

**GROWTH PERFORMANCE OF *Cirrhinus mrigala*
FINGERLINGS FED ON DIFFERENT PROTEIN
SOURCE DIET**



Entry 15

M.Sc. Zoo Dept. *Fish Biology & Aquaculture*

Signature *Raju Shrestha*

Date: *2079-05-10*
26 Aug. 2022

Submitted by:

Raju Shrestha

Roll No: Zoo. 733/075

T.U. Registration No: 5-1-19-675-99

Batch: 2075

A thesis submitted in partial fulfilment of the requirements for the award of the degree of Master of Science in Zoology with special paper Fish Biology and Aquaculture (Zoo.653).

Submitted to:

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu


Nepal

November, 2022

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author or institution.

Date: 2079-04-15 (B.S)
2022-07-31 (A.D)


.....

Raju Shrestha

T.U. Registration No.: 5-1-19-675-99

T.U. Examination Roll No.: 733/075

Batch: 2075



त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY

01-4331896
01-4331896

Email: info@cdztu.edu.np
URL: www.cdztu.edu.np

प्राणी शास्त्र केन्द्रीय विभाग

CENTRAL DEPARTMENT OF ZOOLOGY

कीर्तिपुर, काठमाडौं, नेपाल ।
Kirtipur, Kathmandu, Nepal.



पत्र संख्या :-
च.नं. Ref.No.:-

RECOMMENDATION

This is to recommend that the thesis entitled, “**Growth performance of *Cirrhinus mrigala* fingerlings fed on different protein source diet**” has been carried out by Raju Shrestha for the partial fulfilment of Master’s Degree of Science in Zoology with a special paper Fish Biology and Aquaculture. This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

Date: 2079-05-01 (B.S)
2022-08-17 (A.D)

(Supervisor)

Dr. Archana Prasad

Associate professor

Central Department of Zoology

Tribhuvan University, Kirtipur

Kathmandu, Nepal



त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY

01-4331896
01-4331896

Email: info@cdztu.edu.np
URL: www.cdztu.edu.np

प्राणी शास्त्र केन्द्रीय विभाग

CENTRAL DEPARTMENT OF ZOOLOGY

कीर्तिपुर, काठमाडौं, नेपाल।
Kirtipur, Kathmandu, Nepal.

Central Department of Zoology
T.U., Kirtipur

पत्र संख्या :-
च.नं. Ref.No.:-

LETTER OF APPROVAL

On the recommendation of supervisor "Associate Professor Dr. Archana Prasad" this thesis submitted by Raju Shrestha entitled "**Growth performance of *Cirrhinus mrigala* fingerlings fed on different protein source diet**" is approved for the examination in partial fulfilment of requirements of Master's Degree of Science in Zoology with special paper Fish Biology and Aquaculture.

Date: 2079-05-07 (B.S.)
2022-08-23 (A.D.)

Dr. Tej Bahadur Thapa

Head of Department

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal



त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY

प्राणी शास्त्र केन्द्रीय विभाग

CENTRAL DEPARTMENT OF ZOOLOGY

कीर्तिपुर, काठमाडौं, नेपाल।
Kirtipur, Kathmandu, Nepal.



०१-४३३१८९६
०१-४३३१८९६

Email: info@cdztu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-

च नं. Ref.No. :-

CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Raju Shrestha entitled “Growth performance of *Cirrhinus mrigala* fingerlings fed on different protein source diet” has been accepted as a partial fulfilment for the requirements of Master’s Degree of Science in Zoology with a special paper Fish Biology and Aquaculture.

EVALUATION COMMITTEE

Supervisor

Dr. Archana Prasad

Associate Professor

Central Department of Zoology

Tribhuvan University, Kirtipur

External Examiner

Head of Department

Prof. Dr. Tej Bahadur Thapa

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

Internal Examiner

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

Date of examination: 2079-06-07 (B.S.)
2022-09-23 (A.D.)

ACKNOWLEDGEMENTS

This thesis work has been accomplished with the guidance of my supervisor Dr. Archana Prasad, Associate Professor at the Central Department of Zoology, Institute of Science and Technology, Kirtipur, Kathmandu. My sincere gratitude goes to her for her support and encouragement in the course of this thesis work.

I would like to express my thankfulness to respected Professor Dr. Tej Bahadur Thapa, Department Head, Central Department of Zoology, Kirtipur, for his suggestions. I am equally grateful to all my teachers at the department who provided constructive guidelines, valuable materials and information during my research.

I am thankful to Station Chief Sumitra Laudari, Station chief of Central fisheries promotion and conservation center, Balaju, Kathmandu, Nepal for providing an opportunity and all the facilities to complete this dissertation successfully.

Many thanks are extended to relevant members of CFPPC, Balaju, Kathmandu for their guidance and encouragement during the entire experimental period.

I will not forget to extend my appreciation to my friends and colleagues whom I have indebted for their encouragement, interest, support and advice in successful completion of this dissertation.

Finally, I would like to express profound gratitude to my family for their patience, care, guidance and unconditional love.

Raju Shrestha

TABLE OF CONTENTS

	Pages
DECLARATION	ii
RECOMMENDATION	iii
LETTER OF APPROVAL	iv
CERTIFICATE OF ACCEPTANCE.....	v
ACKNOWLEDGEMENTS	vi
TABLE OF CONTENTS.....	vii
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF PHOTOGRAPHS	xi
LIST OF ABBREVIATION	xii
ABSTRACT.....	xiii
1. INTRODUCTION	1
1.1 Background	1
1.2 <i>Cirrhinus mrigala</i> (Hamilton, 1822) as experimental fish.....	2
1.3 Plant protein supplements in fish feed	3
1.4 Objectives of the study	4
1.5 Limitations of the study.....	4
2. LITERATURE REVIEW	5
3. MATERIALS AND METHODS.....	7
3.1 Experimental site.....	7
3.2 Materials.....	7
3.2.1 Experimental Setup apparatus	7
3.3 Experimental setup.....	7
3.4 Collection of fingerlings.....	8

3.5 Feed formulation	9
3.5.1 Pearson's method to calculate feed formulation	10
3.6 Feeding	10
3.7 Feeding cost.....	10
3.8 Growth measurement	11
3.9 Water quality parameters	11
3.10 Survival and Growth parameters.....	11
3.11 Statistical Analysis	12
4. RESULTS	13
4.1 Water quality parameters of pond	13
4.2 Survival Rate (SR)	14
4.3 Growth Performance	14
4.3.1 Specific growth Rate (SGR):.....	14
4.3.2 Feed Conversion Ratio (FCR):	15
4.3.3 Conditioning Factor (K):	16
5. Discussion.....	17
6. CONCLUSION AND RECOMMENDATION.....	19
6.1 Conclusion.....	19
6.2 Recommendations	19
REFERENCES	20
PHOTOPLATE.....	23

