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Growth and protein profile in head-kidney of *Labeo rohita* (H) treated with varied temperature during climate change study

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Abstract
Fishes are cold blooded affected by surrounding water temperature which directly influences its body temperature, growth rate, food consumption, and other physiological activities. Therefore, this study was conducted to investigate the effect of varied water temperature on growth performance and protein profile on head-kidney of *Labeo rohita* (Hamilton 1822) during climate change study. Four hundred fifty fingerlings of major carp *Labeo rohita* (3.17 ±0.13 g) were introduced at the rate of 25 fish per tank in eighteen glass tanks (14”x12”x36”) for this experiment, divided into six treatments and the temperatures were fixed as T1 (18°-20°C), T2 (20-22°C), T3 (22-24°C), T4 (24-26°C), T5 (26-28°C) and T6 (28-30°C). To maintain the varied climatic conditions of aquatic environment initial temperature fixed in the beginning changed and increased 1°C after a month in all the tanks e.g. in T1 first month was fixed as 18°C followed by 19°C and in last month as 20°C respectively. The experiment was conducted for 3 months and during rearing regular feed were given at the rate of 3% of its body weight and three times (7 am, 12 pm and 5 pm) daily. Temperature, pH and Dissolved Oxygen were monitored on regular and daily basis. At the end of feeding sampling were done for growth and protein profile from the head kidney to understand the effect of temperature on physiology and growth of carp. Average weight gain, specific growth ratio (SGR) found significant high ($P<0.05$) in T4 and T5 fed group while decreasing trends were observed in feed conversion ratio (FCR). Similarly, total protein, albumin, globulin and the ratio of albumin and globulin found significantly increasing ($P<0.05$) in higher the temperature (T4 & T5) and decreasing in lower the temperature (T1 & T2). Similarly, no significant difference was observed in FCR and total protein in head kidney of fish fed in T6 group. In conclusion, it was clear that temperature is prime factor for fish growth and optimum temperature (T4 -T5) is needed to maintain for proper growth and physiology of carp in a particular climatic condition.

Keywords: water temperature, carp, growth, FCR, *Labeo rohita*, physiology

Introduction

Climate change refers to the variation in the global climate since long. It describes changes in the variability of the atmosphere over time scale ranging from decades to millions of years (Emmanuel 2005) [8] on going climate change in predicted to change in life of individual species and hence affecting the population of species and on ecosystem (Porter & Peck 2010) [20]. Climate change directly affect the physical, chemical and biological process in aquatic life that acts on fresh water ecosystem. The climate change in fresh water ecosystem depends largely on temperature and alteration in water chemistry such as nutrient level, dissolve CO_2, DO and particular organic matter (POM). Enhanced temperature creates physiological, ecological and operational change on fishes and fish production system. Average annual temperature in Nepal increases by 0.056 degree Celsius in the past from decades witnessing the climatic change (27 Aug 2017 Kathmandu post). Elevated temperature alters the respiratory rate, food intake, enzyme activities, oxygen consumption and feed metabolism hence affecting growth of fish (Magaawata & IPinjohi 2014). Growth rate of any species determine how fast the species attain maturity, the fecundity recruitment into the exploitable phase of the population and ultimately influence increase in population size (Koeypudsa & Jongjareanjai 2010) [13]. Twelve-week research on *Labeo rohita* reared in fresh water aquariums under controlled temperature (between 24-26) showed the best FCR value and survival rate, as well as total protein in kidney was significantly higher under water temperature 24-26 degree Celsius. This research work seems to be most effective for rearing of *Labeo rohita* proved for better growth and healthy carp fish farmers can be benefitted by using this technique. On the basis of above information it can be anticipated that how temperature affects the performance of fishes. Therefore an experiment was conducted to interrogate the different temperature range on growth and physiology of *Labeo rohita*

Materials and Methods

Table 1: Ingredients and proximate composition of experimental diets for growth of Fishes.

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

The experiment was conducted at fisheries development and training centre Janakpurdham, for which fingerling of *Labeo rohita* was selected for the experiment and a regular diet was prepared using standard procedure and fed at the rate of 3% of fish body weight twice a day at 9 am and 4 pm.

1. Experimental design and feeding trial

A 90 days experiment was conducted to investigate the effects of different water temperature on length gain, feed conversion ratio and protein profile in kidney of Indian major carp rohu (*Labeo rohita* Hamilton 1822) [22] 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 T1 (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 T1 (18-20 °C), T2 (20-22 °C), T3 (22-24°C), T4 (24-26 °C), T5 (26-28 °C) and T6 (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* (3.17±0.13g) 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. Five fingerlings were weighed randomly from each aquarium on every 15 days interval to adjust the feeding status of carp.

