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# Assessment of the effect of water temperature on length gain, feed conversion ratio (FCR) and protein profile in brain of *Labeo rohita* (Hamilton 1822) fed *Nigella sativa* incorporated diets

**Bimlendu Kumar Mishra, Monowar Alam Khalid and Shyam Narayan Labh**

**Abstract**
Temperature is one of the environmental factors with greatest influence on the growth performance of animals. Thus, a 12 week study was conducted to investigate the effects of different water temperature on length gain, feed conversion ratio and protein profile in brain of Indian major carp rohu (*Labeo rohita* Hamilton 1822) in the wet laboratory of Fisheries Development and Training Center, Janakpurdham, Nepal. Altogether six treatments ($T_1$, $T_2$, $T_3$, $T_4$, $T_5$ and $T_6$) of varied temperaturesas18-20 °C, 20-22 °C, 22-24 °C, 24-26 °C, 26-28 °C, 28-30 °C were fixed with the help of aquarium thermometer in 18 aquaria (three replicates). Natural climatic condition was at that time around 17-18 °C hence in T1 tank 18 °C was fixed in the beginning and after a month it was changed to 19 °C and in final month the temperature was fixed as 20°C, thus this tank was considered as control. Similar temperature variations were applied in other treated tanks and after 90 days of feeding final sampling were done. *Nigella sativa* (Kalaunji) a medicinal plant seed was prepared using ethanol extract of Kalaunji along with other ingredients and fed at the rate of 3% of fish body weight wet twice a day. *Labeo rohita* attained significantly higher body length (10.59±0.06 cm) and length gain % (151.28±0.68) under water temperature range of 24-26 °C fed in fish group of T4 aquaria. The fish reared in water having temperature between 24-26 °C showed cent per cent survival rate and the best FCR value (1.18±0.13). Similarly, total protein in brain (15.98±0.41g) was significantly higher in T4 group under water temperature range of 24-26 °C. Thus, it has been concluded that water temperature ranging from 24-26 °C seemed to be the most effective for rearing of *Labeo rohita* proved for better growth and healthy carp. Aquafarmers can be benefitted by using this technique.

**Keywords:** Water temperature, length gain, FCR, *Labeo rohita*, *Nigella sativa*, brain

## 1. Introduction
Increase in water temperature has a direct impact on aquaculture. Fish and other aquatic animals are susceptible to changing environment. Increase in the temperature affects physiological processes, such as metabolism, growth, reproduction etc. which results in a loss in the productivity [1, 2]. Fish survives well in the optimal temperature in between 15-25 $^0$C [3]. Different fish species are resistant to different temperatures, beyond the particular temperature, it becomes harmful for growth and damage physiological processes [4]. Temperature has multiple effects on fishes, such as controlling, masking, limiting, directing, and as a lethal agent, i.e., too much or too little can destroy the integrity of a fish causing its death [5]. Some fish which shows physiological adaptations can survive well in changing environment [6].
Rohu (*Labeo rohita* Hamilton 1822) is the most important among the three Indian major carp species used in carp polyculture systems [7]. This graceful Indo-Gangetic riverine species is the natural inhabitant of the riverine system of northern and central India, and the rivers of Nepal, Pakistan, Bangladesh and Myanmar. In India, it is almost present all rivers [8]. The species has also been introduced in many other countries, including Sri Lanka, the former USSR, Japan, China, Philippines, Malaysia, and some countries of Africa. Its growth rate shows reduction with decrease in the temperature [9]. Nigella sativa, commonly known as black cumin seed is an important herb belongs to family Ranuculaceae has a great medical importance. A number of researchers have used black cumin seeds as enhancer for performance, growth and immune system of some fish species.

Keeping in view the information given above, it can be envisaged that by understanding how temperature affects the performance of fish, particularly during winter season, a farmer can maximize his profit by exploiting maximum production potential of local fish species. However, information regarding the effect of water temperature on various species of fish in Nepal is limited. Therefore, an experiment was conducted to investigate the effect of different temperature ranges on the growth performance and feed conversion ratio of *Labeo rohita*.

