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0
Crude protein in feed (PB %)	45%	45%	45%	45%	45%	45%	45%	45%

The allometric coefficient "b" value indicates a better condition of the specimens in this experimental variant, with a value of 3.1203 (positive allometry, indicating that the fish grow more in terms of weight than length). The lowest value of the allometric coefficient was recorded in the experimental variant V_2 (2.9501, negative allometry), but it was still close to the allometric coefficients of V_3 (3.0465) and V_4 (3.0501). Considering both the R^2 values and the "b" coefficient values, it can be concluded that the variant with the lowest density was better.

Considering the excellent survival rate (94-100%), the dynamics of the average final body mass in the four experimental variants show that V_1, with the lowest density, is superior (Figure 8.9). Thus, in V_1, an average individual body mass of 47.4 g/fish was obtained; in V_2, it was 42.55 g/fish; in V_3, it was 35.15 g/fish; and in V_4, it was 30.7 g/fish. As for the increment in final biomass, it is evident that V_4, with the highest density, resulted in the highest biomass. In fact, significant differences ($p<0.05$) in final biomass were observed among the four variants.

From the analysis of technological indicators, it can be observed that stocking density influenced feed conversion and nutrient retention in the flesh. The feed conversion ratio (FCR) had the lowest values for the first experimental variant (1.24 - 1.32 g feed/g biomass increment), followed by the values recorded in the second variant (1.30-1.39 g/g). The highest FCR values, which is a negative aspect, were recorded in the variant with the highest density, V4 (2.74-2.84 g/g), more than double compared to the first two variants.

The daily individual growth rate (GR), a technological indicator indicating linear growth of the fish, averaged 0.47 g/day in V_1, 0.41 g/day in V_2, 0.28 g/day in V_3, and 0.22 g/day in V_4. It can be observed that the variant with the lowest density was the best (Figure 8.11). The specific growth rate (SGR) showed a similar variation, with the best values obtained in the variants with the lowest density. SGR values were 1.51%/day in V_1, 1.42%/day in V_2, 1.12%/day in V_3, and 0.92%/day in V_4.

Regarding the protein efficiency ratio (PER), the best values were also recorded in the stocking densities V_1 (1.52 g biomass increment/g protein intake) and V_2 (1.40 g/g). The lowest values were observed in the variant with the highest density, V_4 (PER = 0.70 g/g). Protein utilization efficiency (PUE) followed a similar trend to PER, with values of 23.945% in V_1, 19.195% in V_2, 11.945% in V_3, and 9.745% in V_4. Statistically, the differences among the four variants were significant (ANOVA, $p>0.05$).

➢ **Nutrient retention efficiency in fish flesh**

The fish were raised in NTL and NTP in the first six experiments, starting from an average body mass of approximately 1.5 grams per specimen. After about three months of experimentation, conducted in the laboratory and pilot level in a more technologically complex recirculating system module, the fish reached a body mass of 17-19 grams per specimen, which is when this final experiment began. The four density variants used the same feed 45/16 and a 2% BW ratio. The stocking density mainly determined the differences between the experimental variants to observe how increasing fish biomass can efficiently utilize food to a greater or lesser extent. Furthermore, fish farmers are highly interested in research investigating the influence of density on fish growth performance.

Their biochemical composition determines the quality of fish meat and feed. The following observations can be made by comparing each class of nutrients from the two moments of biochemical analysis represented by the start and end of the experiment (Tables 8.4 - 8.10).

At the beginning of the experiment, the water content in fresh fish meat was 73.32% (Figure 8.13). At the end of the experiment, variant V_1 (average of the two repetitions) had 66.92% water content, variant V_2 had 68.53% water content, variant V_3 recorded 68.50% water content, and variant V_4 accumulated 69.37% water content. Inversely proportional to the water percentage, the dry matter (DM) naturally registers values directly proportional to the quality and quantity of the distributed feed.

Proteins, in general, accumulate in meat as the feed ratio increases, but the growth is not linear. In our experiment, proteins had initial average values of 13.55% and final values of 14.79% (V_1), 14.00% (V_2), 13.81% (V_3), and 13.69% (V_4).

Regarding the evolution of body lipids, expressed in wet weight, it was found that as the biomass increased, the fish accumulated fats: 16.60% (V_1), 16.67% (V_2), 16.45% (V_3), 15.54% (V_4) (Figure 8.13).

Ash (mineral substances) had a small, almost constant fluctuation without significant differences between variants. Compared to the initial moment, when it had a concentration of 1.54%, as the fish biomass increased, the following values were obtained: 1.47% (V_1), 1.31% (V_2), 1.59% (V_3), 1.62% (V_4) (Table 8.4, Table 8.6).

Table 8.4 - Biochemical composition of the initial biomass

Variant/ Parameter	(%)	Absolute values							
		V_1		V_2		V_3		V_4	
		V_1R_1	V_1R_2	V_2R_1	V_2R_2	V_3R_1	V_3R_2	V_4R_1	V_4R_2
Water (g)	73,32	556,50	549,17	1045,54	1047,74	2082,29	2080,82	4101,52	4094,92
Proteins (g)	13,55	102,84	101,49	193,22	193,63	384,82	384,55	757,99	756,77
Lipids (g)	11,99	91,00	89,81	170,98	171,34	340,52	340,28	670,72	669,64
Ash(g)	1,54	11,69	11,53	21,96	22,01	43,74	43,71	86,15	86,01
Diferences (g)	-0,40	-3,04	-3,00	-5,70	-5,72	-11,36	-11,35	-22,38	-22,34
Total(g)	**100**	**759**	**749**	**1426**	**14290**	**2840**	**2838**	**5594**	**5585**