2. Examination Procedures

1. Weight gain and FCR profiles

Before harvesting, fingerlings were fasted for 24 hours and then final weight 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. Kidney 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. Kidney 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 kidney tissues were stored at -20 °C for further analysis. Total protein content was determined by Biuret method developed by Doumas using a kit where as albumin was determined by BCG (Bromo Cresol Green) method developed by Doumas and globulin was calculated by the deduction of albumin from total protein while albumin - globulin ratio was calculated by dividing albumin values by globulin values.

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.

Results and Discussion

1. Survival and growth performances

Survival and growth rate of a fish depends upon the temperature and other environmental factors of aquatic environment. In this study fingerlings of *Labeo rohita* were selected and treated with varied temperatures in which *Cent per cent* survival were observed 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, Saeed *et al.* and Abid and Ahmed 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 of fish has all the differential organs already developed so energy from feed is directly utilized for fish growth after maintaining all the physiological activities. 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 weight (13.91± 0.075 g) and weight gain % (345.01±0.155) under water temperature range of 24-26 °C fed in fish group of T4 aquaria. 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 weight gain was observed in the fish maintained on 24-26 °C. Houlihan *et al.*, Britz *et al.* and Azevedo *et al* [4]. observed that fish were markedly influenced by the temperature of water in which they lived. The fish reared in water having temperature between 24-26 °C showed the best FCR value (1.18±0.58). 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.

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*., Tareque *et al*., 2009 [23] for other common carp species but not satisfactory for Indian major carps (Singh *et al.*; Pramanik *et al.*, and grass carp (Dabrowski) [22, 19, 18], need further study.

Goolish and Adelman 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 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) [4] 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,

2. Protein profile in Kidney of carp

Protein is the major dietary nutrient affecting performance of fish. It provides the essential and nonessential amino acids which are necessary for muscle formation and enzymatic function and in part provides energy for maintenance. Several experiments have been conducted to determine the optimal level of protein for various fish species (Ogino and Saito, Dabrowski, Hepher, De Silva and Anderson, Pramanik *el al*,. 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 temperatureT4 (24-26 °C) Increase in the total serum protein, albumin and globulin levels in kidney 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.

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. 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. Water temperature has been identified as the major abiotic factor affecting the physiology and growth of fish. However, although the effects of water temperature on rates of protein synthesis in fish have been well studied (reviewed by Haschemeyer; McCarthy and Houlihan), there are very few examples where rates of protein synthesis and growth have both been measured for the same animals.
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. 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 to 10 °C. (Reid *et al*. 1995, 1997).

Conclusion and Recommendation

In the present experiment, the weight gain, weight gain percentages and feed conversion ratio (FCR) was found comparatively better in 24-26 °C than that of other treated groups. Protein concentrations in kidney 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.