## 2. Materials and Methods

### 2.1 Preparation of Kalaunji seeds incorporated diet

Kalaunji *Nigella sativa* (Family Ranunculaceae) is a widely used medicinal plant. The seeds of *N. sativa* have been widely used in the treatment of different diseases and ailments. In Islamic literature, it is considered as one of the greatest forms of healing medicine. It has been recommended for using on regular basis in Tibb-e-Nabwi (Prophetic Medicine). It has been widely used as antihypertensive, liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, and analgesics, anti-bacterial and in skin disorders. Hence, feed was prepared using extract of Kalaunji along with other ingredients and fed at the rate of 3% of fish body weight wet twice a day at 9 am and 4 pm.

**Fig 1:** Showing encapsulation of Kalaunji seeds in garden

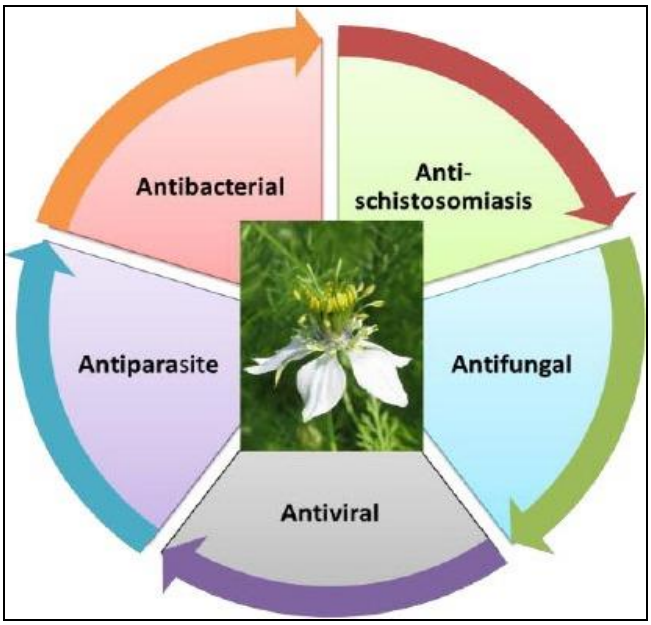


**Fig 2:** Flowers of Kalaunji showing multi-health properties.

**Fig 3:** Refined seeds ready for extractions

As described in Labh *et al.* [10] with slight modifications; black seeds of Kalaunji collected from Kathmandu local market were identified by National Herbarium and Plant laboratory, Department of Plant Resources, Ministry of Forests and Soil Conservation, Government of Nepal. After identification, the samples were transported to the Laboratory of Fish Nutrition, Department of Zoology, Amrit Campus, Tribhuvan University, and Kathmandu, Nepal. The crude extracts were prepared separately by using ethanol (80%) and distilled water. The seeds were properly washed with clean tap water and spread over plain sheet of paper for air drying under shade. After drying, the samples were powdered using warring blender. A known quantity (20 g) of Kalaunji powder was taken in a 250 ml conical flask and added with 100 ml of ethanol (80%).

The ethanol with Kalaunji powdered mixtures were kept for 48 hrs on orbital shaker and then, the extract was filtered using muslin cloth to exclude the residues. The extraction was repeated two times, the filtrate pulled together was centrifuged at 10,000 x g for 5 min. and the supernatant was

collected. Further, the supernatant was concentrated using water bath at temperature of 70 °C. A greasy final crude material obtained was transferred to screw-cap bottle, labeled and stored under refrigerated (4 °C) condition until use. Finally an experimental diet having 40% crude protein was prepared by mixing different feed ingredients (Table 1). The feed was offered at the rate of 3% of wet body weight of the fish twice a day in feeding aquaria.