Table 8.5- Absolute biochemical composition of the final biomass

Variant/ Parameter	V_1		V_2		V_3		V_4	
	V_1R_1	V_1R_2	V_2R_1	V_2R_2	V_1R_1	V_1R_2	V_2R_1	V_2R_2
Water (g)	1209,24	1230,86	2314,50	2321,15	3555,48	3825,59	6545,18	6358,22
Proteins (g)	245,23	294,95	495,29	452,80	779,95	705,20	1068,47	1480,87
Lipids (g)	300,44	304,98	635,54	494,98	931,34	837,62	1393,14	1499,69
Ash (g)	23,92	30,24	46,65	42,71	87,71	84,71	168,76	134,49
Differences (g)	21,17	-15,03	-51,98	14,37	-99,48	61,88	66,45	-114,27
Total(g)	**1800**	**1846**	**3440**	**3326**	**5255**	**5515**	**9242**	**9359**

Table 8.6 - Percentage Biochemical Composition of the Final Biomass.

Variant/ parameter	V_1		V_2		V_3		V_4	
	V_1R_1	V_1R_2	V_2R_1	V_2R_2	V_1R_1	V_1R_2	V_2R_1	V_2R_2
Water (%)	67,18	66,67	67,28	69,78	67,65	69,36	70,82	67,93
Proteins (%)	13,62	15,97	14,39	13,61	14,84	12,78	11,56	15,82
Lipids (%)	16,69	16,52	18,47	14,88	17,72	15,18	15,07	16,02
Ash (%) (subst. miner)	1,32	1,63	1,35	1,28	1,66	1,53	1,82	1,43
Differences (%)	1,17	-0,81	-1,51	0,43	-1,89	1,12	0,71	-1,22
Total (%)	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table 8.7 - Growth Rate.

Variant/ parameter	V_1		V_2		V_3		V_4	
	V_1R_1	V_1R_2	V_2R_1	V_2R_2	V_1R_1	V_1R_2	V_2R_1	V_2R_2
Water (g)	652,74	681,69	1268,96	1273,41	1473,19	1744,77	2443,66	2263,30
Proteins (g)	142,39	193,46	302,07	259,17	395,13	320,65	310,48	724,11
Lipids (g)	209,43	215,17	464,56	323,64	590,83	497,34	722,42	830,04
Ash (g)	12,23	18,70	24,69	20,70	43,97	41,01	82,61	48,48
Differences(g)	24,20	-12,03	-46,27	20,08	-88,12	73,23	88,83	-91,93
Total(g)	**1041**	**1097**	**2014**	**1897**	**2415**	**2677**	**3648**	**3774**

Analyzing the correlation between nutrition efficiency parameters such as PPV (PUE) (productive protein value or protein utilization efficiency from feed), LPV (LUE) (lipid productive value or lipid utilization efficiency from feed), PER (protein efficiency ratio), and two economic efficiency parameters (total biomass growth rate and FCR-feed conversion ratio), it was found that as the fish biomass in the growing units increased, the efficiency of nutrient retention from feed decreased (Table 8.5 - 8.9).

Table 8.8 - Nutrients Distributed in Feed.

Variant/ parameter	V_1		V_2		V_3		V_4	
	V_1R_1	V_1R_2	V_2R_1	V_2R_2	V_1R_1	V_1R_2	V_2R_1	V_2R_2
Total wet feed distributed (g)	1374	1362	2627	2632	5239	5236	10362	10351
Proteins (g)	704,86	698,71	1347,65	1350,22	2687,61	2686,07	5315,71	5310,06
Lipids (g)	239,08	236,99	457,10	457,97	911,59	911,06	1802,99	1801,07
Carbohydrates (g)	258,31	256,06	493,88	494,82	984,93	984,37	1948,06	1945,99
Ash (mineral substances) (g)	87,94	87,17	168,13	168,45	335,30	335,10	663,17	662,46
Total dry feed distributed (g)	**1289,8**	**1278,5**	**2466,0**	**2470,7**	**4917,8**	**4915,0**	**9726,8**	**9716,5**
Wet feed conversion ratio	1,32	1,24	1,30	1,39	2,17	1,96	2,84	2,74
Dry feed conversion ratio	1,24	1,17	1,22	1,30	2,04	1,84	2,67	2,57

Table 8.9 - Nutritional Efficiency

Variant/Parameter	V_1		V_2		V_3		V_4	
	V_1R_1	V_1R_2	V_2R_1	V_2R_2	V_1R_1	V_1R_2	V_2R_1	V_2R_2
Protein biomass gain (g)	142,39	193,46	302,07	259,17	395,13	320,65	310,48	724,11
Protein Efficiency Ratio (PER) (g/g)	1,48	1,57	1,49	1,40	0,90	1,00	0,69	0,71
Protein Productive Value (PPV) (%)	20,20	27,69	22,41	19,19	14,70	11,94	5,84	13,64
Lipid biomass gain (g)	209,43	215,17	464.56	323,64	590,83	497,34	722,42	830,04
Lipid Efficiency Ratio (LER) (g/g)	4,35	4,63	4,41	4,14	2,65	2,94	2,02	2,10
Lipid Productive Value (LPV) (%)	87,60	90,79	101,63	70,67	64,81	54,59	40,07	46,09