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Charts

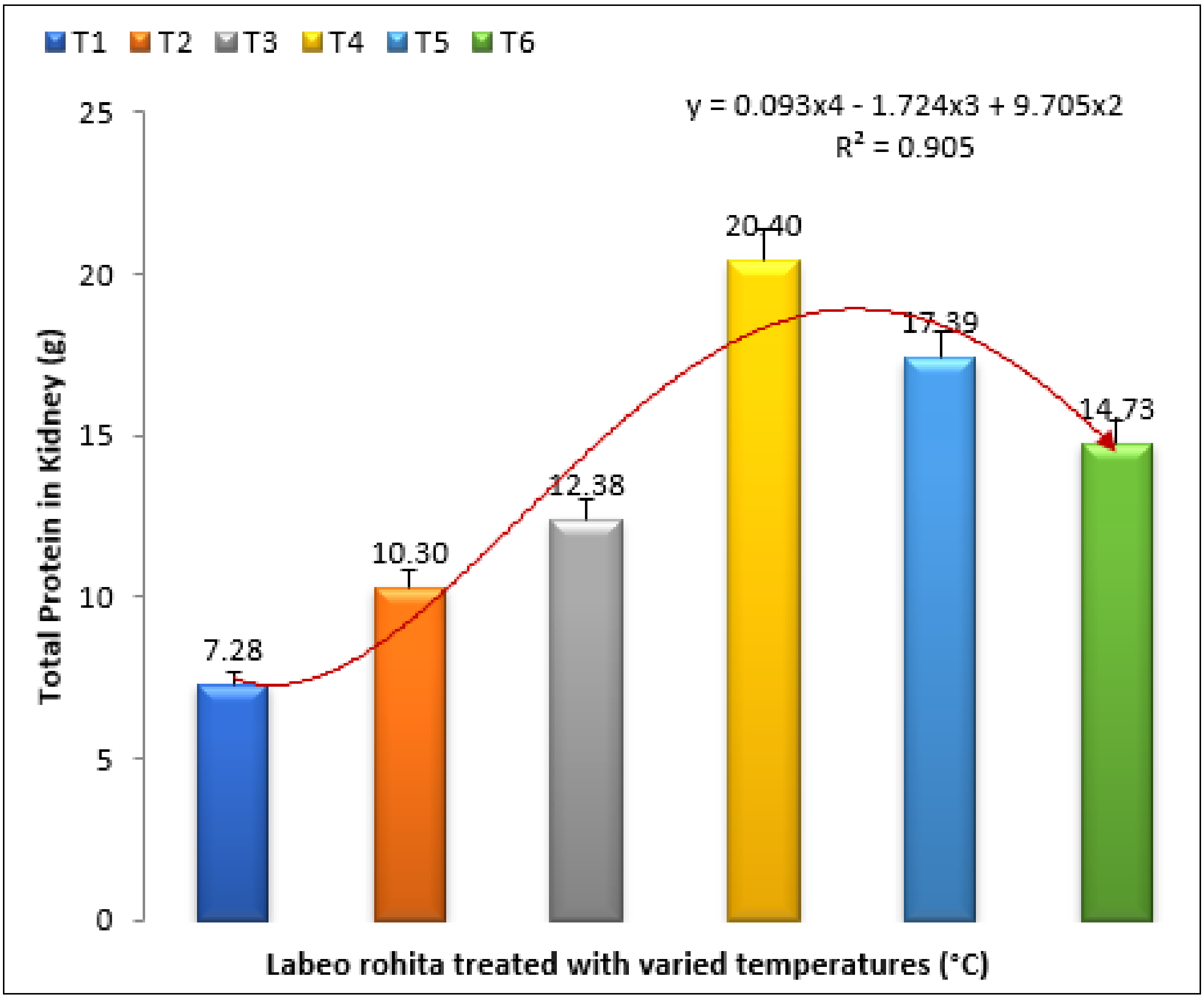