LIST OF TABLES

Table	Title of Table	Page
1	Experimental designs of happa	8
2	Experimental setup of 4 treatments	8
3	Feeds (with components) used in different treatment groups	9
4	Crude Protein in different plant protein source	10
5	Different protein source meal used in fish feed	11
6	Effect of feed with different protein source fed on growth performance of <i>C.mrigala</i>	14

LIST OF FIGURES

Figure	Title of Figure	Page
1	Central fisheries promotion and conservation center, Balaju, Kathmandu	7
2	Calculating feed formulation using Pearson's Method	10
3	Water quality parameters of the pond water	13
4	Specific Growth Rate under different treatments fed with different proportion of protein in <i>Cirrhinus Mrigala</i>	15
5	Feed Conversion Ratio under different treatments fed with different proportion of protein in <i>Cirrhinus Mrigala</i>	15
6	Conditioning factor (K) under different treatments fed with different proportion of protein in <i>Cirrhinus Mrigala</i>	16

LIST OF PHOTOGRAPHS

Photograph	Title of Photograph	Page
1	Feed Preparation	23
2	Preparation of 12 treatment ponds	23
3	Selection of fingerlings	24
4	Measurement of length and weight	24
5	Introduction of fingerlings in the treatment ponds	25
6	Feeding the fish in the pond	25
7	Measuring of water parameters of the ponds by using Multimeter	26
8	Algal bloom in the pond	26
9	Cleaning the pond	26
10	Study of fishes that died due to various reasons	27
11	Inlet and Outlet of the experimental pond	27
12	Aeration of the pond	27
13	Harvesting of the fishes	28
14	Measurement of length and weight after harvesting	28

LIST OF ABBREVIATION

Abbreviated form	Details of abbreviations
CA	Commercially available
CF	Conditioning Factor
CFPCC	Central Fisheries Promotion and Conservation Center
CP	Crude Protein
DO	Dissolved Oxygen
FCR	Feed Conversion Ratio
FG	Fish Gut
FM	Fish Meal
FML	Final Mean Length
FMW	Final Mean Weight
FRS	Fisheries Research Station
IML	Initial Mean Length
IMW	Initial Mean Weight
PER	Protein Efficiency Ratio
PM	Plant Meal
SGR	Specific Growth Rate
SR	Survival Rate
WG	Weight Gain

ABSTRACT

This study work evaluates the efficacy of different plant protein sources as substitutes to the expensive and costly commercial fish feed on the survival, growth and development of *Cirrhinus mrigala*. The experiment was conducted in happas in Central Fisheries Promotion and conservation center (CFPCC) from October 2021 to January 2022. *Cirrhinus mrigala* fingerlings were obtained from nursery pond of CFPCC. Average length of 5.06 ± 0.5 cm and average weight of 1.5 ± 0.25 gm fishes were selected. Total six hundred fishes were stocked in 12 happas (50 in each happa). The fishes were fed with three different plant protein sources like soybean, mustard oil cake, fish meal and denoted T1, T2 and T3; while T4 was commercial feed. Length and weight of the fishes were recorded at the interval of every 30 days. There was no significant difference ($P > 0.05$) observed in the growth in different treatment groups. However, Food Conversion Ratio (FCR) was recorded increasing all throughout the experiment. Conditioning factor (K) were recorded ranging from 0.99 to 1.44 under different treatments. pH and DO of water were recorded suitable while the water temperature was slightly lower than optimum for fish growth. Though, overall growth of fingerlings in different treatments was not encouraging but showed almost the same pattern, so plant protein can be used as a source of protein in fingerling feed.

1. INTRODUCTION

1.1 Background

Aquaculture refers to as farming of aquatic organisms by various methods to improve the production of the stock being cultivated. The contribution of this aquaculture to world food production, pharmaceutical use, raw materials for industrial and for stocking or trade for ornamental purposes has increased significantly in recent decades (FAO, 2000). The annual world food fish production from aquaculture rose yearly by 5.8% to 70.5 million metric tons in 2013 (Moffitt, 2014). The result to such big extent of intensification of fish farming has resulted in technology based on very high protein pelleted feeds which are energy intensive as well as high capital investments and require high degree of management practices (Islam, 2002). This has caused the fish farming to be a rather immoderately costly agricultural field.

Today around half of the operating cost in fish farming i.e. 50% of it, is represented by fish feeding, in which protein is the most costly dietary source. In the traditional way of developing of commercial feed, fish meal (FM) is used as the main protein source because of its greater protein content as well as balance EAA (Essential Amino Acid) profile. FM is also considered to have amazing source of EFA (Essential Fatty Acids), vitamins, minerals and also digestible energy (Albert G.J.Tacon, 1998). Coupled with nonstop market demand and competition for its use and shortage in global production in recent time has led to increase in FM prices. If this continues to go on, it is very much likely that developing countries would not be able to depend on FM as major protein source in their aquaculture feeds. That's why many attempts has been made in past and also at present time to try to minimize use of FM by partial or total replacement of it with less expensive, locally available protein sources.

FM is an increasingly expensive as well as scarce source of protein for fish feeds. This alone explains why we need to replace protein source from FM by other cheap alternatives (Delbert M Gatlin III, 2007). Almost all of the studies done on this topic has showed that the partial replacement of FM can be achieved successfully with respect to health and nutritional view (Gomes et al., 1995; Kaushik et al., 1995; Bransden et al., 2001; Refstie et al., 2001; Torstensen et al., 2008). A possible and viable replacement to FM should have certain nutritional specifications which include low fiber levels, great nutrient digestibility and also a relatively great protein percentage

with reasonable palatability (Gatlin et al., 2007). According to Glencross et al. (2007), in ingredient assessment, major role is played by the ingredient functionality as a key parameter.

According to M. El-Sayed (1999), due to the shortage of FM which is main conventional source of protein, production in world that is again coupled by increase in demand of it is likely to reduce dependence of FM as an only protein source in fish feeds. So many attempts to partially or totally replace FM with less costly and locally found protein source will be very helpful in aquaculture.