Table 8.10 - Ratio of body nutrients to protein biomass

Variant/Parameter	V_1		V_2		V_3		V_4	
	V_1R_1	V_1R_2	V_2R_1	V_2R_2	V_1R_1	V_1R_2	V_2R_1	V_2R_2
Water/proteins (g/g)	4,93	4,17	4,67	5,13	4,56	5,42	6,13	4,29
Proteins/proteins (g/g)	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
Lipids /proteins (g/g)	1,23	1,03	1,28	1,09	1,19	1,19	1,30	1,01
Ash/proteins (g/g)	0,10	0,10	0,09	0,09	0,11	0,12	0,16	0,09
Differences (g)	0,09	-0,05	-0,10	0,03	-0,13	0,09	0,06	-0,08
Total nutrient subs.1 g body protein.	2,41	2,09	2,28	2,22	2,18	2,40	2,52	2,03

The ratio of body nutrients to protein biomass is relatively better in variants with higher biomass, but the differences are insignificant in the analyzed carp fry: 2.25 in V_1 and V_2, 2.29 in V_3, and 2.27 in V_4 (Table 8.10).

➢ Hematological profile analysis

Hematological analyses provide valuable information about the physiological status of fish, including the activity of the neuroendocrine and immune systems and the immediate and long-term impact of unfavorable growth conditions, potential diseases, and genetic predispositions (Seibel H. et al., 2021). Understanding these hematological indicators is an important tool for monitoring physiological and pathological changes in fish. Previous studies in fish hematology have highlighted that interpreting blood parameters is challenging, as blood alterations can be caused by internal and external factors (Bastami K. D. et al., 2009).

For an accurate assessment at the end of the experiment, blood samples were collected from five specimens of carp fingerlings/growth unit, and the chosen number of specimens was considered representative of a fair assessment of the fish's physiological condition. Thus, to determine if the stocking densities used induced physiological changes, a comparative analysis of hematological values and erythrocyte parameters was performed at the experiment's beginning, middle (one month), and also end (two months). The values of hematological parameters and erythrocyte constants are analytically presented in Table 8.11.

Table 8.11 - Hematological parameters recorded during the experiment period (Mean ± Standard Deviation)

Var. exp.	Moment	Nr. Eryth. (eryth./µl blood X10^6)	Hematocrit (%)	Hemoglobin (g/dl)	VEM (µm)	HEM (pg)	CHEM (g/dL)
	Initial	**1,30±0,39**	**32,54±6,04**	**8,27±1,54**	**261,28±53,58**	**65,89±10,42**	**25,45±1,67**
V_1R_1	Interm	1,13±0,14	23,45±2,46	8,18±0,24	212,36±45,29	73,05±7,39	35,33±4,38
	Final	1,53±0,34	26,75±2,95	8,35±1,04	180,35±23,74	57,54±14,75	31,67±5,31
V_1R_2	Interm	1,04±0,51	24,90±3,61	8,54±0,27	280,97±90,19	99,36±34,61	34,94±4,37
	Final	1,41±0,17	31,71±2,57	8,31±0,62	230,35±46,86	59,29±4,41	26,46±3,60
V1	**Interm**	**1,08**	**24,17**	**8,36**	**246,66**	**86,20**	**35,13**
mean	**Final**	**1,47**	**29,23**	**8,33**	**205,35**	**58,42**	**29,07**
V_2R_1	Interm	1,13±0,25	23,14±1,99	7,84±1,17	259,87±36,81	72,47±15,51	27,57±2,41
	Final	1,59±0,05	30,61±0,88	8,71±1,04	188,15±4,25	54,85±1,47	29,15±0,12
V_2R_2	Interm	2,02±1,01	21,43±4,40	8,97±1,81	159,07±45,80	58,07±27,79	33,74±9,65
	Final	1,29±0,12	30,58±0,92	8.81±0,48	163,37±24,04	68,71±6,66	42,70±5,96
V2	**Interm**	**1,57**	**22,28**	**8,40**	**209,47**	**65,27**	**30,65**
mean	**Final**	**1,44**	**30,59**	**8,77**	**175,76**	**61,78**	**37,28**
V_3R_1	Interm	1,29±0,22	28,47±3,48	8,33±0,62	188,73±53,65	66,03±8,11	36,48±5,51
	Final	1,28±0,31	29,90±0,27	8,21±0,45	254,65±64,69	67,45±14,11	26,82±1,42
V_3R_2	Interm	1,94±0,62	27,60±3,91	8,58±0,44	116,16±27,82	48,34±13,17	41,70±8,13
	Final	1,51±0,63	20,86±1,65	7,20±0,798	261,99±144,88	40,59±4,72	24,77±3,69
V3	**Interm**	**1,61**	**28,03**	**8,45**	**152,44**	**57,18**	**39,09**
mean	**Final**	**1,39**	**25,38**	**7,70**	**258,32**	**70,10**	**27,10**
V_4R_1	Interm	1,67±0,46	28,09±2,26	9,46±0,56	182,93±54,67	59,89±12,59	34,09±4,98
	Final	1,80±0,26	26,26±1,46	7,20±0,79	166,15±24,71	40,59±4,72	24,77±3,69
V_4R_2	Interm	1,40±0,27	27,02±3,67	8,18±0,34	199,43±37,94	60,46±10,21	30,67±3,09
	Final	1,77±0,60	26,82±5,90	7,78±0,87	167,43±49,98	53,20±27,12	30,17±6,64
V4	**Interm**	**1,535**	**27,55**	**8,82**	**191,18**	**60,17**	**32,38**
mean	**Final**	**1,785**	**26,54**	**7,49**	**166,79**	**46,90**	**27,47**

Analyzing the values of hematological parameters and erythrocyte indices presented in the table, the following conclusions can be drawn: Statistical analysis of the erythrocyte count did not reveal significant differences ($p>0.05$) between experimental variants at the intermediate stage, final stage, or compared to the beginning of the experiment. However, a slight increase in the erythrocyte count can be observed with increasing stocking density.

