

## Comparative Analysis Of Biochemical Constituents In Labeo Rohita (Ham.) From Normal And Alkaline Soil Ponds

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### ABSTRACT

The present study investigates the age-related variations in the biochemical composition of *Labeo rohita* (Hamilton) under contrasting soil pH conditions. Conducted at the Department of Fisheries and Agricultural Biochemistry, Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj), Faizabad, Uttar Pradesh, the research focuses on fish from a 5-year-old pond with normal soil (pH 7.0) and a newly constructed pond with alkaline soil (pH 9.5). Fish specimens were categorized into four age groups: 6, 18, 36, and 60 months, with age determination following the methodology of Johal and Tandon (1996). Biochemical analyses were performed on muscle tissue to assess moisture, fat, protein, and carbohydrate content. A Complete Randomized Design (CRD) was employed to evaluate the significance of age and soil pH on these parameters. Preliminary findings suggest that both age and environmental factors, such as soil pH, influence the biochemical composition of *Labeo rohita*. These insights are crucial for optimizing aquaculture practices and enhancing the nutritional quality of fish.

**Keywords:** *Labeo rohita*, soil pH, biochemical composition, nutritional quality.

### 1. INTRODUCTION

India is a major maritime state and a key player in global aquaculture, ranking as the world's second-largest fish-producing country after China. India achieved a record fish production of 18.4 million metric tons, including 13.1 million metric tons from inland fisheries and 4.4 million metric tons from marine fisheries (CEIC, 2024). This accounts for approximately 8% of global fish output. The country also stands out as a significant exporter of fish and fishery products, contributing around 2.4% of world's fish trade. The United States remains India's largest seafood importer, accounting for an estimated 43.45% of total export value (Department of Fisheries, 2023). Per capita fish consumption in India increased substantially from 7 kg in 2011–12 to 13 kg in 2022–23 (NCAER, 2023).

Growth in fish production is about more than just body size—it represents the harmonious development of tissues like bones, connective tissue, muscles, and adipose tissue, which form the bulk of edible fish. Growth reflects the assimilation and retention of chemical constituents—proteins, lipids, carbohydrates, and minerals—resulting in both typical and altered tissue development and changes in composition. The rearing environment is a key factor influencing the growth of body

components and chemical composition (Benoit et al., 1995). After first feeding, fish undergo significant changes in body composition: internal organs—including gut and visceral lipids—grow faster than the body itself (Denton & Yousef, 1976; Weatherly & Gill, 1983). Each organ grows at its own rate, but for a given overall fish size, each organ generally comprises a consistent percentage of body weight. This organ-specific growth, independent of growth rate, leads to predictable increments in protein and ash content with increasing fish size (Vanstone & Market, 1968; Groves, 1970; Weatherly & Gill, 1983). During the juvenile stage, fish focus on increasing size rather than storing energy (Gardiner & Geddes, 1980).

Fish must continuously acclimatize to environmental changes stemming from variations in water quality. Therefore, factors like temperature, light, dissolved oxygen, nitrogen, and pH are essential to sustaining healthy growth. *Labeo rohita* (Hamilton), commonly known as “Rohu,” is a prominent Indian major carp valued for its excellent protein quality (Mukundan et al., 1986) and considered one of the tastiest fish by consumers. However, there is scarce information on how body traits and biochemical composition vary with age in saline-soil ponds. If pond water remains alkaline rather than neutral, growth patterns, body characteristics, and biochemical composition of fish may differ compared to neutral-water ponds—this is the central focus of the present study.

## 2. MATERIAL AND METHODS

The details of the materials and standard methods used in the present investigation are as follows:

### Experimental materials:

Indian major carp *Labeo rohita* was selected for the experiments. Fish of different age groups were used during the observations:

S. No	Fish Group	Approximate age (in months)
1.	Below one Year	6
2.	Above one and below two years	18
3.	Above two and below three years	36
4.	Above four and below five years	60

No. of Samples-12

No of replications-3

### Experimental Site

Samples of fish from different age groups were collected from the newly constructed ponds (soil pH 9.5) and a lime-activated 5-year-old pond (soil pH 7.0) at the College of Fisheries. Biochemical analyses of muscle tissue and body traits were conducted in the laboratories of the Department of Fisheries and Agricultural Biochemistry, N.D.U.A.T.Kumarganj, Faizabad(U.P).