1.2 *Cirrhinus mrigala* (Hamilton, 1822) as experimental fish

Cirrhinus mrigala (Hamilton, 1822) is a member of Indian major carp group. This species of carp is considered to have a commercial significance because of its aquaculture potential and great preference by the consumers. I chose *Cirrhinus mrigala* as my experimental fish because it was easily available in ponds of Central fisheries and promotion center. Also they were widely used to make ‘chadhi macha’ which are fingerlings weighing up to 50 grams used for frying and eating. The two attributes of major importance in getting to fish population and their response to various aspects of management measures are age and growth rates. Mrigal carp also known as White carp (*Cirrhinus mrigala*) is a ray-finned fish. It is a native species to streams and rivers in Indian continent. It is also popular as a food fish and an important aquacultured freshwater species throughout South Asia. Along with *Labeo Rohita* and the *Catla*, this species is very widely cultured as a polyculture system component of the 3 Indian major carps. Due to their failure in breeding naturally in ponds, mrigal are thus breed using induced breeding. Mrigal is the mostly bottom feeder or benthopelagic and migratory i.e. potamodromous plankton feeder. It generally lives in quick flowing currents of streams and rivers, but they can also endure great levels of salinity. (Wikipedia-*C.mrigala*, 2022)

Determining of the rate of amount of fish that can be produced over time is the purpose of the growth studies. In understanding the dynamics of fish population, the ability to age of fish accurately is considered to be very essential. The annual variance in a fish aquaculture does depend on its pattern of growth. Isolating the catch on the basis of age group is often the best desired way to know about vulnerability of any such group. Many fisheries of world has often used age composition of the catch to determine the

prediction of available stocks in future. Age composition data on sexes is used to study on the differential growth rates in both males and females and in the sample as a whole. The spawning of mrigal occurs in marginal areas of the water bodies in around a depth of 50-100 cm (20 to 39 in) over a substrate of sand or clay. A 6-kg (13 lb) female can lay about a million eggs. (FAO-*C.mrigala*, 2022) The introduced populations of this species today exist outside its native range as well. Thus today, it is known to be a widely aquafarmed species.

1.3 Plant protein supplements in fish feed

Fish Meal (FM) being costly, plant protein supplements can be added in place of meal to lower the overall cost in feed of Mrigal carp. Protein sources like soybean as well as mustard oil cake are cheap and using them in replacement of FM proves to be quite a good solution from the economic point of view. Beside it, these protein sources also help to meet all protein needs of the target fish. According to El-Sayed (1999), despite biological performance of most of these feeds being similar to that of commercial feed, the analyses in cost benefit has indicated them to be better from economical point of view. Economic evaluation of cotton seed meal (El-Sayed, 1990), corn gluten feed and meal (Wu et al., 1995) and animal by-product meal (Rodriguez-Serna et al., 1996); (El-Sayed, 1998) as sources of protein for Nile tilapia; brewery waste (Oduro-Boatenga and Bart-Plange, 1988) for *Tilapia busumana* and cocoa cake (Fagbenro, 1988) for *Tilapia guineensis* concluded with an indication that final cost and profit indices of these sources were far better than them with FM-based feeds.

1.4 Objectives of the study

This research's main objective was to evaluate the effect of plant protein source in growth performance of fingerlings of *Cirrhinus mrigala* while the specific objectives were:

- ❖ To determine Survival Rate (SR), Specific Growth Factor (SGF), Conditioning Factor (K), Feed Conversion Ratio (FCR) of *Cirrhinus mrigala* fed with different proportion of plant protein from different sources.
- ❖ To determine the cost efficient alternative feed for fingerlings of *Cirrhinus mrigala*.

1.5 Limitations of the study

As we know, all and every research has its limitations. This study too faced challenges on its way. Some of them are listed down below:

- ❖ The experiment was carried out in winter although *C.mrigala* is a warm water fish.
- ❖ The experiment was carried out with two plant protein sources only.
- ❖ Algal bloom caused oxygen deficiency effecting growth rate of fingerlings.

2. LITERATURE REVIEW

The aim of aquaculture industry is to produce high quality fish along with the optimization of growth and performance of the fishes. Along with this comes the problem of fish feed. The commercial feed is very costly and is not an optimal choice. Many research have been done on attempt to replace the commercial fish feed with feed that contains locally available plant protein sources. In context of *Cirrhinus mrigala*, a research by (Paul et al. 2021), showed better growth and high survival rate obtained when fed with 10% of their body weight (Paul, 2021). *Cirrhinus mrigala* is a bottom feeder fish feeding mainly on decayed vegetation at the bottom of its habitat (Jhingran, 1979). So, many research conducted to fully or partially replace FM diet with locally available plant protein sources, (Wanjie cai et al. 2022), concluded that fish meal in gibel carp diet can be totally replaced by plant Meal (PM) with no or negligible negative impact on growth overall and muscle along with performance related activities at the transcriptional level, which provided the requirement of digestible protein and balanced amino acid profile is satisfied (Cai, Liu, & Han, 2022). Attempt to partially replace fish meal also has been done. Growth performance in *Cirrhinus mrigala* is seen well when 20% of their FM diet was replaced with a locally available canola seed meal (Zafar & Ahmed, 2008). Soybean meal is an easily available plant protein source and is very cost effective in fish feed for Indian major carps. 350-400 g/kg overall protein included in soybean meal can yield good growth response and survival in mrigal (Jose, 2006). Furthermore, heat processed soybean gave better results than raw soybean. The fish fed with processed soybean diet when compared to the fish fed with raw soybean diet, showed that mrigal fingerlings fed on diet with 25% autoclaved soybean meal and 75% fishmeal showed maximum increase in growth. Minimum growth parameters were observed in fish fed on raw soybean based diet (Rani, 2017). Herbs and Herbal product supplementation also resulted for better fish production (Shakhya, 2017). Enzyme treated soybean meal shows significantly higher growth performance than raw soybean meal for Rainbow trout (Kumar, Lee, & Nicholas, 2019). No significant difference in SGR, FCR, PER of the fish was seen when FM was replaced up to 50% by soybean meal in diet of *L.rohita* fingerlings (Jahan & Hussain, 2012). So soybean is one of the promising ingredients to replace FM without amino acid supplement. According to a research (Mazid, 1997), FM with partial replacement by mustard oil cake showed better results than just using FM or just using mustard oil cake in the fish feed. Between

soybean and mustard oil cake based diet for rearing carp fish fingerlings, soybean base diet gave better result in SGR, lower FCR, Protein efficacy ratio (PER) and apparent net protein utilization (ANPU%) and survival of fingerlings feed than mustard oil cake based fish (Ladha Hussain, 2013). Maximum FM replacement is possible at high feed moisture content (Dragoanovic, 2011). Alternative sources of protein for *Tilapia* fish with emphasis on fishery byproducts, terrestrial animal byproducts, oilseed plants, aquatic plants, single cell proteins, grain legumes, cereal byproducts which are locally available and less expensive to prepare fish feed can be used for better growth performance in them (El-Sayed, 1999).