Chart 1

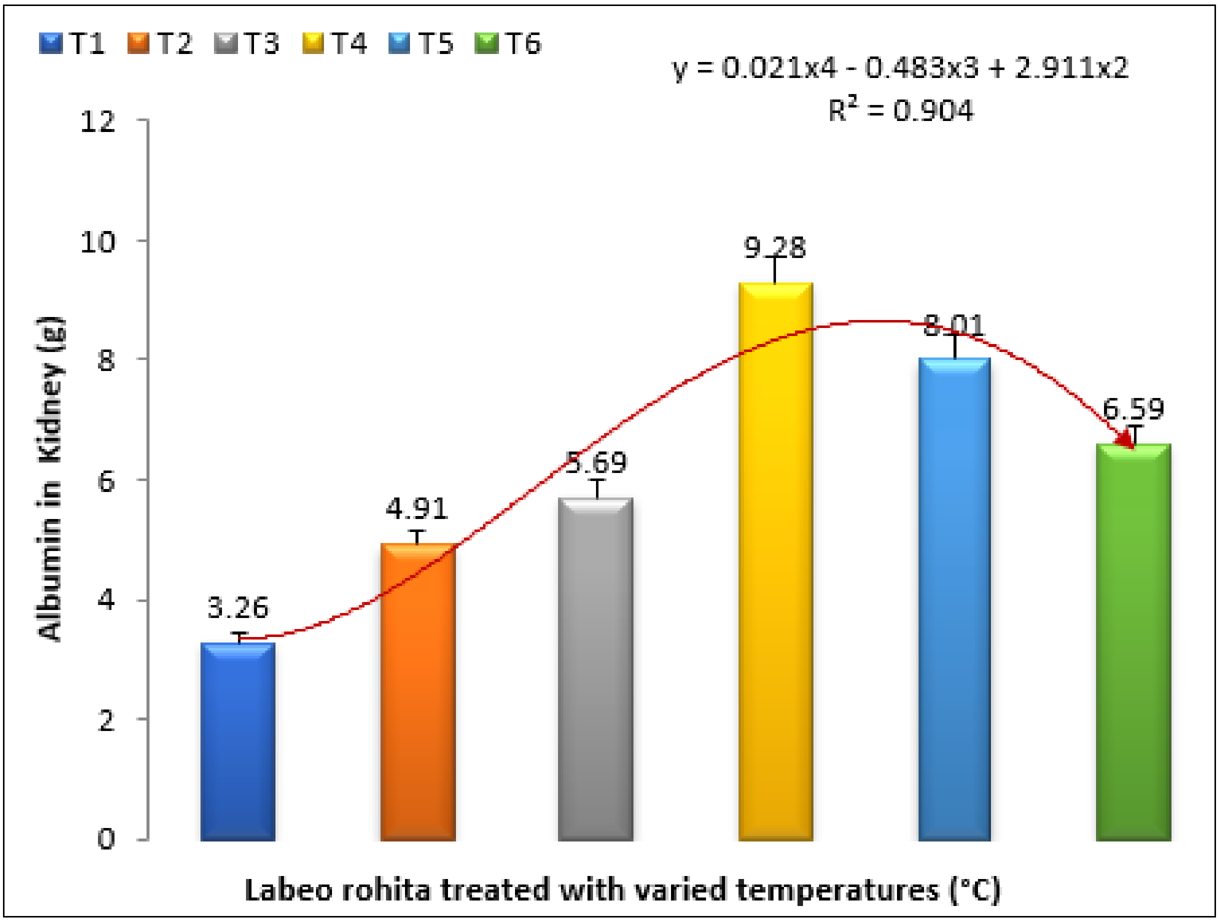


Chart 2