### Biochemical Analysis

Representative flesh samples were taken from various body parts using a sharp knife and scissors. Intramuscular spines were carefully removed using scissors, knife, and forceps. Samples were weighed for wet weight, then placed in an oven at 70 °C for 6–8 hours. After turning the samples, the temperature was controlled at approximately 45 °C for 15–16 hours to ensure complete drying. Once fully dried, samples were removed and placed in desiccators to avoid atmospheric moisture. Before biochemical analysis, samples were ground to a fine powder using a pestle and mortar and stored in airtight bottles.

#### 1. Moisture Content

Determined by drying a known amount of sample at 60–70 °C for 8–10 hours. Moisture content was calculated as the difference between fresh and dry weights and expressed as percent fresh weight.

#### 2. Protein Content

Measured using Lowry’s (1951) method, which relies on the development of color from protein reacting with alkaline copper reagent. A 10% homogenate was prepared in water and centrifuged at 5,000 rpm for 20 minutes. Then:

- 1 ml of the supernatant was mixed with 1 ml of 10% TCA and left for 30 minutes.
- After centrifugation, the supernatant was discarded and the residue dissolved in 0.1 N NaOH.
- 0.5 ml of this solution was diluted to 1 ml with distilled water; 5 ml alkaline copper reagent was added and left for 5–10 minutes.
- Next, 5 ml Folin’s reagent was added, tubes were shaken, and color intensity was measured at 650 nm

using a Spectronic-20 spectrophotometer against a blank.

### 3. Fat Content

Estimated using the Soxhlet method (A.O.A.C., 1970). Powdered samples were placed in a pre-weighed Soxhlet extractor, and fat was extracted with petroleum ether (40–60 °C boiling point) on a water bath for 8–10 hours. After extraction, the solvent was distilled from the receiving flask (100 ml capacity), leaving behind pure fat. The flask was weighed again, and fat content was expressed as a percentage of the sample's weight.

### 4. Carbohydrate Content

Determined following Yam and Willis (1954):

- A 1 g fried sample was placed in a 100 ml stoppered measuring cylinder.
- 10 ml of distilled water was added and stirred to disperse the sample.
- 13 ml of 52% perchloric acid was added, and the mixture stirred frequently for 20 minutes.
- The volume was made up to 100 ml with distilled water, mixed thoroughly, and filtered into a 250 ml volumetric flask; the final volume was adjusted with distilled water.
- For analysis, three test tubes were prepared:
  1. 1 ml sample in the first tube.
  2. 1 ml distilled water as a blank in the second tube.
  3. 1 ml of 100 mg/ml glucose solution for the standard.
- To each tube, 5 ml anthrone reagent was added. After heating in a water bath for 12 minutes, tubes were cooled to room temperature and the intensity of the green-blue color was measured at 620 nm against the blank using the Spectronic-20.

## 3. RESULTS

### 1. Moisture content:

Average moisture content was recorded in different age groups of fishes of soil ponds (pH 7 and 9.5, shown in table 1)

The maximum moisture content in alkaline pond fish was 78.4% in six months old fishes and minimum 76.73% in sixty-month-old fishes as compared to 79.81 and 77.60% pH soil pond respectively.

**Table-1: Variation in moisture percent in experimental fish rohu at different age groups.**

S. No	Months	Moisture content (%)	
		pH9.5	pH7.0
1.	6 Months	78.41	79.81
2.	18 Months	78.82	79.22
3.	36 Months	77.20	78.30
4.	60Months	76.73	77.60
	C.D at 5%	NS	NS

### 2. Protein Content:

The Protein content in the experimental fish Rohu is presented in table 2. The protein content of fish grown in saline pond was lower than normal soil pond and ranged between 18.10 to 15.8% during the observations. The maximum 18.10% protein was in the six months old fish followed by 17.31, 16.56, and 15.82 % in eighteen, thirty-six, and sixty-months old fish respectively. Minimum 15.82 % protein was recorded in sixty months old fish.