According to a research (Bhuiyan, 2018), the lipid containment of soybean (18.5%) and mustard oil cake (15.12%) were high. And carbohydrate were also high (15.67% and 39.65% respectively in soybean and mustard oil cake). Among protein, fat and carbohydrate supplemented diet maintaining same dietary energy, growth performance and various parameter of body composition (ash, fat, carbohydrate) is found better in diets with increasing carbohydrate level than other in case of *Labeo rohita*. Carbohydrate has high protein sparing efficiency and results in food profitability (Umer & Ali, 2014). Lipid also plays a great role in protein sparing effect (Ellis & Reigh, 1991). Protein sparing mechanism is used in several fish species with carp (Erfanullah & Jafri, 1995). Protein is the main source of energy for growth and lipid & carbohydrate are supplied in to maintain the non-protein dependent metabolic process (Tacon & Cowey, 1985). The feed with more carbohydrate will significantly reduce the feed cost than feed with more protein and fat (Keshavanath, Manjappa, & Gangadhara, 2002).

3. MATERIALS AND METHODS

3.1 Experimental site

The experiment of this research was conducted on Central fisheries promotion and conservation center, Balaju, Kathmandu.

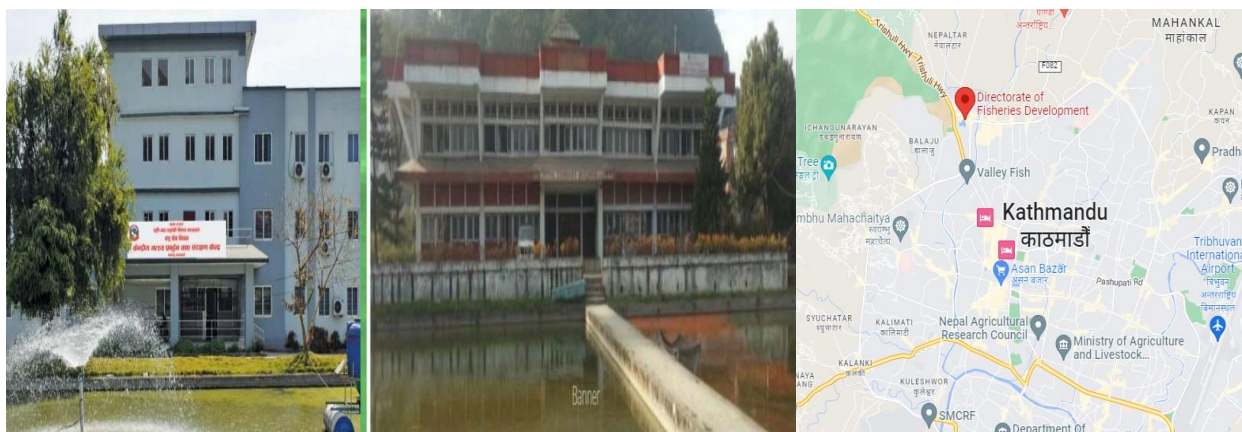


Fig 1: Central fisheries promotion and conservation center, Balaju, Kathmandu

3.2 Materials

3.2.1 Experimental Setup apparatus

- ❖ Happas (5m×1m×1m), Multiparameter meter (Hanna company) to measure pH, DO and temperature, Scoop net, Measuring scale, Electronic weight balance, Bucket

3.3 Experimental setup

The Central fisheries center had twelve ponds. Among the 12 available ponds there, pond number 3 was chosen for research activity. Twelve rectangular happas of equal dimension (5×1×1m) were placed on the edge of the pond. Furthermore, 12 treatment groups were created using happas of dimensions 5x1x1m³. The optimum stocking density for any Indian major carp fingerlings is 2-3 lakhs/ha i.e. 20-30/m³ (Singh, 2001). In our case, 50 fishes were kept in each happa i.e. 10/m³. They were fed with 4 different feeds with different protein sources with equal amount of protein i.e. 28%, to find out which resulted in best growth in the fingerlings.

50 new fingerlings (1.5 gm and 5.06 cm on average) were introduced in each one of them and they were fed with 4 different types of feeds. Commercial feeds were used in T1 (1,2,3) and T2 (4,5,6) were given feed with soybean + wheat flour + rice bran

(soybean protein source) and T3 (7,8,9) were given feed with mustard oil cake+ wheat flour + rice bran (mustard protein source) and T4 (10,11,12) were given feed with fish meal+ wheat flour + rice bran (fish meal protein source). Although feed with different protein source was used in different happas, they all had equal protein percentage (28%).

Fishes, when first introduced to the pond, weighed 1.5gm each on average with an average length of 5.06 cm. Then everyday fishes were given feed at the rate of 2.5-3 % of their body weight. Water parameters, Temperature, pH and DO (Dissolved Oxygen) were measured every day for each happa in the pond.

Table 1: Experimental designs of happa

Treatments	T1	T2	T3	T4
Happa number	1	4	7	10
	2	5	8	11
	3	6	9	12

Table 2: Experimental setup of 4 treatments

Fish Group	No of fish	Treatment	Replicates	Protein source	Feeding% per body wt
Group 1	150	T1	R1, R2, R3	commercial feed	2.5-3%
Group 2	150	T2	R1, R2, R3	soybean	2.5-3%
Group 3	150	T3	R1, R2, R3	mustard oil cake	2.5-3%
Group 4	150	T4	R1, R2, R3	fish meal	2.5-3%

3.4 Collection of fingerlings

The fingerlings of *Cirrhinus mrigala* were netted out from the pond of Fish Research Center by using seine net. Fingerlings were sorted and only uniform sized with an

average weight of 1.5 ± 0.25 gm average length of 5.06 ± 0.5 cm were selected for stocking. The fishes were then dipped in table salt solution for 15 minutes to prevent any possible fungal growth or destroy them if present. Then the weight of six hundred fingerlings were taken and stocked into happa.

3.5 Feed formulation

The feed was prepared in Central fisheries promotion and conservation center, Balaju, Kathmandu using different protein sources. Then it was fed to all 4 treatment groups as in Table 3.