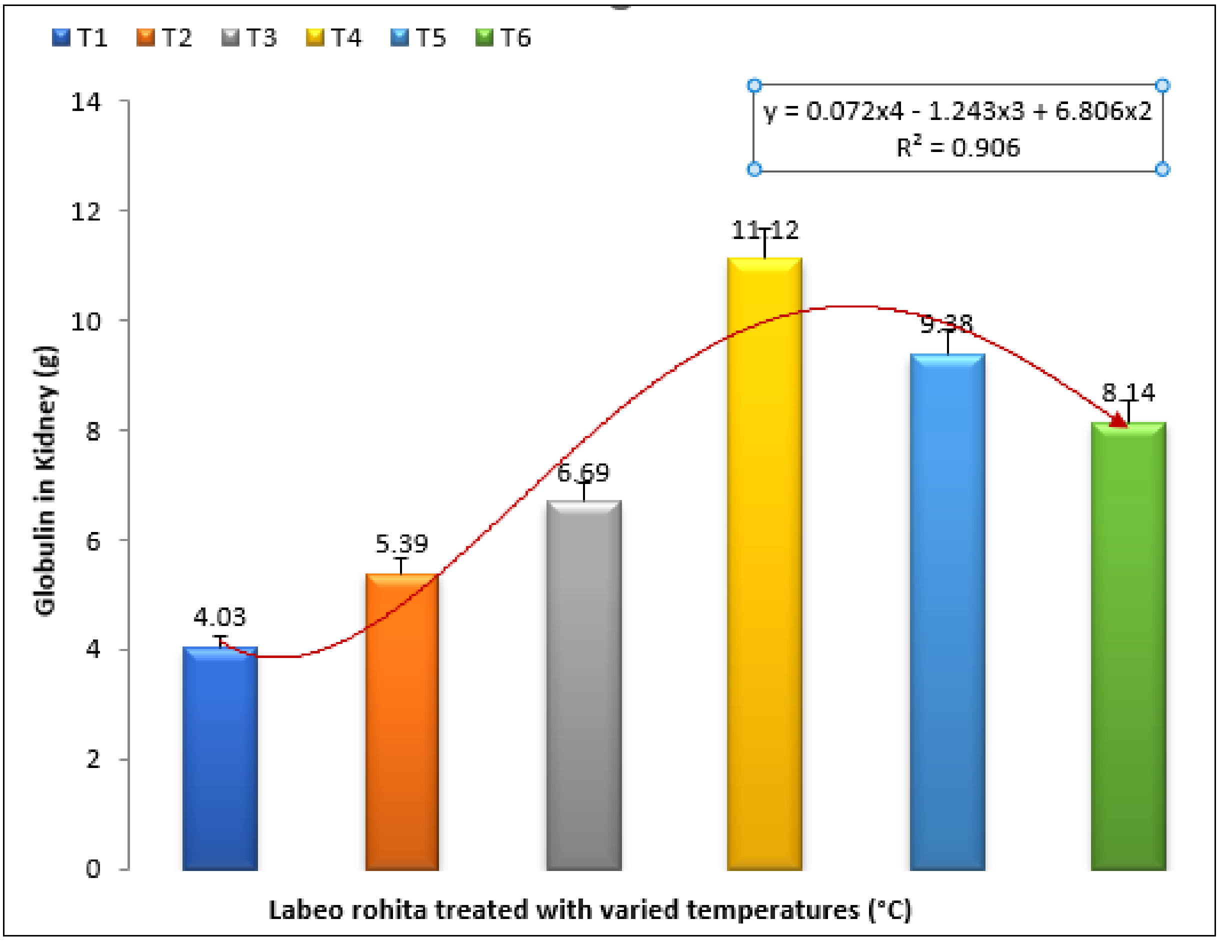
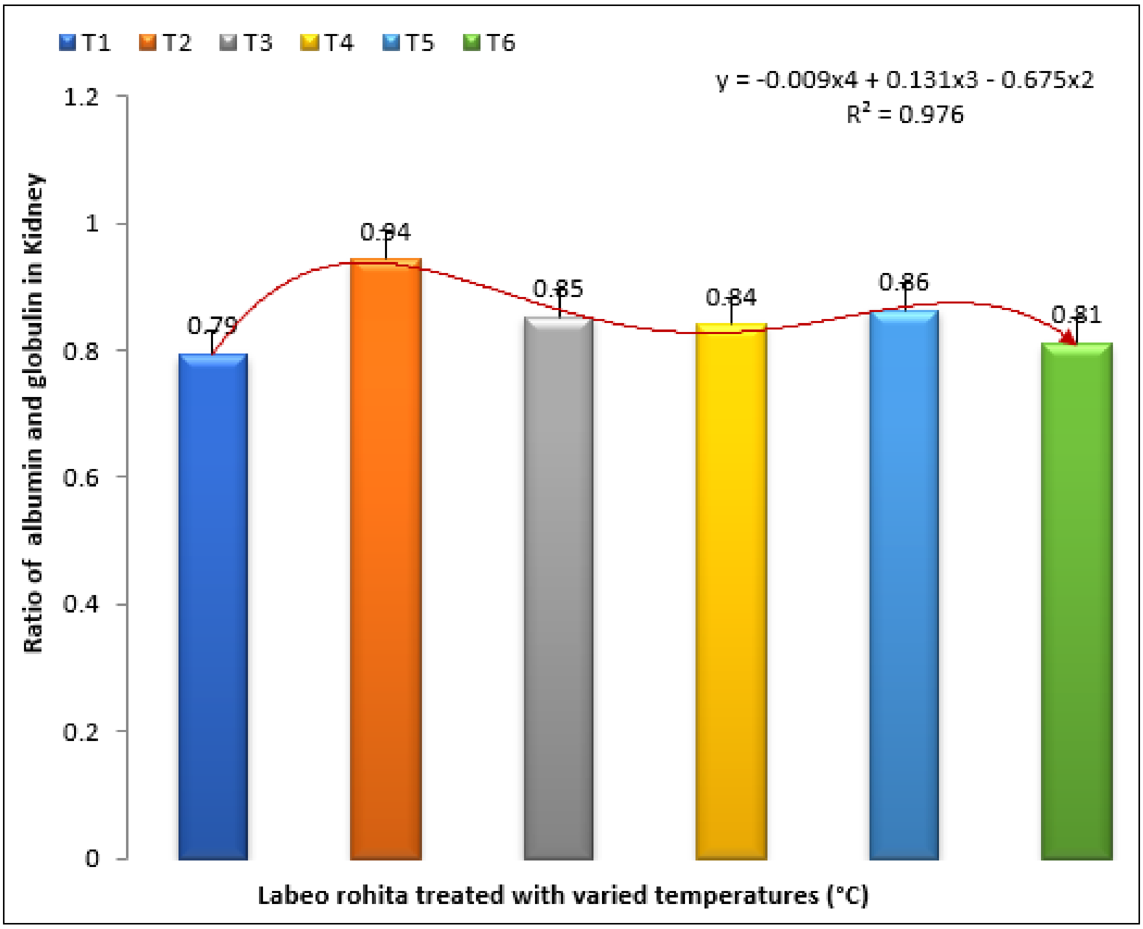