**Table 1:** Preparation of black cumin incorporated experimental diets for growth

| Ingredients | Experimental diets (% inclusion) | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| Fish Meal† | 29.31 | 29.31 | 29.31 | 29.31 | 29.31 | 29.31 |
| Soya meal‡ | 14.52 | 14.52 | 14.52 | 14.52 | 14.52 | 14.52 |
| Groundnut oil cake† | 9.17 | 9.17 | 9.17 | 9.17 | 9.17 | 9.17 |
| Rice Powder† | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 |
| Wheat Flour† | 14.43 | 14.43 | 14.43 | 14.43 | 14.43 | 14.43 |
| Corn flour† | 11.37 | 11.37 | 11.37 | 11.37 | 11.37 | 11.37 |
| Sunflower oil† | 3 | 3 | 3 | 3 | 3 | 3 |
| Cod liver oil† | 2 | 2 | 2 | 2 | 2 | 2 |
| Vitamin & Mineral Premix§ | 1 | 1 | 1 | 1 | 1 | 1 |
| *N. sativa Seeds* | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Betain Hydrochloride†† | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| BHT(Butylatedhydroxytoluene)†† | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| CMC (Carboxymethylcellulose) †† | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |

†Ingredients like fish meal, soya meal, groundnut oil cake, rice powder, wheat flour, corn flour, sunflower oil and Cod Liver Oil were procured from local market of Kathmandu Valley.

‡Ruchi Soya Industries, Raigad, India.

§Composition of vitamin mineral mix (EMIX PLUS) (quantity 2.5kg -1)

Vitamin A 55,00,000 IU; Vitamin D3 11,00,000 IU; Vitamin B2 2,000 mg; Vitamin E 750 mg; Vitamin K 1,000 mg; Vitamin B6 1,000 mg; Vitamin B12 6 µg; Calcium Pantothenate 2,500 mg; Nicotinamide 10 g; Choline Chloride 150 g; Mn 27,000 mg; I 1,000 mg; Fe 7,500 mg; Zn 5,000 mg; Cu 2,000 mg; Co 450 mg; Ca 500 g; P 300g; L- lysine 10 g; DL-Methionine 10 g; Selenium 50 mgl-1; Selenium 50 mgl-1; Satwari 250 mgl-1; (Lactobacillus 120 million units and Yeast Culture 3000 crore units).

†Black cuminwere obtained locally and then extracts were prepared.

††Himedia Laboratories, Mumbai, India.

### 2.2 Experimental design and feeding trial

A 12 week study was conducted to investigate the effects of different water temperature on length gain, feed conversion ratio and protein profile in brain of Indian major carp rohu (*Labeo rohita* Hamilton 1822) in the wet laboratory of Fisheries Development and Training Center, Janakpurdham, Nepal. The experiment was conducted during October/November and continued up to mid February so natural climatic condition at that time was around 17-18 °C hence 18 °C was fixed in T1 tank in the beginning of feeding trial and after a month it was changed to 19 °C followed after a month by 20 °C and then this tank was considered as control having $T_1$ (18-20 °C). Similar temperature variations were applied in other treated tanks in which after a month in each tank 1°C temperature was increased in each aquarium using aquarium heater manually. Thus, experimental rearing system consisted of18 (three replicates) glass aquaria (14"x12"x30") for six treatments $T_1$ (18-20 °C), $T_2$ (20-22 °C), $T_3$ (22-24°C), $T_4$ (24-26 °C), $T_5$ (26-28 °C) and $T_6$ (28-30 °C) for this experiment and in each aquarium temperature of water were fixed with the help of aquarium heater. Four hundred fifty fingerlings of *Labeo rohita* (7.25±0.27cm) were randomly distributed in six treatment groups in triplicates following a completely randomized design (CRD). pH ranged from 7.53 to 7.92 throughout the study. DO was maintained above 5 gm/l with the help of aerators. A randomly 5 fingerlings were weighed randomly from each aquarium on every 15 days interval to adjust the feeding status of carp.