Table 3: Feeds (with components) used in different treatment groups

Feed Types	Feed name with main protein source	Components	Total protein %	Treatment ponds number
I	Commercial	Rice bran+ Wheat flour+ Soya bean meal+ Mustard oil cake+ Soya cake+ Vitamins+ Minerals+ Toxin Binders	28%	1, 2 & 3
II	Soya bean meal	Soya bean meal +Rice bran+ Wheat flour	28%	4, 5 & 6
III	Mustard oil cake	Mustard oil cake + Rice bran+ Wheat flour	28%	7, 8 & 9
IV	Fish meal	Fish meal + Rice bran+ Wheat flour	28%	10, 11 & 12

3.5.1 Pearson's method to calculate feed formulation

Feed preparation is done by Pearson's method (Fig. 2)

Table 4: Crude Protein in different plant protein source

Source	Crude Protein %
Rice Bran	12
Wheat Flour	15
Mustard Oil Cake	40
Soybean meal	45
Fish meal	65

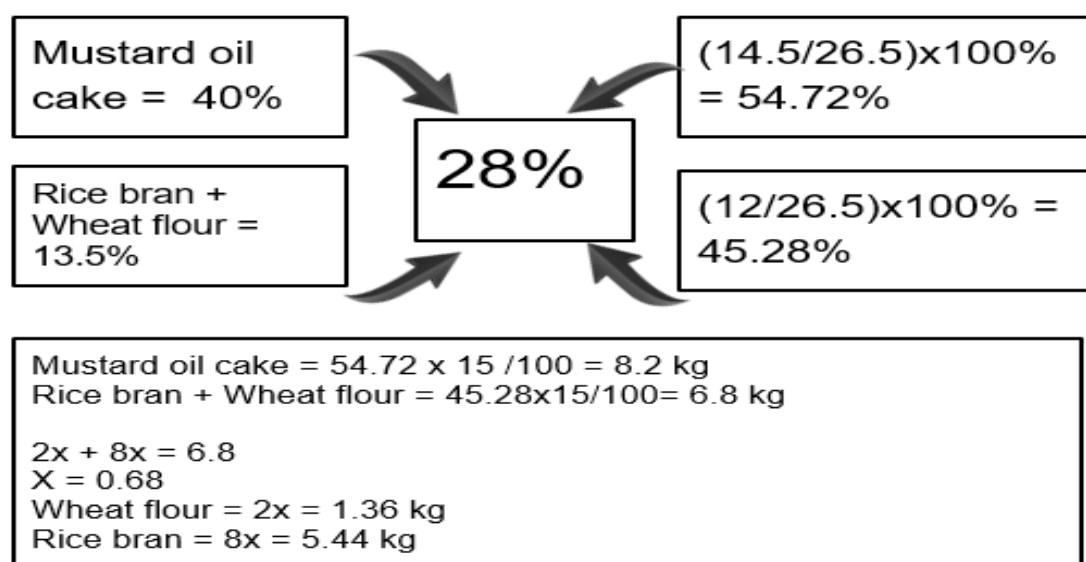


Fig 2: Calculating feed formulation using Pearson's Method

3.6 Feeding

The experimental fish were fed at 2.5-3% of their body weight per day for three months continuously. Feeding was performed twice daily at 11am and 4pm for first month. Then for the next 2 months, fish were fed only once at 11am.

3.7 Feeding cost

The cost of feed was calculated as given below in Table 5.

Table 5: Different protein source meal used in fish feed

S.No	Name of Ingredients	Cost in Rs./kg
1	Commercial Feed	100
2	Soybean meal	70
3	Mustard oil cake meal	50
4	Fish meal	200

3.8 Growth measurement

From every happa, 10 fishes were scooped out using scoop net for growth checkup. Growth checkup was performed at every 30 days interval during which length and weight of individual fish were recorded by digital balance and measuring scale.

3.9 Water quality parameters

The water quality parameters like water pH, DO, temperature was recorded using a Multiparameter daily during 11 to 12 noon.

3.10 Survival and Growth parameters

Length and weight were measured at an interval of 30 days of the experimental ponds and water quality parameters were measured. Experimental data collected during the trial was used to determine SGR, FCR, Survival rate, Conditioning Factor (K) using following formulae:

$$\text{Survival Rate (\%)} = \frac{\text{Number of fish survived}}{\text{Total number of fish stocked}} \times 100\%$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Quantity of feed intake(g)}}{\text{Net weight gain (g)}}$$

$$\text{Conditioning factor (K)} = \frac{\text{Weight of fish in gram}}{(\text{Length of fish in cm})^3} \times 100\%$$

$$\text{Specific Growth Rate (SGR)} = \frac{\ln(\text{FMW}) - \ln(\text{IMW})}{\text{Culture periods(Days)}} \times 100\%$$

Where,

FMW= Final mean weight of the fish

IMW= Initial mean weight of the fish

ln = Natural Logarithm

3.11 Statistical Analysis

The differences between the groups means of SGR, FCR, CF, SR was tested by one-way analysis of variance (ANOVA). All statistical tests were performed using statistical package R software. The comparison was made at 5% level of significance. Experimental data were evaluated as mean \pm SE. Mean and standard error were calculated using Microsoft Excel 2013.

4. RESULTS

4.1 Water quality parameters of pond

Water temperature was recorded 26⁰C, 17⁰C and 13⁰C respectively in three different study months. Dissolved Oxygen ranged around 9 mg/l, 10 mg/l and 10 mg/l respectively and average pH of water was found to be 8.0 - 8.5 (Fig. 3).