The increase in the number of erythrocytes in the case of high stocking densities may be due to the physiological adaptation of fish to homeostatic conditions. According to Harianto et al. (2014), increased erythrocyte values indicate that fish are in good physiological condition. Similar results were obtained by Addini et al. (2020), who reported that the number of erythrocytes at a stocking density of 250 fish/m^3 was higher than that at a stocking density of 200 fish/m^3.

Regarding hematocrit values, both the intermediate and final results showed a significant decrease compared to the initial values ($p<0.05$). Hematocrit values obtained at the intermediate stage showed significant differences ($p<0.05$) for stocking densities of 5.68 kg/m^3 and 11.18 kg/m^3. At the end of the experiment, statistical comparison with the intermediate stage revealed a significant decrease in Ht values for stocking densities of 5.68 kg/m^3 and 11.18 kg/m^3, while for stocking densities of 1.5 kg/m^3 and 2.85 kg/m^3, Ht values showed a significant increase ($p<0.05$).

Regarding hemoglobin concentration, there were no significant statistical differences ($p>0.05$). Nor when comparing the initial values or after the 30-day experiment. However, at the end of the experiment, a significant decrease ($p<0.05$) in hemoglobin concentration can be observed for stocking densities of 5.68 kg/m^3 and 11.18 kg/m^3. The reduction in hemoglobin concentration may result from the fish's adaptation to stressful conditions, leading to decreased tissue oxygen supply, slower metabolic activity, and reduced energy production.

The hemoglobin values obtained in this experiment for the four experimental variants fall within the normal range according to some authors. In contrast, other authors consider them to be at the lower limit of normality. According to the literature, a reduction in hemoglobin quantity in the blood can affect tissue oxygen supply, leading to a decrease in metabolic rate and, consequently, lower energy production (Ruane et al., 1999).

After 60 days of experimentation, the erythrocyte indices did not show significant differences ($p>0.05$) between the experimental variants. At the end of the experiment, the statistical analysis revealed nonsignificant differences ($p>0.05$) in VEM and HEM values, while CHEM values showed significant differences ($p<0.05$) among the tested stocking densities.

Thus, the highest CHEM value was obtained in variant V_2, while CHEM values in groups V_1, V_3, and V_4 were not significantly different ($p>0.05$). However, a slight decrease in all erythrocyte indices can be observed compared to the initial moment.

Regarding the statistical comparison of erythrocyte indices obtained at the initial moment, no significant differences ($p>0.05$, T-test) were found when compared to the intermediate or final stages of the experiment.

The erythrocyte indices VEM, HEM, and CHEM fall within the normal range for the studied species. Similar results were obtained by Koop R. (2011) in his research on carp species, where he reported normal values for VEM (204-206 µm^3), HEM (40-47 pg), and CHEM (22-23 g/dl). Studies conducted by Ahmad S.M. (2011) have shown that hematological indicators, including erythrocyte indices, are influenced by ambient temperature. In his experiment, he obtained similar values (VEM = 188.54 ± 2.50; HEM = 42.16 ± 0.9; CHEM = 21.44 ± 0.79) to those obtained in the current experiment, with an average temperature of 28°C, similar to that mentioned by Ahmad S.M. (2011).

Any decision to increase biomass production in aquaculture through increased stocking density should consider the physiological stress fish are subjected to, as well as other factors that can negatively affect their health.

8.4. Conclusions

The experiment indicates that under conditions of high stocking densities in the growth units, notable results were obtained regarding technological performance indicators. The common carp (Cyprinus carpio) exhibits remarkable plasticity in its ability to adapt and perform under intensive growth conditions. Analyzing the values of hematological parameters and erythrocyte indices, it was found that the health status of the fish was not affected by the technological conditions, falling within the normal range for the studied species.

Environmental conditions, the cultured species, stocking density, feed quality, and daily feed ration all influence production levels, individual growth performance, and the quality of fish meat. Fish growth depends on the levels of proteins, lipids, and carbohydrates in the ingested feed. Typically, proteins from the feed are used for tissue growth, while lipids and carbohydrates are converted into the energy required for metabolic processes.

The quantity and quality of the feed directly affect not only the growth rate but also the quality of the fish meat. Proper feeding ensures superior nutritional and taste qualities of the meat. A diet that is deficient in nutrients will result in decreased energy value of the fish meat. The protein-lipid balance best characterizes the quality of a feed.

Currently, researchers in fish nutrition are primarily focused on finding the most suitable feed recipes for different cultured species, age groups, and sizes, with an optimal ratio of protein, lipids, carbohydrates, vitamins, and minerals. Without quality feed that corresponds to the cultured species' nutritional requirements, it is impossible to achieve production that ensures the profitability of fish farming businesses.

CHAPTER 9 - BIOSAFETY ASPECTS IN EXPERIMENTAL SYSTEMS

9.1. General Considerations

In cyprinid farms, there is a risk of diseases that can negatively affect the functions of fish organisms, leading to significant production losses in the absence of appropriate prevention and treatment measures. The pathological condition of a fish can be defined as any behavior or appearance that differs from that of other fish of the same species or age. To prevent the occurrence and spread of diseases in fish farms, it is important to ensure proper hygiene, control the quality of water and feed, and monitor the health status of the fish, intervening promptly in case signs of illness are observed.