### 2.3 Examination Procedures

#### 2.3.1 Length gain and FCR profiles

Before harvesting, fingerlings were fasted for 24 hours and then final length of each and individual carp were measured for growth profiles. To understand the feed conversion ratio (FCR) standard formula were used as FCR=F/(Wf-Wo); Where F is the weight of food supplied to fish during the experimental period; Wo is the live weight of fish at the beginning of the experimental period; Wf is the live weight of fish at the end of the experimental period.

#### 2.3.2 Brain tissue collection for protein estimation

At the end of the feeding trial, three fish from each of the control and experimental groups were anaesthetized with tricaine methane sulfonate (MS-222) (5 mg l-1) for 2-3 minutes. Brain tissues were collected through surgical dissection. The tissues samples were homogenized and then transferred immediately to eppendorf tubes, allowed to settle for a while then centrifuged for 5 min at 3000×g.Thus collected brain tissues were stored at -20 °C for further analysis. Total protein content was determined by Biuret method developed by Doumas [11] using a kit where as albumin was determined by BCG (Bromo Cresol Green) method developed by Doumas [11]. Globulin was calculated by the deduction of albumin from total protein while albumin - globulin ratio was calculated by dividing albumin values by globulin values.

#### 2.3.3 Statistical Analysis

Value for each parameter measured has been expressed as mean ± standard error of mean. The results were analyzed by one-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test. Significance was tested at $P<0.05$ level.

## 3. Results and Discussion

### 3.1 Survival and growth performances

Survival rate and growth of a fish depends upon the temperature and other environmental factors of aquatic environment where fish live. In the present study fingerlings of *Labeo rohita* were selected and treated with varied temperatures in which *Cent per cent* survival were observed (Figure 4 and Table 2) in fish group treated with water temperature T4 (24-26 °C) while in other treated groups were 99.6, 98.4, 97.3, 93.7 and 93.1%, respectively. To study the survival of fingerlings *Labeo rohita* was selected by Ali and Salim [12], Saeed *et al.* [13] and Abid and Ahmed [14] for their research work and found similar results. Fish requires diet as a fuel for physical activities, body maintenance and excess of intake diet is utilized for fish growth and sexual maturation. Fish farmer desires maximum growth of fish by development of muscle tissue for their economic benefit. Fish growth rate depends upon a number of factors like species, age, health, genetic potential, food and physicochemical properties of the water. Better growth rate occurs in fry and fingerling as compared to latter stage of fish because these do not show sexual maturation. Fingerling stage offish has all the differential organs already developed so energy from feed is directly utilized for fish growth after maintaining all the physiological activities.

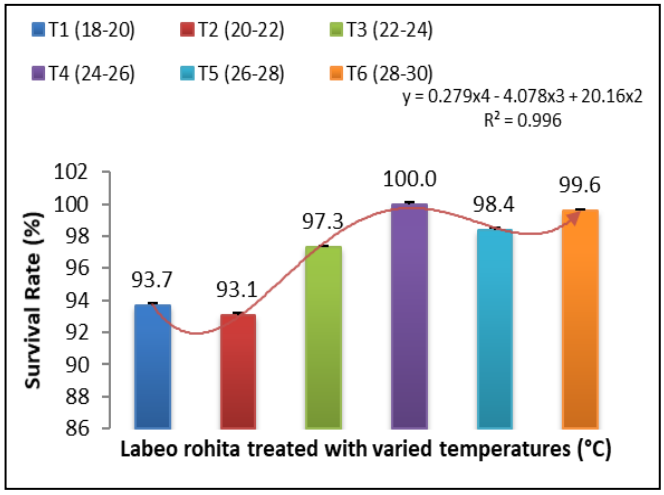


**Fig 4:** Survival rate performance of *Labeo rohita* fed Kalaunji (*Nigella sativa*) and treated by different temperature ranges.

