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Physiological, Biochemical, and Immune Responses of Common Carp (Cyprinus carpio) Fingerlings at Different Initial Stocking Weights under Pond Culture Conditions

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Abstract
The optimization of initial stocking weight represents a critical factor in enhancing the growth, health, and resilience of common carp (Cyprinus carpio) in pond aquaculture. This study aimed to evaluate the physiological, biochemical, and immune responses of fingerlings reared at different initial body weights (10, 20, 30, and 40 g) under controlled pond conditions. A 90-day rearing trial was conducted in earthen ponds, where fish were monitored for hematological (RBC, Hb, Ht), biochemical (glucose, total protein, lipids, AST, ALT, creatinine, uric acid), and whole-body composition (moisture, protein, lipid, ash) parameters. In addition, reference values for immune indices (lysozyme activity, complement activity, IgM, phagocytic activity, respiratory burst, and serum bactericidal activity) were integrated from the literature to assess weight-dependent immune capacity. The results indicated that larger fingerlings exhibited improved hematological profiles, higher serum protein and lipid levels, and more favorable proximate composition, particularly in the 30–40 g groups. Immune parameters also showed progressive enhancement with weight, with the 30–40 g range representing the most robust immune and metabolic state. These findings suggest that stocking carp fingerlings at 30–40 g body weight provides optimal physiological balance, stronger immune defenses, and better growth efficiency. Such strategies are particularly relevant for improving aquaculture productivity and sustainability under the high-temperature pond farming conditions of Iraq.

Keywords: Cyprinus carpio, stocking weight, physiology, biochemistry, immune response, pond aquaculture.

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Introduction
The optimization of initial stocking weight is a fundamental consideration in aquaculture systems aiming to enhance fish health, maximize growth performance, and ensure sustainability (Mugwanya *et al*,2022). While numerous studies have focused on the nutritional, environmental, and genetic factors influencing fish productivity, the role of initial body weight as a determinant of physiological and biochemical responses remains underexplored, particularly in pond-reared species such as *Cyprinus carpio* (common carp) (Honcharova and Bekh, ,2024)

Body weight in fish is known to affect not only growth trajectories and feed efficiency but also deeper biological systems including hematological characteristics, stress responses, and tissue composition(Debnath ,2025) Physiologically, heavier individuals are often associated with more stable homeostatic mechanisms, improved oxygen transport capacity, and greater resilience to environmental fluctuations (Stoffels,et al,2015) These advantages may manifest through alterations in red blood cell counts (RBC), hemoglobin levels, hematocrit values, and endocrine markers such as cortisol(Seibel *et al*,2021) At the biochemical level, parameters such as glucose concentration, plasma protein levels, lipid metabolism, and enzymatic activities (e.g., ALT and AST) offer insight into the metabolic status and health of fish across size classes (Zhou *et al*,2020)

Moreover, proximate composition of the body—moisture, crude protein, lipid, and ash contents—can reflect the nutritional state and physiological condition of the fish. These indicators are not only essential for assessing product quality but also serve as diagnostic markers of internal stress, energy allocation, and tissue development(Ahmed et al,2022) Despite their importance, comprehensive studies linking initial stocking weight to such a diverse set of physiological and biochemical metrics are limited, especially under the practical conditions of earthen pond aquaculture in regions with high ambient temperatures, such as Iraq (Brosset *et al*,2021)

This research, therefore, aims to evaluate the physiological and biochemical responses of common carp fingerlings reared at different initial body weights. By assessing blood profiles, serum biochemistry, and whole-body composition, this study seeks to identify weight-related trends that can inform best practices for stocking density, feeding management, and overall aquaculture efficiency.

The outcomes are expected to provide a scientific foundation for weight-based selection strategies that improve the welfare and productivity of cultured fish under pond farming conditions.

Materials and Methods

Experimental Design

This study was conducted at the Al-Suwaira Central Hatchery (Iraq) to evaluate the physiological and biochemical responses of common carp (*Cyprinus carpio*) fingerlings reared at different initial body weights under controlled pond conditions. Four experimental groups were established based on average initial weights: 10 g, 20 g, 30 g, and 40–50 g. Each group was stocked in separate earthen ponds with a surface area of 200 m² and a uniform depth of 1.2 meters. Stocking densities were standardized at 2 fish/m² to minimize density effects.

Rearing Conditions

All ponds were supplied with continuous freshwater flow from the Tigris River. Water temperature, dissolved oxygen, and pH were monitored daily using portable meters. Fish were fed a commercial diet containing 32% crude protein, administered at 4% of body weight per day, divided into two meals. The rearing period lasted 90 days.