**Table-2: Variation in Protein percent in experimental fish rohu at different age groups.**

S. No	Months	Protein content (%)	
		pH 9.5	pH 7.0
1.	6 Months	18.40	18.10
2.	18 Months	17.87	17.31
3.	36 Months	17.19	16.56
4.	60 Months	16.60	15.82
	C.D at 5%	NS	NS

### 3. Fat Content:

Fat content in different age groups of fishes in soil pond pH 7.0 & 9.5 has been shown in table-3. The fat content (pH 9.5) ranged between 1.60 to 2.89% during the study. Maximum fat was 2.89% in sixty months old fishes followed by 2.65, 1.78 and 1.60 in thirty-six, eighteen-, and six-months old fishes, respectively.

**Table-3: Variation in Fat percent in experimental fish rohu at different age groups.**

S. No	Months	Fat content (%)	
		pH9.5	pH7.0
1.	6 Months	1.60	1.60
2.	18 Months	1.78	1.88
3.	36 Months	2.65	2.73
4.	60 Months	2.89	2.98
	C.D at 5%	68.73	71.65
	C.D at 5%	NS	NS

### 4. Carbohydrate Content:

Carbohydrates content in experimental fish Rohu (*Labeo rohita*) grown in soil pond pH 7.0 & 9.5 has been shown in table-4. The Carbohydrate content of fishes of soil pond (pH 9.5) ranged from 3.87 to 4.89 as compared to normal pH range 4.2 to 5.45.

The maximum Carbohydrates were 4.89% in sixty-month-old fish followed by 4.76, 4.43 & 3.87% in thirty-six, eighteen, and six-months old fishes, respectively. Higher pH non-significantly reduced the carbohydrates % in test fishes.

**Table-4: Variation in carbohydrates content percentage in experimental fish Rohu at different age group.**

S. No	Months	Carbohydrates content (%)	
		pH 9.5	pH 7.0
1.	6 Months	3.87	4.20
2.	18 Months	4.43	4.90
3.	36 Months	4.76	5.25
4.	60Months	4.89	5.45
	C.D at 5%	NS	NS

#### 4. DISCUSSION

The **moisture content** declined with age but did not differ significantly across age groups. The highest moisture content—78.4%—was observed in six-month-old fish from the alkaline pond (pH 9.5), while normal-soil pond fish exhibited 79.81% moisture at the same age. The lowest value (76.73%) occurred in sixty-month-old fish. Across both pond types, moisture content decreased as fat content increased, indicating an inverse relationship between moisture and fat during aging.

The **fat content** increased significantly with age in both groups. Among fish from the alkaline pond, fat rose from 1.60% at six months to 2.89% at sixty months. This regular increment may result from reduced moisture and higher food intake in later life stages, a trend also noted by Benoit et al. (1995) in carp.

The **protein content** showed a declining trend with age, though differences weren't statistically significant. In alkaline-soil fish, it decreased from 18.10% (six months) to 15.82% (sixty months), compared to 18.40% and 16.60% in control pond fish. This decline aligns with findings by Gopakumar (1997) and reflects a typical pattern in carp ontogeny, where protein levels plateau between 15–18%. Literature indicates that approximately 70–72% of flesh protein is myofibrillar, which undergoes qualitative changes as fish develop.

The **carbohydrate content** increased with age in both groups: from 3.87% to 4.89% in alkaline-pond fish, and from 4.20% to 5.45% in normal-pond fish. This rise parallels the increasing fat content and mirrors observations in Indian major carps (Sanker & Ramachandran, 2001).

#### 5. CONCLUSION

Based on the results of this study, it can be concluded that although the overall biochemical composition of muscle did not change significantly with age, a decrease in protein and an increase in fat content were observed as the fish aged. Nonetheless, fish from the alkaline-soil pond consistently showed lower protein and fat values compared to those from the normal-soil pond.

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#### 7. CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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