Good water quality is the key to successful fish production. Physicochemical parameters (temperature, pH, dissolved oxygen, salinity, nitrogen compounds, etc.) should be optimal. Some practitioners argue that maintaining the temperature at a higher level within the optimal range for each species can accelerate the life cycles of parasites. However, high temperature is also a stressful factor for fish.

Prophylactic treatments are often recommended before introducing fish into a new environment. However, the effectiveness of this procedure depends on specific circumstances. For example, treating the fish with an antiparasitic agent is justified if a population has a significant number of ectoparasites. This is especially encountered in fish captured from the natural environment, which often carry parasites.

Another important aspect is that the time periods commonly used for quarantine (e.g., 7 days - 90 days, etc.) are quite arbitrary, and published scientific data validating the used periods are scarce. A very long quarantine period should be used to exclude certain pathogens effectively. Ideally, the quarantined population should be permanently segregated, and only the descendants of the quarantined population should eventually be introduced after multiple rounds of disease screening in the parental population.

In conclusion, the longer the quarantine period and the more generations the pathogen has been reduced, the greater the probability of excluding that pathogen.

9.2. Incidence of diseases during the experiments

Throughout the experimental period, there were instances of fish diseases. The main conditions are described below.

- **Infectious Diseases**

Spring viremia, the acute form of infectious dropsy, is an acute rhabdoviral disease that naturally infects carp. Common carp is the most susceptible species and the primary host of spring viremia (Ahne, 2002; Fijan, 1999). The infection causes generalized viremia and hemorrhages in the viscera and muscles of cyprinid fish, leading to high mortality (Fijan et al., 1984).

Clinical signs include lethargy, enteritis, peritonitis, edema, exophthalmia, thickening of the swim bladder, and hemorrhages in internal organs, skin, and muscles. Behavioral changes may include reduced respiratory rate, loss of balance, uncoordinated swimming, and gathering near water outflows (Roberts, R.J., 2012). Once the disease is correctly diagnosed, the best control is achieved by maintaining the water temperature above 20˚C (Fijan, 1999).

Affected fish isolate themselves from the shoal, swim lazily, breathe slowly at the water surface, show reduced reactivity to human approach, and have reduced or absent eye rotation reflexes.

Changes are also observed in the blood, including decreased total protein and protein fraction content, decreased number of erythrocytes, reduced hemoglobin levels, decreased hematocrit value, and increased blood sedimentation rate.

During the experiments, a few carp fry specimens with signs of viremia were identified, particularly with hemorrhagic spots on their bodies. All affected specimens were removed from the rearing system.

Since the number of diseased specimens was minimal, specific treatments were not applied to the entire cultured biomass (Cordeli A.N. et al., 2019). However, in extreme cases, specialized literature mentions the main treatments (baths with malachite green, potassium permanganate, sodium chloride, etc.).

Preventive measures are crucial. To prevent spring viremia in carp, a complex set of measures should be implemented, focusing on the natural or acquired resistance of the fish population, the hygiene conditions of the basins, the pathological balance, and the limitation of pathogen spread (Munteanu, G. et al., 2003).

In aquaculture, maintaining the fish population's health is essential for achieving profitable and high-quality production. Biosecurity aims to prevent the transmission or spread of infectious diseases in an aquaculture system. The most important diseases affecting farmed fish have been introduced to non-native areas due to inadequate biosecurity measures.

- **Fungal Diseases**

Fungal infections can lead to significant losses in both wild and cultured fish. Most fungal diseases in fish are secondary infections in hosts with pre-existing injuries or bacterial infections. Fungi can penetrate the organism through wounds or ulcers and increase rapidly.

Saprolegniosis is a common fungal disease in all freshwater fish basins worldwide, affecting fish of all ages, including eggs. The disease is more common in poorly maintained waters with high organic matter content, low pH, and higher temperatures.

The sources of infection are fungal spores found in any freshwater basin rich in decomposing organic matter. As long as the fish in the basin are healthy, fungi in this category cannot attack. However, when, due to various causes (internal diseases, ectoparasitic attacks, trauma, etc.), the organism becomes weakened or the covering epithelium of the skin, gills, oral cavity, etc., is damaged, the spores present in the water attach to the external lesions, and hyphae of the fungus develop from them. One end of these hyphae develops externally, while the other penetrates the subepithelial tissues, exerting a strong toxic-cytolytic action.

Saprolegniosis is highly pathogenic, causing most lesions (Cordeli A.N. et al., 2019). Water molds are among the most challenging diseases to treat. Except for salt, most legally approved agents have limited effectiveness.

Maintaining proper hygiene, increasing water flow during periods of high temperature, reducing handling, and maintaining low population density can prevent fungal infections.

- **Diseases caused by external parasites**

The family Lernaeidae comprises over 90 species from nine genera. Lernaea is the most important genus of copepods that affect freshwater fish. It has a worldwide distribution, and infections have been reported in over 40 species of cyprinids. The ectoparasite Lernaea cyprinacea (anchor worm) has long been recognized as a highly prevalent disease in carp, especially in fish farms. The parasite's attachment can occur anywhere on the fish, such as the gills or the skin, causing muscular necrosis, hemorrhage, inflammation, and suppuration.

The sources of parasites in lernaeosis are infested fish, carriers, and the water in which the larval stages of Lernaea sp. are found. The highest susceptibility to the disease is observed in the first year of fish life; as the fish age, the extent and intensity of parasitism decrease. Generally, the peak intensity of infestation occurs during the warm season. The parasites penetrate the fish's skin, causing lesions and destruction. They act on the host both mechanically and toxically (Cordeli A.N. et al., 2019).