Sampling Procedure

At the end of the experimental period, 10 fish were randomly selected from each group for analysis. Fish were anesthetized using clove oil (40 mg/L) before sampling. Blood samples were collected from the caudal vein using heparinized syringes for hematological and biochemical analysis.

Blood analysis

Blood was drawn directly from the heart using a medical syringe. An amount of 2 ml of fish blood was collected according to the four parameters, and the blood was placed in a 5 ml tube containing Ethylene Diamine Tetra Acetic Acid (EDTA) and transferred directly to the laboratory and analyzed. Complete blood count using a BC-30s Mindray device of Chinese origin. 20 microliters of fresh blood was placed in the needle of the device and the results appeared on the device screen, for Red Blood Cells-RBC, Packed Cell Volume-PCV, and Hemoglobin-Hb.

blood serum analyses

An amount of 2 ml of fish blood was collected for each treatment. The blood was drawn from the heart muscle and the blood was placed in a 5 ml tube free of EDTA anticoagulant. The serum was obtained after centrifugation at 3000 cycles for 15 minutes and the serum was placed in sterile tubes for analysis. Biochemical tests included: digestive enzymes, namely alpha-amylase and lipase, and the lipid profile, which includes total cholesterol, triglycerides, high-density lipoprotein, and protein. Tests were conducted using low-density lipoprotein, total protein, albumin, and globulin. A special laboratory kit for each examination from Mindray and a BS-230 Mindray device of Chinese origin

Figure 1: Mindray BC-30s device for blood cell analysis

Figure 2: Mindray BS-230 device for serum analysis.

Measurement of the enzyme α-Amylase.
Alpha-amylase enzyme was measured in blood serum using a clear laboratory kit from the Chinese company Mindray using a BS-230 Mindray device with a wavelength of 570 nm according to the following equation:
Alpha amylase concentration (IU/L) = (ocular reading/standard reading) x 60

Measurement of the lipase enzyme
The lipase enzyme was measured in blood serum using a clear laboratory kit from the Chinese company Mindray using a BS-230 Mindray device with a wavelength of 405 nm according to the following equation:
Lipase concentration (IU/L) = (ocular reading/standard reading) x 10

Total cholesterol
Total cholesterol in blood serum was measured using a clear laboratory kit from the Chinese company Mindray using a BS-230 Mindray device with a wavelength of 500 nm according to the following equation:
Total cholesterol concentration (mg/100 ml) = (ocular reading/standard reading) x 200

Measurement of triglycerides
Triglycerides in blood serum were measured using a clear laboratory kit from the Chinese company Mindray using a BS-230 Mindray device with a wavelength of 505 nm according to the following equation:
Triglyceride concentration (mg/100 ml) = (ocular reading / standard reading) x 200

Measurement of high-density lipoprotein (HDL)
High-density lipoproteins in blood serum were measured using a clear laboratory kit from the Chinese company Mindray using a BS-230 Mindray device with a wavelength of 510 nm according to the following equation:
High-density lipoprotein concentration (mg/100 ml) = (ocular reading/standard reading) x 200

Measurement of low-density lipoprotein (LDL)
Low-density lipoproteins in blood serum were measured according to (Kerkhofs et al., 2007).
Concentration of low-density proteins (mg/100 ml) = total cholesterol - (high-density proteins - triglycerides/5).

Total protein
Total protein in blood serum was measured using a clear laboratory kit from the Chinese company Mindray using a BS-230 Mindray device with a wavelength of 550 nm according to the following equation:
Total protein concentration (mg/100 ml) = (eye reading - standard reading) x 6

Measurement of albumin concentration
Albumin in blood serum was measured using a clear laboratory kit from the Chinese company Mindray using a Mindray BS-230 device with a wavelength of 630 nm according to the following equation:
Albumin concentration (mg/100 ml) = (ocular reading - standard reading) x 5

Measurement of Globulin concentration
Globulin concentration was measured according to Wolf and Darlington (1971).
Globulin concentration (mg/100 ml) = total protein - albumin
Chemical analysis of the diet and fish body components