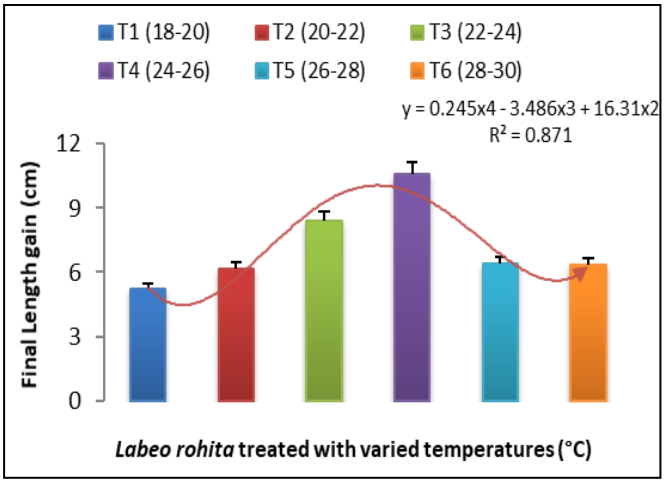


**Fig 5:** Final length gain (cm) of *Labeo rohita* fed Kalaunji (*Nigella sativa*) and treated by different temperature ranges.

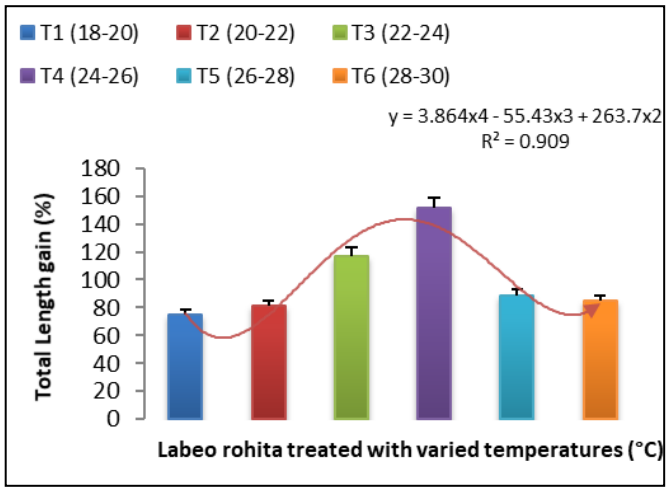


**Fig 6:** Length gain (%) of *Labeo rohita* fed Kalaunji (*Nigella sativa*) and treated by different temperature ranges.

Fingerlings are commonly cultured by fish farmers due to high growth rate and high survival rate which results in to more profits. *Labeo rohita* attained significantly higher body length (10.59± 0.06 cm) and length gain % (151.28±0.68) under water temperature range of 24-26 °C fed in fish group of T4 aquaria (Figure 5, 6 and Table 2). Feeding management plays an important role in fish farming for making it efficient and profitable for fish farmers. Feeding management was scientifically monitored throughout the study periods. The highest length gain was observed in the fish maintained on 24-26 °C. Houlihan *et al.* [15], Britz *et al.* [16] and Azevedo *et al.* [17] observed that fish were markedly influenced by the temperature of water in which they lived. Increased length has also been reported in *Labeo rohita* reared in polyhouse at average temperature of 19 °C as compared with those in outdoor tanks at average temperature of 14.8 °C [18]. he fish reared in water having temperature between 24-26 °C showed the best FCR value (1.18±0.13). The best FCR was observed in the fish kept at 24-26 °C temperature range group by those maintained at 22-24 and 20-22 °C. Similar results were recorded by Andrews and Stickney (1972), who reported that channel catfish, *Ictalurus Punctatus*, fingerlings reared at a temperature range of 18-34 °C registered improvement in FCR, with the best values obtained at 30 °C. Similar results were also recorded by Osborne and Riddle [19].

The food consumption and wet weight production play an important role in the increase or decrease of FCR. The FCR values of the present study are comparable to the findings of Rahman *et al.*, [20]; Tareque *et al.*, 2009 for other common carp species but not satisfactory for Indian major carps (Singh *et al.* [21]; Pramanik *et al.*, [22] and grass carp (Dabrowski) [23], need further study.