Chart 3



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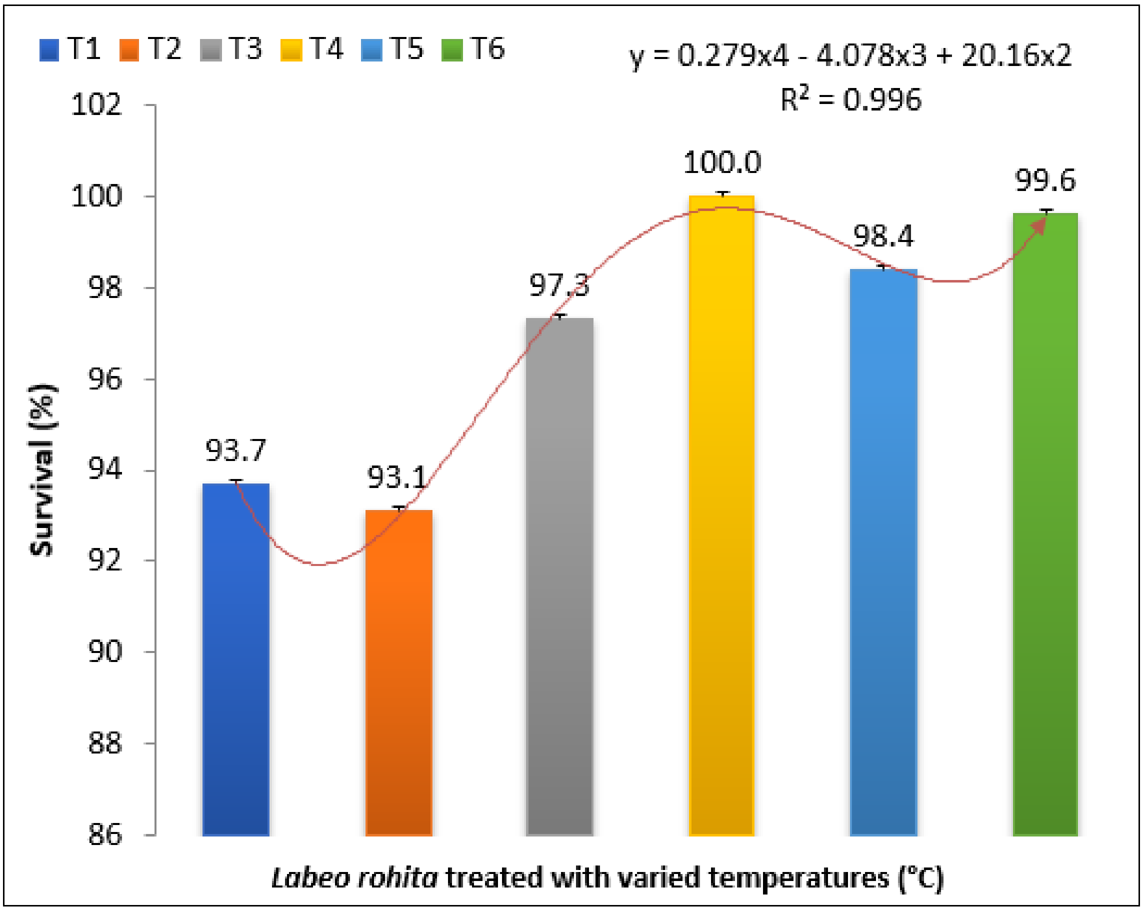