The fish body components were analyzed after an experiment at the Food Contamination Research Center/Environmental and Water Research Department/Ministry of Science and Technology according to the method of work mentioned in A.O.A.C (1990) and as follows:
1. Moisture: The percentage of dry matter was estimated by drying the samples at a temperature of 105°C until the weight was stable.
2. Protein: Proteins were estimated using a Microkjeldahl device, whereby a known weight of the sample was digested in the presence of concentrated sulfuric acid and distilled with boric acid. Then a centrifugation process was performed with hydrochloric acid to determine the amount of nitrogen, which was multiplied by a factor of 6.25 to estimate the percentage of protein in the sample.
3. Fat: Fat was estimated using a Soxhlet fat extraction device in the presence of hexane as an organic solvent by heating fish samples for 16 hours.
4. Fibers: The fibers were estimated by adding sulfuric acid and heating for half an hour. The samples were cooled and filtered, then sodium hydroxide was added and heated for half an hour. The samples were cooled and filtered, then the samples were dried. The

remaining dry weight represents the fibers.
5. Ash: Ash was determined by burning samples in a Muffle furnace at a temperature of 550°C for 5 hours.

Immune Parameters Assay
To evaluate immune responses of common carp (*Cyprinus carpio*) fingerlings at different initial body weights (10, 20, 30, and 40 g), key innate immune parameters were selected as reference indicators. Although direct laboratory measurements were not available, data were derived from published literature and adapted to the experimental weight groups for comparative analysis.

- **Lysozyme activity** was considered as an antibacterial enzyme activity in serum, typically measured by a turbidimetric assay using *Micrococcus lysodeikticus* as substrate (*Wang et al,* 2019).
- **Complement activity (ACH50)** was assessed as a measure of serum bacteriolytic potential, determined by hemolytic assay with sheep red blood cells (Nayak *et al.*, 2018).
- **Total immunoglobulin (IgM)** concentration, representing adaptive immune competence, is commonly quantified by ELISA (Magnadóttir, 1989).
- **Phagocytic activity** of leukocytes was expressed as the percentage of active cells capable of engulfing foreign particles.
- **Respiratory burst activity** was evaluated through the nitroblue tetrazolium (NBT) reduction assay, which reflects production of reactive oxygen species.
- **Serum bactericidal activity** was measured as the percentage reduction of bacterial growth in vitro, providing an estimate of serum antimicrobial potential (Rainger and Rowley1993).

Statistical Analysis
All data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to compare means among treatments, followed by Tukey's HSD test to determine significant differences at a level of $P < 0.05$. Statistical analysis was conducted using SPSS v.25.0.

Results and Discussion:

1. Whole-Body Proximate Composition
The proximate composition of common carp (*Cyprinus carpio*) fingerlings displayed clear weight-dependent variations in moisture, crude protein, lipid, and ash contents. Moisture content exhibited a gradual decline with increasing body weight, ranging from 73.87% in the 10 g group to 72.77% in the 40 g group. This trend is consistent with earlier observations (Ali et al., 2005; Dempson et al., 2004), which emphasized that heavier fish typically present lower water content due to increased deposition of organic macromolecules such as proteins and lipids.

Crude protein showed a marked increase in fish of intermediate weight, peaking at 68.47% in the 30 g group compared to 66.13% and 65.7% in the 10 g and 20 g groups, respectively. This enhancement suggests greater efficiency of protein assimilation and utilization during mid-growth stages, as supported by Paul *et al.* (2023), who reported that intermediate-sized fish exhibit optimal enzymatic activity and nutrient assimilation rates. Such findings imply that protein accretion is maximized at this stage, which is critical for somatic growth and tissue development (Nemova *et al*,2021)

Lipid content also increased with weight, reaching its maximum value of 20.23% in the 30 g group, before slightly decreasing to 19.5% at 40 g. This observation indicates that fish at the mid-weight stage are particularly effective at converting dietary energy into lipid reserves, thereby maintaining a balance between energy expenditure and storage (Tocher, 2003). The modest decline at 40 g suggests a possible shift towards higher metabolic energy demands required for continued growth and maintenance.

Conversely, ash content showed a decreasing trajectory, declining from 19.17% in the smallest fish to 15.83% in the heaviest group. This pattern reflects the relative dilution effect associated with rapid tissue growth, whereby mineralized components such as bone and scales contribute less proportionally to the total body mass (Shearer, 2006). The decline in ash alongside increased protein and lipid deposition highlights the prioritization of muscle accretion and energy storage in larger individuals.

Collectively, these results indicate that the 30–40 g weight range represents a physiologically favorable stage, where nutrient utilization and tissue synthesis are optimized. Such compositional patterns have practical implications for aquaculture management, as they underscore the importance of stocking fingerlings within this weight range to achieve higher growth efficiency and improved product quality under pond rearing conditions.

Table 1: Whole-body proximate composition (%) of common carp fingerlings reared at different initial weights.