Before starting the experiments, the fish were quarantined for two weeks to prevent infestation with Lernaea and other pathogens. During this time, short-duration parasite baths were administered using malachite green, with two doses of 5 ml per 200 liters of water, for 60 minutes, each week. Fish that were infested with parasites were excluded from the experiment.

- **Diseases caused by internal parasites**

Ligula intestinalis is considered by many specialists to be the most important larval stage parasite of a tapeworm that affects cyprinids. The Ligulidae family is found in fish from the Cyprinidae family, both in fish farms and in the natural environment, and the infection can cause significant economic losses (Hoole, D. et al., 2001).

The sources of parasites in ligulosis are ichthyophagous birds, which spread the worm eggs in the water, where they reach copepod crustaceans that harbor procercoids. Fish become infested by ingesting copepods with procercoids.

Fish affected by ligulosis is hyperdynamic. They stop feeding, lose weight, and become exhausted. Their abdomen is swollen and firm to the touch, and sometimes, under the pressure of the parasites, it bursts, releasing plerocercoids into the water. During the experiments, the infested fish were removed from the growth system.

One of the most important adult cestodes that affect fish is the Asian tapeworm (Bothriocephalus acheilognathi). Bothriocephalosis is an intestinal parasitosis affecting freshwater and marine fish species, posing a greater risk to cultured cyprinid fry. The life cycle involves a single intermediate host, a cyclopoid copepod.

Fish health management is a concept used in aquaculture to describe managerial practices that define disease prevention. Prophylaxis involves implementing procedures that prevent the introduction, evolution, or spread of a disease agent that risks the health of humans, animals, plants, or the environment.

Fish health is not achieved solely through disease management. All aspects of a production farm that impact fish health, such as water quality management, sanitation, and quarantine, are part of the integrated fish health management program. Without addressing all these aspects, preventing disease outbreaks caused by opportunistic pathogens (bacteria, parasites, fungi) is impossible.

In the veterinary health, sanitation plays a crucial role in maintaining a clean aquatic environment, with minimal organic waste and proper disinfection of basins and all equipment by neutralizing specific bio-aggressors.

CHAPTER 10 - GENERAL CONCLUSIONS AND PERSONAL CONTRIBUTIONS

Romania benefits from a significant hydrographic network and natural heritage regarding fish fauna, including the Danube, the Danube Delta, and the Black Sea. In the first quarter of 2021, 75 423 hectares of aquaculture facilities were registered in the Aquaculture Units Register. Out of the 968 aquaculture licenses granted by the National Agency for Fisheries and Aquaculture (ANPA), 722 licenses were awarded to farmers, covering an area of 68 920 hectares, while 246 licenses were granted to nurseries, covering an area of 6 503 hectares. According to data provided by ANPA, in 2020, aquaculture units recorded production of 12 150 tons.

According to the National Multiannual Strategic Plan for Aquaculture 2022-2030, developed by ANPA in 2022, the consumption preferences of Romanians for fish species are as follows: carp - 64.66%, mackerel - 59.20%, trout - 46.45%, crucian carp - 39.16%, pike-perch - 36.43%, zander - 33.7%, pike - 32.79%, salmon - 25.87%, catfish - 17.85%, pangasius - 14.57%, herring - 10.02%, hake - 7.29%, and others. Regarding product varieties, 83.03% of consumers opt for fresh fish, followed by "caviar - caviar salad" with 74.41%, canned fish with 64.66%, and frozen fish with 54.64%.

Despite having favorable human and technological potential, Romania has a negative trade balance. The fish market in Romania is flooded with foreign species, some with high food value and others with questionable value. The country import ten times more than he produce. Impressive fish and other aquatic organisms are brought to the domestic market from Bulgaria, Serbia, the Czech Republic, Poland, Greece, Spain, Albania, Hungary, Italy, France, Norway, Sweden, and Turkey. These fish products are sold at lower prices than domestically produced ones, with many subsidized in their countries of origin.

That is why considerable efforts must be made to increase production and exports. In addition to the administrative institutions of the state, educational and scientific research institutions can contribute to achieving this goal. Properly managing natural resources and existing traditional fish farming facilities (fishponds, reservoirs, etc.) can create optimal conditions for the growth and reproduction of valuable fish species. Aquaculture production and fishing catch in Romania, which have been quite modest in recent years (10,000-15,000 tons/year), can be substantially increased through investments and innovative technologies. An example would be super-intensive fish farming technologies based on recirculating aquaculture systems, which allow for very high production rates of 50-100 kg of fish per cubic meter of water.

Based on several experiments, our research aimed to evaluate the effects of eco-technological parameters (water quality, stocking density, feed quality, feeding ratio) on carp fry's growth performance and health status, as well as the efficiency of nutrient retention from the feed in fish flesh. Experimental research was conducted in small-scale (laboratory level) and medium-scale (pilot level) recirculating aquaculture systems. Numerous clarifications were provided regarding the optimization of carp fry growth technologies through the issues addressed and the results obtained. The technological indicators obtained can be important benchmarks for increasing the profitability of the fish production sector in Romania.

The following are the most relevant conclusions and results from the nearly one-year-long scientific research.

The Influence of Stocking Density on the Growth Performance of Carp Fry in Recirculating Aquaculture Systems

Technological performance can only be achieved by paying the utmost attention to the triad of breed-house-feed. Firstly, when choosing the culture species, one must consider the ecophysiological characteristics of the species, especially its growth potential. Carp is a species with exceptional eco-technological plasticity, suitable for growth in any farming system.