Chart 5

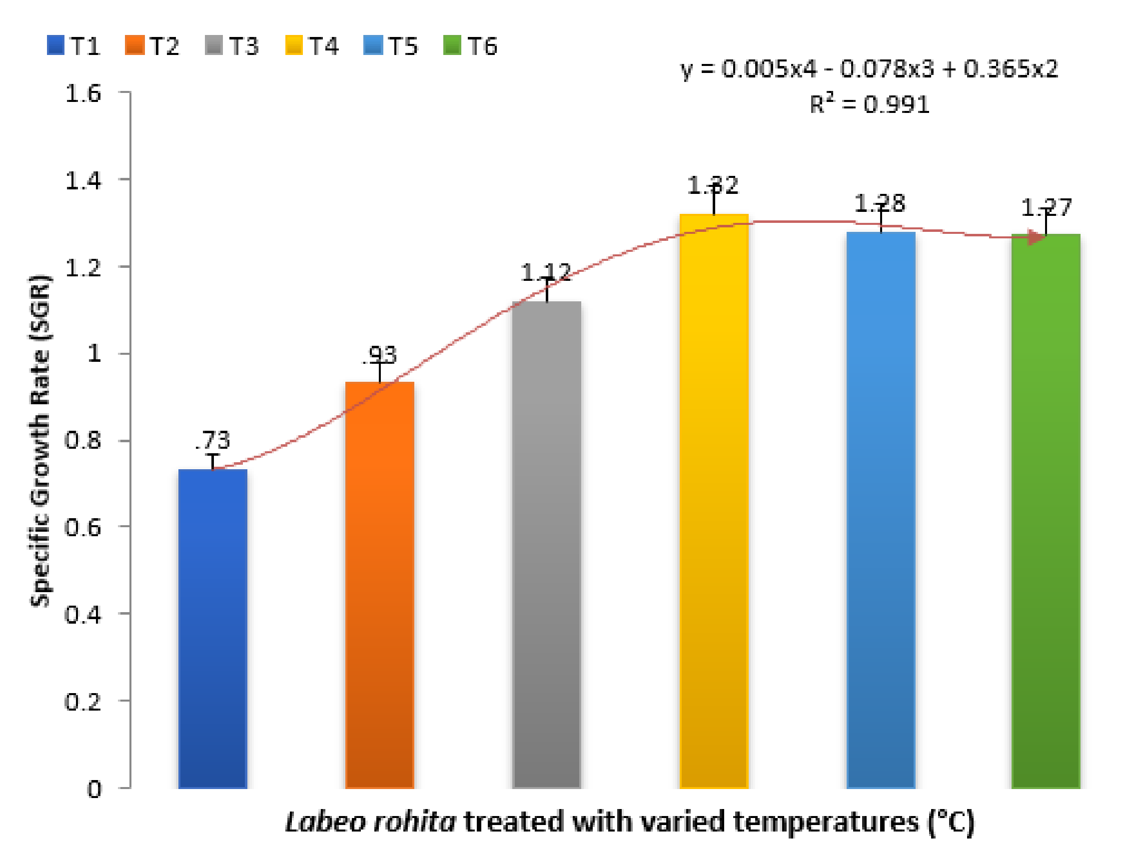

Chart 6

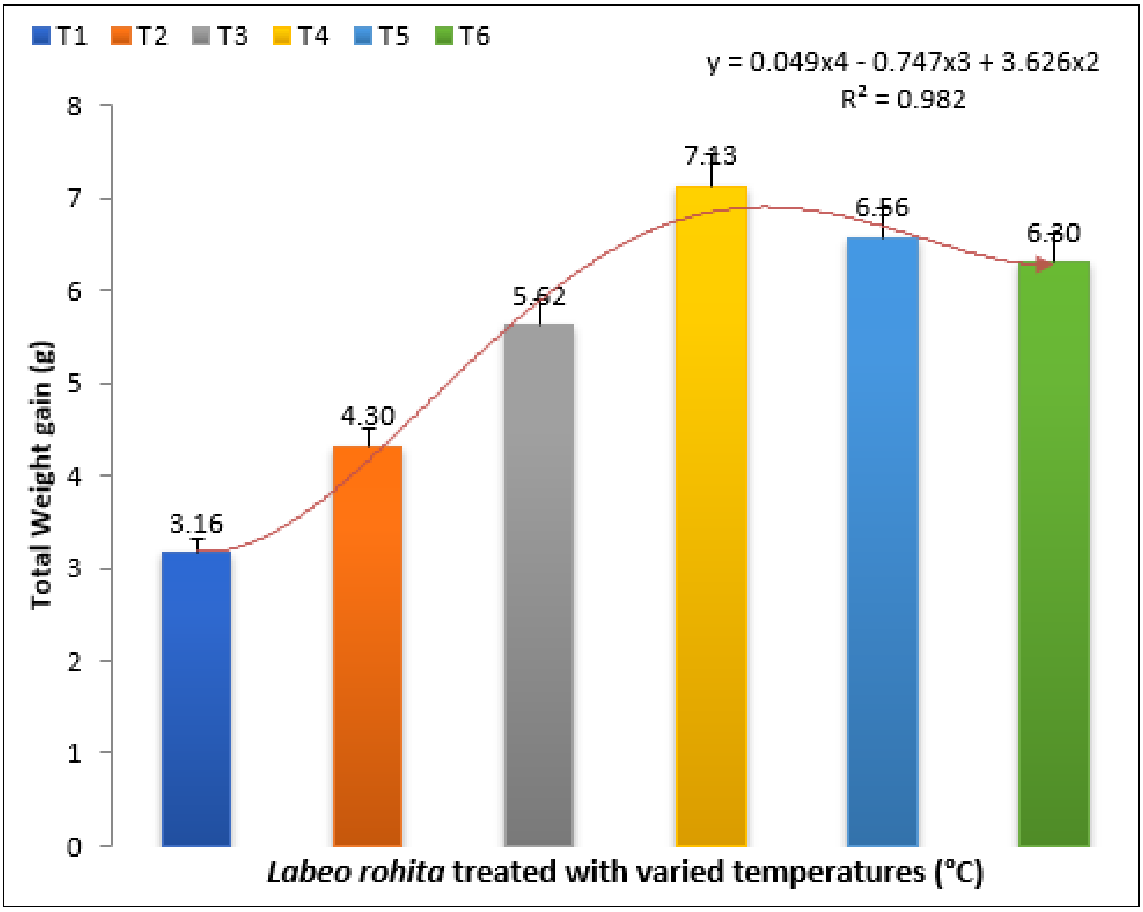

Chart 7