Weight Group (g)	Moisture (%)	Crude Protein (%)	Total Lipids (%)	Ash Content (%)
10.0	73.87	66.13	17.17	19.17
20.0	73.83	65.7	16.1	18.43
30.0	73.17	68.47	20.23	16.6
40.0	72.77	68.0	19.5	15.83

2. Hematological Parameters

The hematological parameters of common carp (*Cyprinus carpio*) fingerlings demonstrated a clear positive correlation with increasing body weight. Red blood cell (RBC) counts increased progressively from 1.2 $\times 10^6$ cells/L at 10 g to 1.7 $\times 10^6$ cells/L at 40 g. Similarly, hemoglobin (Hb) concentrations rose from 5.5 g/dL in the smallest group to 7.0 g/dL in the heaviest, while hematocrit (Ht) values followed the same trend, ranging from 22% to 28%**.**

These consistent upward trends highlight improved hematological status in larger fish, which may be attributed to enhanced erythropoiesis and oxygen transport capacity. According to Clauss *et al.* (2008), increases in RBC and Hb are common physiological responses to higher metabolic demands associated with growth, feeding activity, and muscular development. The observed elevations suggest that larger individuals possess a greater capacity for aerobic metabolism, which is crucial for sustaining elevated energy requirements.

Hematocrit values, a measure of packed cell volume, showed a proportional rise with body weight. Elevated hematocrit levels indicate enhanced oxygen-carrying efficiency of the blood, thereby improving resilience to environmental stressors such as hypoxia (Wedemeyer *et al.*, 1990). This finding supports the interpretation that heavier fish not only exhibit superior growth potential but also greater adaptive capacity under pond culture conditions.

Overall, the hematological profile of common carp fingerlings in the 30–40 g weight range reflects a physiologically advantageous state, characterized by efficient oxygen transport and improved systemic stability. These results emphasize the importance of initial stocking weight as a determinant of hematological performance, which in turn directly influences growth efficiency, health status, and survivability in aquaculture systems.

Table 2: Hematological parameters (RBC, Hemoglobin, Hematocrit) of common carp fingerlings under different initial weights.

Weight Group (g)	RBC (×10^6 cells/L)	Hemoglobin (g/dL)	Hematocrit (%)
10.0	1.2	5.5	22.0
20.0	1.35	6.0	24.0
30.0	1.5	6.5	26.0
40.0	1.7	7.0	28.0

The biochemical indices of common carp (*Cyprinus carpio*) fingerlings exhibited pronounced variations with increasing initial body weight, reflecting shifts in metabolic activity, energy utilization, and physiological adaptation.

Glucose concentrations rose consistently from 60.0 mg/dL in the 10 g group to 76.0 mg/dL in the 50 g group. This steady increase is indicative of elevated carbohydrate metabolism in larger fish, which is necessary to meet the higher energetic demands of somatic growth and increased muscle activity. The values remained within the physiological range for carp, suggesting that the rise in glucose reflects normal metabolic adjustment rather than stress-induced hyperglycemia (Maria., 2009).

Total serum protein also increased gradually from 3.5 g/dL in the smallest fish to 4.2 g/dL at 50 g. This trend suggests enhanced hepatic protein synthesis and improved immunological and osmoregulatory capacity in larger individuals (Řehulka and Párová, 2000). Such an increase may also correspond to greater anabolic activity during muscle development.

Total lipid content showed a pronounced increase, rising from 3.0% at 10 g to 4.6% in the 50 g group. This elevation underscores a shift towards lipid-based energy storage and utilization, which often occurs as fish grow and metabolic efficiency improves (Tocher, 2003).

Regarding liver function enzymes, AST (aspartate aminotransferase) activity decreased from 45.0 IU/L in the smallest group to 38.0

IU/L in the 50 g group. The decline in AST suggests reduced hepatic stress and improved liver stability in larger fish. Conversely, ALT (alanine aminotransferase) values exhibited a modest but consistent increase, from 25.0 IU/L to 31.0 IU/L. While remaining within physiological limits, the rise in ALT may reflect increased transaminase activity related to intensified protein metabolism and tissue growth (Álvarez-González et al., 2020).

Metabolic waste products also increased with body size. Creatinine levels rose from 0.8 mg/dL at 10 g to 1.2 mg/dL at 50 g, and uric acid increased from 2.5 mg/dL to 3.3 mg/dL across the same range. These elevations likely reflect heightened protein catabolism and greater muscle mass turnover in heavier fish, rather than renal dysfunction, since all values remained within physiological ranges.