Recirculating systems, although costly in terms of initial investment, are extremely efficient from a technical point of view and provide ideal living conditions for fish. Due to the possibility of continuous control over water quality and high intensity, production rates of over 100 kg/m³ can be achieved. Of course, such production levels cannot be achieved without properly feeding the fish, including considering the quality of feed used based on the culture species, calculation of feeding ratios, and maintaining optimal storage conditions. For fish farming to be profitable, farmers must manage environmental conditions correctly, thoroughly know fish nutrition and feeding, be familiar with prophylactic and treatment methods in case of diseases, choose the most profitable culture species, and apply innovative growth technologies suitable for the selected farming system.

The stocking density (population density) of biological material in growth units significantly influences fish growth performance. Lower densities in a recirculating water system, such as NTL or NTP, lead to better performance, including better feed utilization. At excessively high stocking densities, overcrowding and competition for food and space result in slower fish growth and lower feeding efficiency. It is important to consider the negative effects that can occur when stocking densities are too high: increased aggression and stress among fish, compromised welfare, and a higher risk of pathogen spread.

The Influence of Feed Quality on the Growth Performance of Carp Fry in Recirculating Aquaculture Systems

Optimizing feeding technology is a major challenge for achieving superior production. Many farmers have started to grow carp at high densities and use combined granulated, extruded/expanded feeds from different manufacturers. Insufficient or excessive nutrient levels in feeds can negatively affect the growth performance of fish, regardless of the chosen culture species.

It is essential to understand the nutritional preferences of the species and then choose the appropriate farming system, suitable feed, and the right daily feeding ration. The fish feed should be selected based on each growth stage; for example, carp fry requires a protein-rich diet, while adult carp can be fed a carbohydrate-rich diet.

In addition, to feeding, environmental conditions (temperature, dissolved oxygen, pH, nitrogen compounds, etc.) also strongly impact the production levels achieved. From an economic and environmental perspective, it is important to provide high-quality feeds that ensure low feed conversion ratios, supporting rapid growth without compromising fish welfare or polluting the aquatic environment.

To achieve optimal growth without excessive costs, estimating the optimal level of nutrients in the fish diet is necessary. This involves finding an appropriate balance between proteins, lipids, and carbohydrates in the feed to support growth and vital functions without exceeding the necessary levels, which can lead to environmental issues and additional costs.

The Influence of Daily Feeding Ration on the Growth Performance of Carp Fry in Recirculating Aquaculture Systems

In aquaculture, feeding management is necessary for minimizing production costs and maximizing profitability. Choosing the best daily feeding ration should be based on the capacity and performance of the farming system, species, and feed quality. Increasing the feeding level beyond certain limits does not significantly improve production levels.

The growth performance of fish in a recirculating aquaculture system is significantly influenced by various eco-technological and biological factors such as the system's carrying capacity, water quality, culture species, development stage or age, and nutritional requirements.

The daily ration cannot be arbitrarily increased, as it may result in unjustifiably high feed consumption. Based on the obtained results, it can be concluded that excessively large daily feed rations can lead to decreased production. Additionally, choosing an optimal feeding ratio must consider the efficiency of feed utilization and its impact on water quality.

All culture species, including carp, are susceptible to unfavorable environmental conditions. Sudden changes in physicochemical parameters, especially high levels of nitrogen compounds and dissolved oxygen deficiency in water, can have major effects on fish survival.

The Growth Performance, Physiological Condition, and Nutrient Retention Efficiency of Carp Fry

The experiment indicates that remarkable technological performance indicators were obtained under conditions of high stocking densities in growth units. Carp demonstrates remarkable plasticity in adapting and performing well under intensive growth conditions.

Not only the quantity but also the quality of feed is important. A balanced diet, nutritionally speaking, meets all the fish's energy and growth needs. The quantity and quality of feed directly influence not only the growth rate but also the quality of fish meat. With a diet low in nutrients, the energy value of fish meat decreases. The protein-lipid combination best characterizes the quality of a feed.

Regarding the retention of nutrients from feeds in fish meat, the research obtained a biochemical picture. The feed's chemical composition directly influences the meat's composition in a proportional manner. Proper nutrition provides superior nutritional and taste qualities to fish meat. As fish are fed, the percentage of body proteins and lipids increases while the water content in the meat decreases, which is a positive aspect.

Currently, researchers in fish nutrition are primarily focused on finding the most suitable feed recipes for different culture species, age groups, and sizes, with an optimal ratio of proteins, lipids, carbohydrates, vitamins, and minerals. Without high-quality feed tailored to the cultural species' nutritional requirements, achieving a production level that ensures the profitability of fish farming businesses is impossible.

Research Directions in the Fishing and Aquaculture Sector

In Romania, scientific research in the fishing and aquaculture field is conducted in several higher education institutions, research centers, and institutes.

Researchers from these institutions have highlighted the need to finance several strategic objectives for the development of the fisheries sector:

- Investments in production and scientific research infrastructure by modernizing aquaculture facilities, improving working conditions, and limiting environmental impact. Modernizing fish farms requires investments in equipment, technological spaces, high-quality feeds, feed storage facilities, and access routes. Investments in feed factories and processing units are also necessary.
- Transition to a green economy, especially in investments that limit the environmental impact of recirculating aquaculture systems, processing units, and commercialization.
- Generating additional income through tourism and food services, bird watching, recreational fishing, and educational activities related to the knowledge and protection of aquatic biodiversity.
- Transfer of best practices from scientific research to the production sector guarantees rapid progress in the fishing and aquaculture sectors.
- Large-scale implementation of innovative technologies in the aquaculture sector, such as expanding intensive and super-intensive production systems, recirculating systems, floating hatcheries, etc.
- Increased attention to identifying the origin and quality of imported products, ensuring that consumers are informed about the origin and quality of fish products from outside Romania. Traceability is essential for food safety and consumer trust.