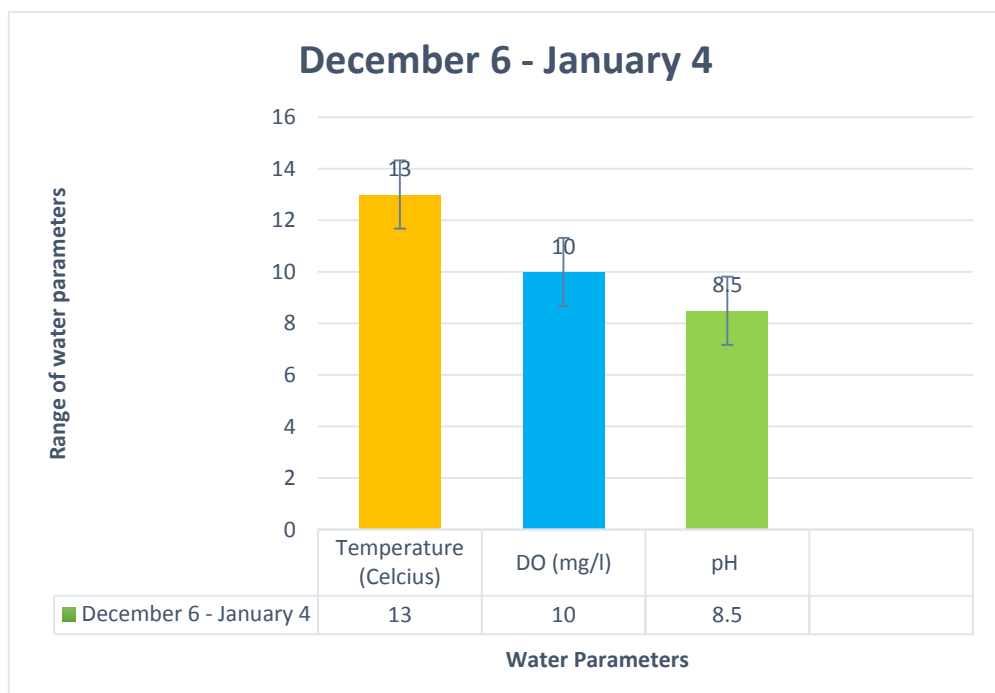
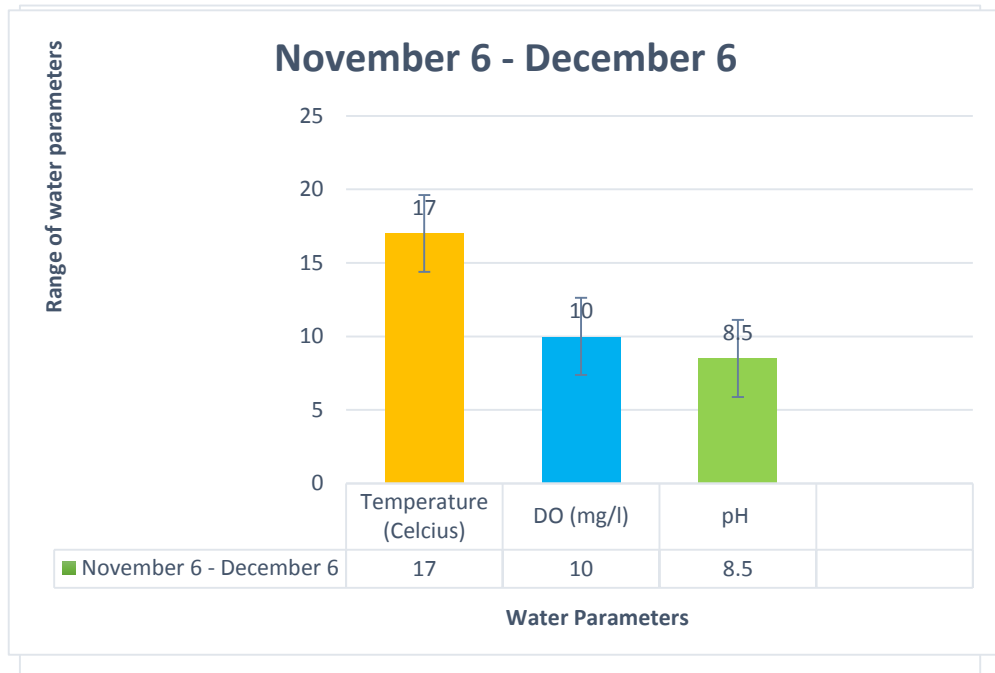


Fig 3: Water quality parameters of the pond water

4.2 Survival Rate (SR)

During the experiment, the survivality rate of the fish was not significantly different among all treatment groups ($P > 0.05$).

4.3 Growth Performance

The growth performance of all treatment groups is presented. (Table 6)

Table 6: Effect of feed with different protein source fed on growth performance of *C.mrigala*. Values are presented as Mean \pm SE

Parameters	Treatment 1 (T1)	Treatment 2 (T2)	Treatment 3 (T3)	Treatment 4 (T4)
IMW(g)	1.74 \pm 0.7118	1.35 \pm 0.2407	1.4 \pm 0.7087	1.4 \pm 0.7584
FMW(g)	3 \pm 0.7767	2.96 \pm 0.83719	4.03 \pm 0.5555	3.3 \pm 0.4973
IML(cm)	5.3 \pm 0.4417	4.66 \pm 0.1039	5.06 \pm 0.1559	5.22 \pm 0.2577
FML(cm)	7.03 \pm 0.1379	7.17 \pm 0.1719	7.46 \pm 0.1318	7.16 \pm 0.1145
SGR (%)	0.61 \pm 0.0834	0.870.0966	1.17 \pm 0.0751	0.95 \pm 0.0674
FCR (%)	5.64 \pm 1.1113	4.01 \pm 0.9211	3.09 \pm 1.1689	3.71 \pm 1.3247
CF(K)	1.44 \pm 0.0377	1.04 \pm 0.0134	1.11 \pm 0.02458	0.99 \pm 0.0178
SR (%)	90%	88%	88%	94%

4.3.1 Specific growth Rate (SGR):

Up to three months, SGR was recorded in ascending order in all treatments. But at the end of study period, T3 had the highest SGR while T1 had the lowest SGR (Fig. 4).

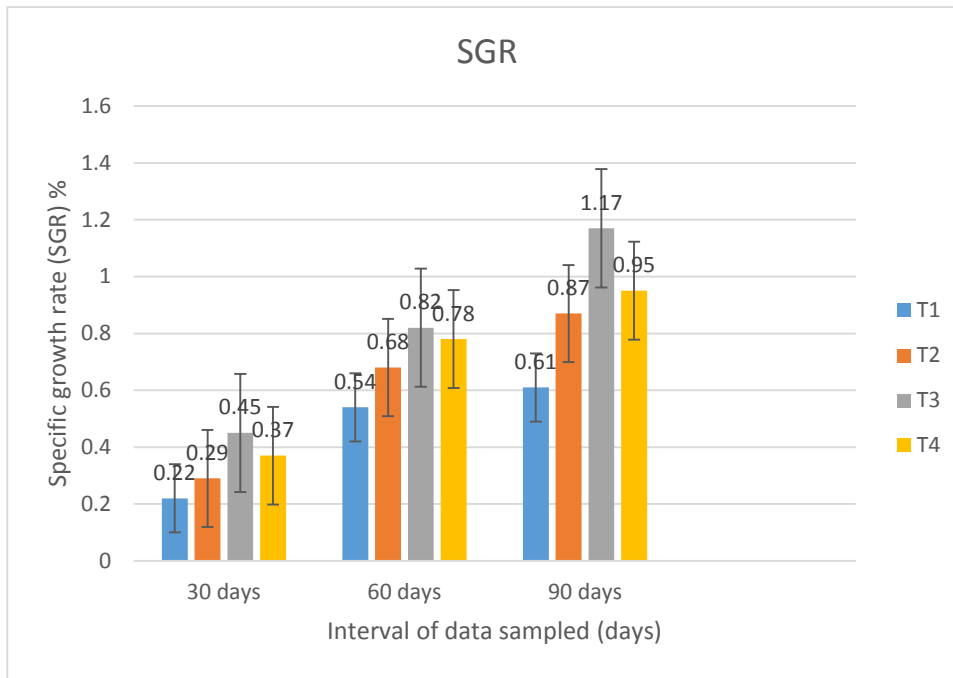


Fig 4: Specific growth rate under different treatments fed with different proportion of protein in *Cirrhinus mrigala*.