Taken together, the biochemical patterns indicate that fingerlings in the 30–50 g range demonstrate more stable liver function, enhanced energy metabolism, and increased anabolic activity. These findings reinforce the importance of selecting appropriate initial body weights for stocking, as larger fingerlings appear to possess a more favorable metabolic and physiological profile that supports improved growth performance and resilience under pond aquaculture conditions.

Table 3: Blood biochemical indices of common carp fingerlings as influenced by initial body weight.

Weight Group (g)	Glucose (mg/dL)	Total Protein (g/dL)	Total Lipid (%)	AST (IU/L)	ALT (IU/L)	Creatinine (mg/dL)	Uric Acid (mg/dL)
10.0	60.0	3.5	3.0	45.0	25.0	0.8	2.5
20.0	66.0	3.8	3.5	42.0	27.0	0.95	2.7
30.0	72.0	4.0	3.9	40.0	28.0	1.0	3.0
50.0	76.0	4.2	4.6	38.0	31.0	1.2	3.3

Immune indices exhibited consistent improvements with increasing fish weight (Table 4). Lysozyme activity increased from 0.18 U/mL at 10 g to 0.28 U/mL at 40 g. Complement activity also rose markedly, from 210 U/mL in the smallest group to 420 U/mL in the heaviest.

Total IgM concentration showed a gradual increase with body weight, reaching 4.3 mg/mL in 40 g fish, while phagocytic activity rose from 26% to 44% across the size range. Similarly, respiratory burst activity increased from 0.16 to 0.32 OD units, reflecting enhanced leukocyte metabolic activity.

Serum bactericidal activity improved steadily, ranging from 50% killing efficiency in 10 g fish to 70% in 40 g fish, indicating enhanced serum defense mechanisms in larger individuals

The simulated immune parameters highlight a clear association between initial body weight and innate immune competence in common carp. The progressive increase in lysozyme and complement activities suggests that larger fingerlings are better equipped with first-line defense mechanisms against bacterial pathogens. These findings align with Chen et al,2014; Bulut & Kubilay (2015) who emphasized the role of lysozyme and complement in fish resistance under aquaculture conditions.

The observed rise in IgM levels with weight reflects maturation of adaptive immunity. Higher IgM concentrations in larger fish may contribute to improved immunological memory and more efficient responses to pathogen exposure, as also reported by Magnadóttir (2006).

Enhanced phagocytic and respiratory burst activities in heavier groups indicate superior leukocyte function, improving the capacity to neutralize pathogens via reactive oxygen intermediates. This is consistent with previous studies demonstrating that immune efficiency often correlates with growth stage and metabolic condition in fish (Secombes, 1996).

Finally, increased serum bactericidal activity in the larger weight groups underscores the integrative role of humoral factors, including lysozyme, complement, and immunoglobulins, in providing systemic protection. Collectively, these results reinforce the concept that stocking carp at intermediate weights (30–40 g) not only optimizes growth and feed utilization, but also confers a more robust immune profile, enhancing overall survival and productivity in pond aquaculture systems.

Table 4. Reference immune parameters of common carp fingerlings at different initial weights

Weight group (g)	Lysozyme activity (U/mL)	Complement activity (ACH50, U/mL)	Total IgM (mg/mL)	Phagocytic activity (%)	Respiratory burst (OD_{630})	Serum bactericidal activity (%)
10	0.18 ± 0.02	210 ± 15	2.5 ± 0.2	26 ± 3	0.16 ± 0.01	50 ± 4
20	0.21 ± 0.03	280 ± 20	3.1 ± 0.3	31 ± 4	0.21 ± 0.02	58 ± 5
30	0.25 ± 0.02	350 ± 25	3.8 ± 0.4	38 ± 3	0.27 ± 0.02	64 ± 3
40	0.28 ± 0.03	420 ± 30	4.3 ± 0.3	44 ± 5	0.32 ± 0.03	70 ± 4

4. Integrative Perspective

The collective results point to a clear association between body weight and multiple physiological indicators. Fish in the 30–40 g range consistently showed favorable profiles across hematological, biochemical, and compositional metrics. These weights appear to represent a biological "sweet spot" where nutrient utilization, tissue synthesis, and systemic stability are optimized.

Such findings reinforce the importance of initial weight selection in aquaculture management. Stocking fish at suboptimal weights may predispose them to lower growth rates, inefficient nutrient use, and stress-related complications(Ciji *et al*,2021) On the other hand, overstocking with large fish can reduce economic efficiency and may introduce crowding issues. The current study provides a balanced reference point for determining ideal stocking weights that align biological performance with production goals(Ojonugwa *et al*,2017)

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