Goolish and Adelman [24] observed that fish reared in increased temperature resulted in better utilization of feed in fish than those kept under lower temperature (20.9-24.3 °C). In contrast to the better efficiency of feed utilization at higher temperature range, Alanara [25] did not observe any difference in the feed efficiency of rainbow trout reared at 5 or 15 °C. This discrepancy may be due to difference in water temperature used in these studies. Better feed conversion ratio of the fish maintained at 24-26 °C in this study may be attributed to the increased feed intake of the fish, which spared more nutrients for growth of the fish after meeting the maintenance requirements. However, the findings of Azevedo *et al.* (1998) [17] revealed that water temperature had very little

effect on feed efficiency of rainbow trout (*Oncorhynchus mykiss*). Probable explanation of improved feed efficiency of fish maintained at higher temperature might be the increased feed intake of the fish with increase in water temperature, which resulted in better growth of the fish, leading to better feed conversion ratio. Another probable explanation may be the less energy required for the process of thermoregulation to the fish kept at this temperature, Fig 1-8

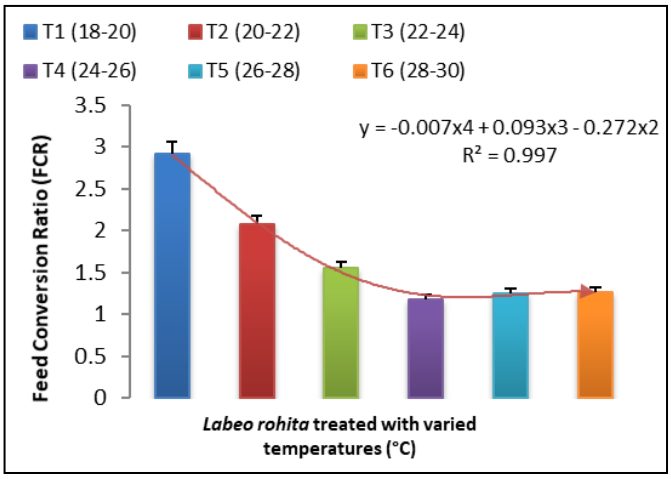


**Fig 7:** Feed conversion ratio (FCR) of *Labeo rohita* fed Kalaunji (*Nigella sativa*) and treated by different temperature ranges.

**Table 2:** Protein profile present in different tissues of *Labeorohita* treated with varied temperature (°C)

| Descriptive | | T1 (18-20 °C) | T2 (20-22 °C) | T3 (22-24 °C) | T4 (24-26 °C) | T5 (26-28 °C) | T6 (28-30 °C) |
|---|---|---|---|---|---|---|---|
| Total Length Gain (TLG) | | 5.21±0.14 | 6.17±0.11 | 8.39±0.26 | 10.59±0.06 | 6.40±0.08 | 6.36±0.05 |
| Length Gain (TLG %) | | 74.70±2.79 | 81.12±1.76 | 117.08±6.57 | 151.28±0.68 | 89.05±1.00 | 84.57±0.98 |
| Feed Conversion Ratio (FCR) | | 2.91±0.00 | 2.08±0.00 | 1.55±0.00 | 1.18±0.00 | 1.25±0.00 | 1.26±0.00 |
| Survival Rate (%) | | 93.74±1.21 | 93.16±1.13 | 97.31±0.15 | 100.00±0.00 | 98.48±132 | 99.56±0.31 |
| Brain | protein | 5.70±0.87 | 8.07±1.09 | 9.69±0.35 | 15.98±0.41 | 13.62±0.47 | 11.54±0.37 |
| | albumin | 2.55±0.49 | 3.85±0.44 | 4.46±0.18 | 7.27±0.15 | 6.27±0.08 | 5.16±0.31 |
| | globulin | 3.15±0.38 | 4.22±0.71 | 5.23±0.26 | 8.71±0.35 | 7.34±0.52 | 6.38±0.06 |
| | AG Ratio | 0.79±0.08 | 0.94±0.12 | 0.85±0.05 | 0.84±0.03 | 0.86±0.07 | 0.81±0.04 |