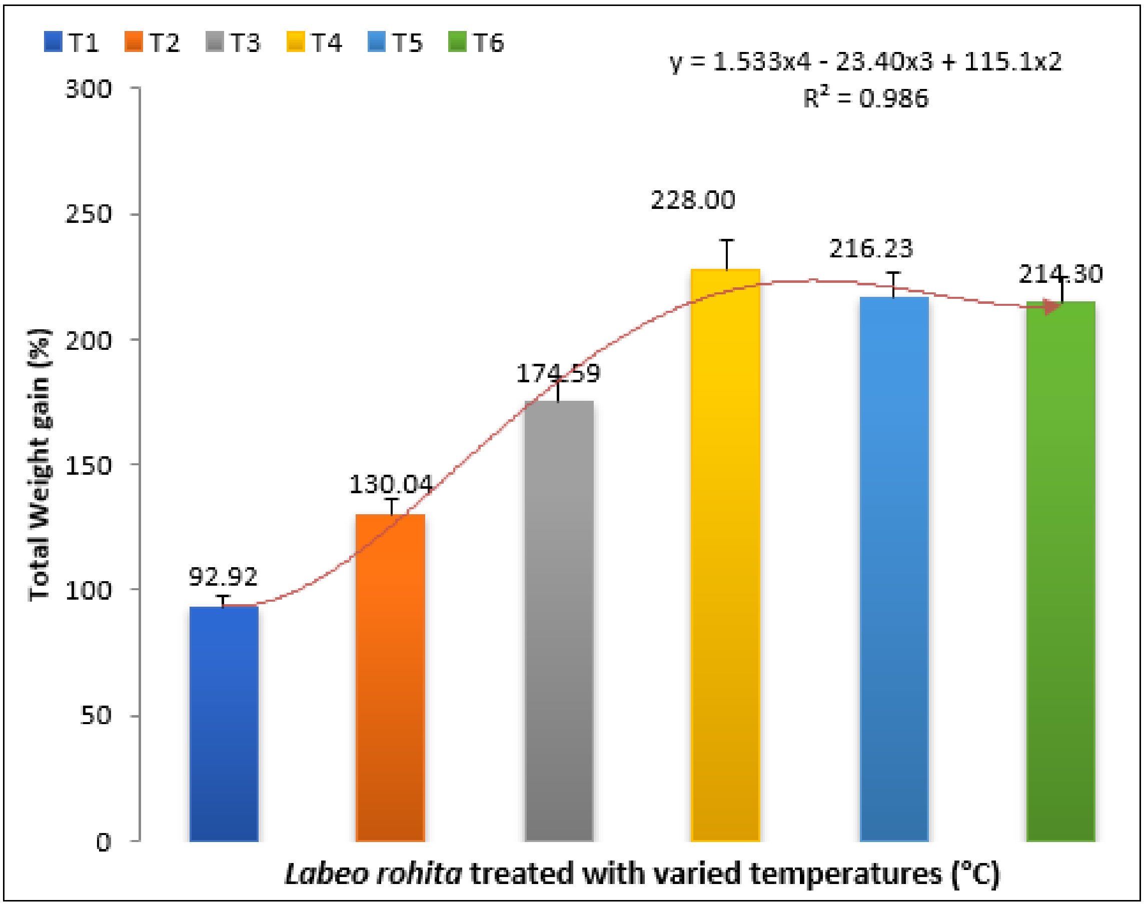

Chart 8

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