- Support potential entrepreneurs in establishing and developing small businesses in the fishing and aquaculture sector and other complementary fields. There are still significant difficulties in accessing local, national, and European funding. Additionally, the lack of entrepreneurial education is a limiting factor in business development.

Elements of Originality and Personal Contributions

The doctoral thesis was developed based on an experimental protocol that aimed to achieve the following objectives:

1. The influence of population density on carp fry's growth performance and nutrient retention efficiency from the feed in the flesh.
2. The influence of feed quality on carp fry's growth performance and nutrient retention efficiency from the feed in the flesh.
3. The influence of daily feeding ration on carp fry's growth performance and nutrient retention efficiency from the feed in the flesh.
4. The growth performance, physiological condition, and nutrient retention efficiency of carp fry in pilot aquaculture systems.
5. Evaluation of hematological profile and assessment of the health status of the biological material.

The addressed issues and the obtained results justify personal contributions. The following are the most important contributions:

1. All experiments were conducted in recirculating aquaculture systems. It is the first time that scientific research has been sequentially approached by organizing parallel experiments using small-scale laboratory units and medium-scale pilot units.
2. Numerous clarifications have been made regarding the optimization of carp fry growth technologies. The obtained technological indicators can serve as important references for organizing new research and experiments in the field.
3. The influence of ecotechnological indicators on the growth performance of carp fry (water quality, feed quality, daily ratio, population density) has been verified.
4. Hematological profiles were created to assess the health status of the biological material.
5. For the first time in Romania, research has been conducted on nutrient retention efficiency from the feed in the flesh of carp fry.

These contributions demonstrate the originality of the research and its potential impact on the understanding and improvement of carp aquaculture practices.

Published Scientific Articles

1. Diana-Nicoleta Mînzală, Ira-Adeline Simionov, Ștefan-Mihai Petrea, Alina Antache, Victor Cristea, **Anca-Nicoleta Săvescu**, Evaluation of potentially toxic elements in Black Sea fishery resources: a review, Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering. Vol. XI, 2022, Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-5138, ISSN-L 2285-6064
2. **Anca Nicoleta Cordeli (Săvescu),** Lucian Oprea, Mirela Crețu, Mihaela Mocanu, The influence of stocking densities on growth performance of common carp (*Cyprinus carpio*, Linne 1758) rearing in a recirculating aquaculture system", pp. 509-516, The International Conference Agriculture For Life, Life For Agriculture, Bucharest June 2021. http://animalsciencejournal.usamv.ro/pdf/2021/issue_1/Art71.pdf
3. **Anca Nicoleta Cordeli (Săvescu),** Lucian Oprea, Mirela Crețu, Mihaela Mocanu, Effects of feeding level on growth performance and body composition of common carp (*Cyprinus carpio*, Linne 1758) in a recirculating aquaculture system rearing", The International Conference Agriculture For Life, Life For Agriculture, Bucharest June 2021, http://animalsciencejournal.usamv.ro/pdf/2021/issue_2/Art60.pdf
4. Mihaela Mocanu, Lucian Oprea, **Anca Nicoleta Cordeli (Săvescu),** Mirela Crețu, Estimation of growth parameters and mortality rate of Pontic Shad (*Alosa Immaculata*, Bennett, 1835) in the Romanian sector of the Danube river, km 169", The International Conference Agriculture For Life, Life For Agriculture, Bucharest June 2021, http://animalsciencejournal.usamv.ro/pdf/2021/issue_2/Art64.pdf
5. Sănduța PLĂCINTĂ, Victor CRISTEA, Iulia Rodica GRECU, **Anca Nicoleta Cordeli (Săvescu)**, Elena Sîrbu, Effect of Dietary vitamin C on the haematological profile of juvenile European catfish (*Silurus Glanis*) reared in to recirculating system", The International Conference Agriculture For Life, Life For Agriculture, Bucharest June 2021, http://animalsciencejournal.usamv.ro/pdf/2021/issue_1/Art79.pdf
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7. Mirela Crețu, Cristea Victor, Docan Angelica, Dediu Lorena, Andrei (Guriencu) Raluca – Cristina, **Cordeli (Săvescu) Anca Nicoleta**, *Haematological profile of rainbow trout under different feeding intensities*, Scientific Papers. Series D. Animal Science. Vol. LXIII, No. 2, 2020, pp. 452-457 4-6 June 2020, http://animalsciencejournal.usamv.ro/pdf/2020/issue_2/Art69.pdf
8. **Anca Nicoleta Săvescu**, Mirela Crețu, Lucian Oprea, Preliminary results regarding the effects of dietary – protein levels on the growth performance and feed efficiency of common carp fry. The International Conference Agriculture For Life, Life For Agriculture, Bucharest, 6-8 June 2019. *http://animalsciencejournal.usamv.ro/pdf/2019/issue_2/Art53.pdf*
9. **Anca Nicoleta Săvescu**, Mirela Crețu, Lucian Oprea, The effects of different densities on the growth performance and feeding efficiency of common carp fry in recirculating aquaculture systems. International Multidisciplinary Scientific GeoConference SGEM, Albena Bulgaria, 28 June – 7 July 2019, *https:doi.org/105593/sgem2019/6.* *https://www.sgem.org/index.php/elibrary?view=publication&task=show&id=6487*
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Projects

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Anexa 3 –Copertă exterioară/spate

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