### 3.2 Protein profile in brain of carp

Protein is the major dietary nutrient affecting performance of fish [26]. It provides the essential and nonessential amino acids which are necessary for muscle formation and enzymatic function and in part provides energy for maintenance [27]. Several experiments have been conducted to determine the optimal level of protein for various fish species (Ogino and Saito, [27]; Dabrowski, [28]; Hepher, [29]; De Silva and Anderson, [30]; Pramanik *el al*., [31]. The total protein, albumin, globulin and ratio of albumin and globulin in brain of *Labeo rohita* found significantly ($P$<0.05) higher in the group of carp treated with temperature$T_4$ (24-26 °C) (Table 2). Increase in the total serum protein, albumin and globulin levels in brain are strongly correlated with the boost of non-specific defense system in fishes. The A/G ratio is a measurable humoral component of innate immune system.

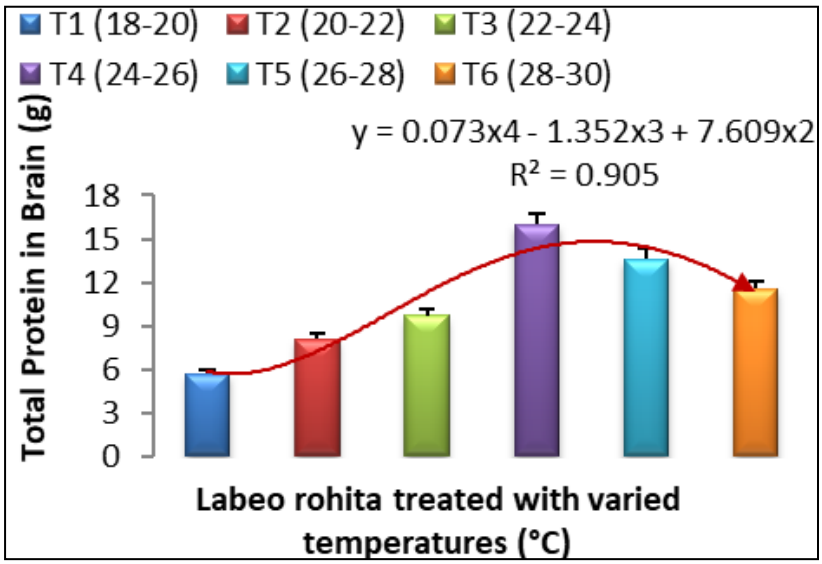


**Fig 8:** Total protein in brain (g) of *Labeo rohita* fed Kalaunji (*Nigella sativa*) and treated by different temperature ranges.

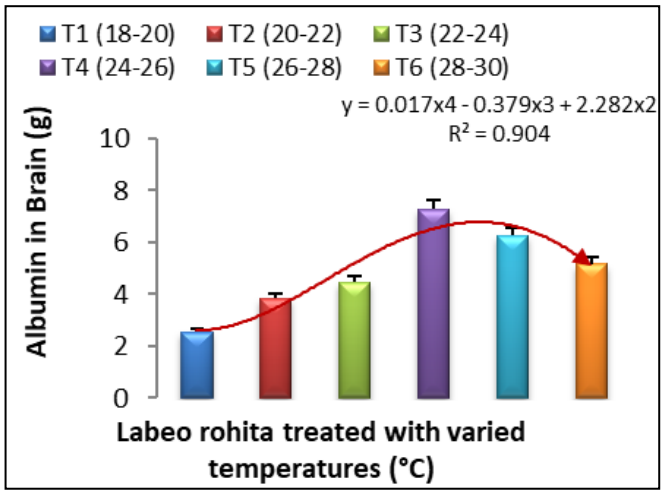


**Fig 9:** Albumin in brain (g) of *Labeo rohita* fed Kalaunji (*Nigella sativa*) and treated by different temperature ranges.