4.3.2 Feed Conversion Ratio (FCR):

In present study, FCR is found in order of Mustard oil cake < FM < Soybean < Commercial feed. The highest FCR was observed in T1 and lowest was in T3 (Fig. 5).

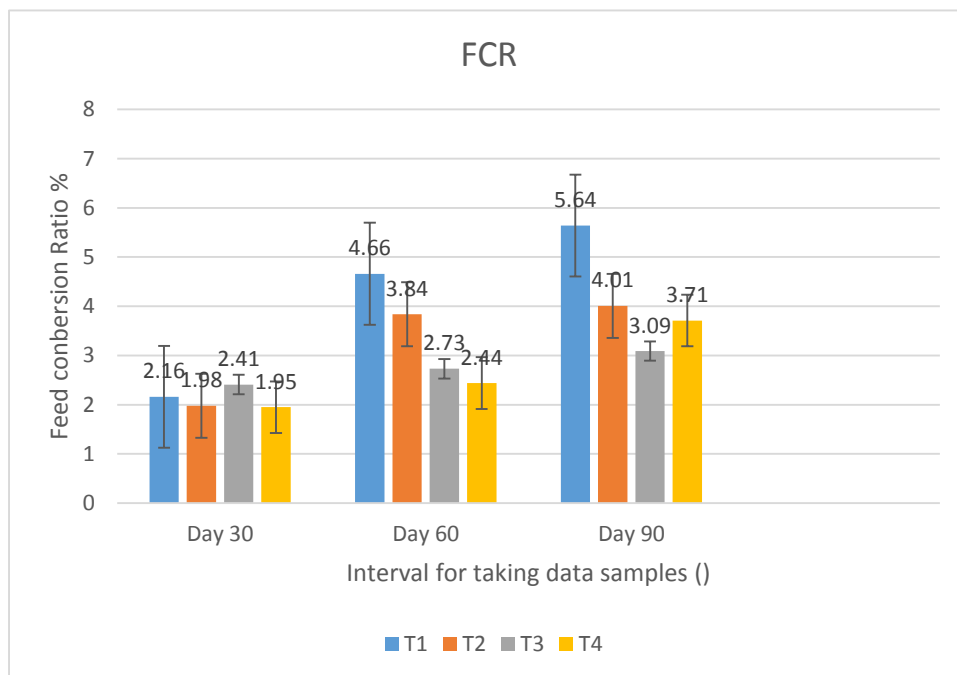


Fig 5: Feed conversion ratio under different treatments fed with different proportion of protein in *Cirrhinus mrigala*.

4.3.3 Conditioning Factor (K):

The value of conditioning factor (K) was found highest on T1 at the end of the study and lowest on T4 (Fig. 6).

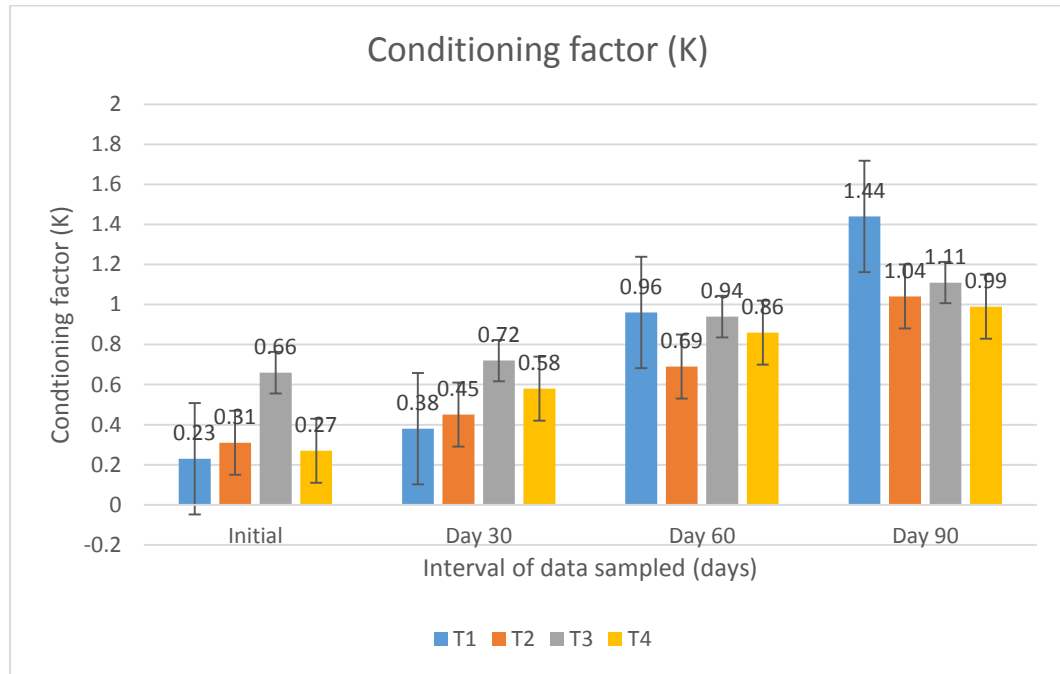


Fig 6: Conditioning Factor under different treatments fed with different proportion of protein in *Cirrhinus mrigala*.

5. Discussion

The current goal of this study was to find better alternative protein source to replace the commercial feed. The fish chosen fish *Cirrhinus mrigala* is a warm water fish. But during our study period, water temperature was recorded below 30°C. The highest body weight gain and lowest feed conversion ratio was recorded at 33°C (Ud Doulaha et al. 2019). This might have affected the growth of fingerlings of *Cirrhinus mrigala*.

During this study, all plant protein source yielded similar better results. In study experiments, no significant difference in SR, SGR, FCR and K was observed in all 4 treatment groups. However, cost of mustard oil cake and soybean as the plant protein source is lower than other, fish meal and commercial feed, and they gave better growth performance in present study. Mustard oil cake gave the best growth performance in our study. Similarly, soybean is also a very good alternative as protein source. The fish fed with processed soybean diet, showed maximum increase in growth of mrigal fingerlings fed on diet with 25% autoclaved soybean meal and 75% fishmeal. Minimum growth parameters were observed in fish fed on raw soybean based diet (Rani, 2017). Furthermore, three feed fish meal, sesame oil cake and soybean meal had greater growth rate and SRG% in a significant fashion than other meals (Rani, 2017).