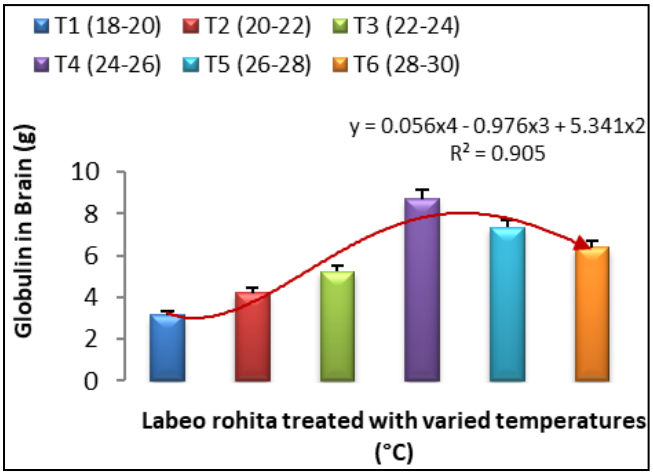


**Fig 10:** Globulin in brain (g) of *Labeo rohita* fed Kalaunji (*Nigella sativa*) and treated by different temperature ranges

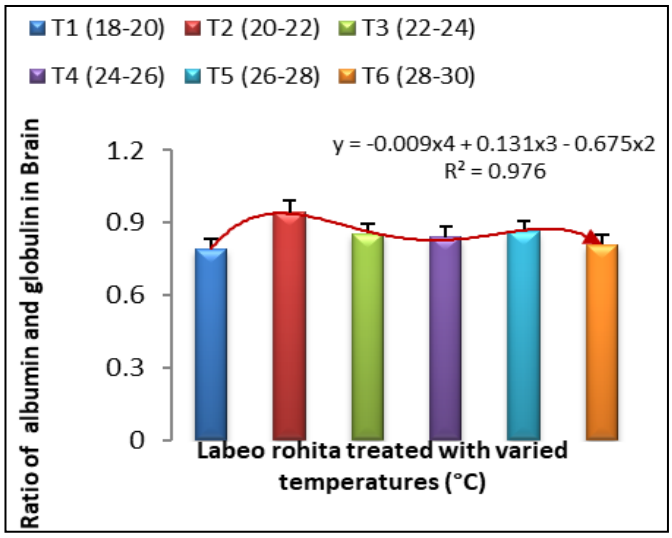


**Fig 11:** Albumin and globulin ratio in brain (g) of *carp* fed Kalaunji (*Nigella sativa*) and treated by different temperature ranges.

In all animals, there is a continual cycle of synthesis and breakdown of protein with growth occurring under conditions where the rate of protein synthesis exceeds protein breakdown [32, 33]. In fish, the effects of various abiotic and nutritional influences on protein synthesis and protein growth have been studied for over 20 years and have been the subject of several reviews [34-37]. Water temperature has been identified as the major abiotic factor affecting the physiology and growth of fish [38, 39]. However, although the effects of water temperature on rates of protein synthesis in fish have been well studied (reviewed by Haschemeyer [40]; McCarthy and Houlihan) [41], there are very few examples where rates of protein synthesis and growth have both been measured for the same animals [42-45].

Our knowledge of the temperature response of protein synthesis and protein growth in fish is still limited in a number of respects. Any fish species exhibits thermal tolerance over a range of water temperatures where feeding and growth will occur and where growth performance (in terms of both rate and efficiency) can vary according to the water temperature [46]. However, previous studies have reared groups of fish at a limited number of water temperatures within the thermal tolerance range for that species, 2 °C (Reid *et al*. 1995, 1997) to 10 °C [47, 48]

## 4. Conclusion and Recommendation

In the present experiment, the length gain percentages and feed conversion ratio (FCR) was found comparatively better in 24-26 °C than that of other treated groups. Protein concentrations in brain also indicated better results in T4 treated group. Thus, it has been concluded that water temperature ranging from 24-26 °C seemed to be the most effective for rearing of *Labeo rohita* proved for better growth and healthy carp. Aquafarmers can be benefitted by using this technique.

## 5. Acknowledgement

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