Fish meal (FM) has traditionally been a major protein source in fish feeds because of its protein quality and palatability but it is very costly. According to a research (Tuladhar, 2003) growth of fish with plant protein source was significant than with fish meal. The higher yield of fish supplemented with Azolla, Soybean and oilcakes was greater than that of fish group supplied with FM diet. No significant difference in SGR, FCR, PER of the fish was seen when FM was replaced up to 50% by soybean meal in diet of *Labeo rohita* fingerlings (Jahan & Hussain, 2012). So soybean is one of the promising ingredients to replace FM without amino acid supplement.

Mustard oil cake is a very effective alternative to protein source in fish feed. Majid (1997) did feed trial experiment, where 4 different feeds like i. fish meal and mustard oil cake, ii. fish meal and sesame oil cake, iii. mustard oil cake and rice bran and iv. mustard oil cake as control diet. The result was significantly higher in first feed followed by second and third in comparison to the control diet. Many other research also suggest mixture of fish meal and plant protein to be more effective (Vhanalakar,

2008). Thus, mixture of fish meal and any plant protein source would have been better for our study as well.

According to Bhuiyan (2018), the lipid contents of soybean (18.5%) and mustard oil cake (15.12%) were high. And carbohydrate content were also high, 15.67% and 39.65%, in soybean and mustard oil cake respectively. Among protein, fat and carbohydrate supplemented diet maintaining same dietary energy, growth performance and various parameter of body composition (ash, fat, carbohydrate) is found better in diets with increasing carbohydrate level than other in case of *Labeo rohita*. Carbohydrate has high protein sparing efficiency and results in food profitability (Umer & Ali, 2014). Lipid also plays a great role in protein sparing effect (Ellis & Reigh, 1991). Protein sparing mechanism is used in several fish species with carp (Erfanullah & Jafri, 1995). Protein is the main source of energy for growth and lipid & carbohydrate are supplied in to maintain the non-protein dependent metabolic process (Tacon & Cowey, 1985). The feed with more carbohydrate will significantly reduce the feed cost than feed with more protein and fat (Keshavanath, Manjappa, & Gangadhara, 2002).

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The result of this experiment demonstrated no significant differences in the growth performance of fishes in all different feed treatment groups. However, our objective was to find a low cost alternative to replace expensive commercial feed. In present study, mustard oil cake as the protein source showed quite good results. Cheaper and local ingredient like Soybean also had similar results. Thus it was concluded that mustard oil cake and soybean as cheaper and good growth performance fish protein source and fish farmers could benefit from these fish feed use. However, further studies including different plant protein sources are needed to develop convincing and decisive knowledge on the improvement of growth of *Cirrhinus mrigala*.

6.2 Recommendations

- ❖ Since only limited option of protein source was used in this experiment, other many possibly better solution can be experimented on for better results.

REFERENCES

- Albert G.J.Tacon, S. S. (1998). Feed preparation and feed management strategies within semi-intensive fish farming systems in the tropics.
- Bhuiyan, M. R. (2018). Proximate composition of fish feed ingredients available in Shipur Upazila, Narsingdi district, Bangladesh.
- Cai, W., Liu, H., & Han, D. (2022). Complete Replacement of Fishmeal With Plant Protein Ingredients in Gibel Carp (*Carassius auratus gibelio*) Diets by Supplementation With Essential Amino Acids Without Negative Impact on Growth Performance and Muscle Growth-Related Biomarkers.
- Delbert M Gatlin III, F. T. (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review.
- Dragoanovic, V. (2011). Assessment of the effects of fish meal, wheat gluten, soy protein concentrate and feed moisture on extruder system parameters and the technical quality of fish feed.
- Ellis, S., & Reigh, R. (1991). Effects of dietary lipid and carbohydrate levels on growth and body composition of juvenile red drum, *Sciaenops ocellatus*.
- El-Sayed, A.-F. M. (1999). Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp.
- Erfanullah, & Jafri. (1995). Protein-sparing effect of dietary carbohydrate in diets for fingerling *Labeo rohita*.
- FAO. (2000). *fao.org*. Retrieved from <https://www.fao.org/3/az998e/az998e.pdf>
- FAO-C.Mrigala*. (2022, 5 8). Retrieved from FAO: https://www.fao.org/fishery/en/culturedspecies/Cirrhinus_mrigala/en
- Islam, M. S. (2002). Evaluation of supplementary feeds for semi-intensive pond culture of mahseer, *Tor putitora* (Hamilton). *Aquaculture* . 212:263-276.
- Jahan, D., & Hussain. (2012). Use of soybean as partial substitute of fish meal in diet of Rohu (*L.rohita*) fry.

- Jhingran, K. (1979). *Synopsis of biological data on the mrigal Cirrhinus mrigala (Hamilton, 1822)*. FAO.
- Jose, S. (2006). Effect of soybean meal based diets on growth and survival rate of indian major carp C.Mrigala.
- Keshavanath, P., Manjappa, K., & Gangadhara, B. (2002). Evaluation of carbohydrate rich diets through common carp culture in manured tanks.
- Kumar, V., Lee, S., & N. R. (2019). Comparative evaluation of processed soybean meal vs regular soybean meal as a fishmeal replacement in diets of rainbow trout (*Oncorhynchus mykiss*) Effects on growth performance and growth-related genes.
- Ladha Hussain, D. J. (2013). Comparative Study of Mustard Oil cake and soybean diet for Rohu Fingerlings.
- Mazid, M. (1997). Formulation of cost effective feeds from locally available ingredients for carp polyculture system for increased production. 14-21.
- Moffitt, C. M.-C. (2014). Blue growth: the 2014 FAO state of world fisheries. 552-553.
- Mohammad Ashaf Ud Doulaha, M. H. (2019). Growth and survival performance of Mrigal *Cirrhinus mrigala* fingerlings in high temperature at laboratory condition.
- Paul, S. K. (2021). Growth Performance, feed conversion and survival of *Cirrhinus Mrigala* with feeding rate in cage system.
- Rani, S. (2017). Effect of alternative protein source soybean meal and sesame oil cake on growth of C.Mrigala.
- Shakhya, S. R. (2017). Effect of Herbs and Herbal products feed supplements on growth in fishes. 1-6.
- Singh, S. (2001). *Fish seed production and nursery management practices of carp*. Ukhrul: SMS (Fisheries).
- Tacon, & Cowey. (1985). *Protein and Amino Acid Requirements*.
- Tuladhar, B. (2003). Comparative Study of fish yields with plant protein source and fish meal.

Umer, & Ali. (2014). Effects of various concentration of protein, fat and carbohydrate in diet on growth and body composition of *Labeo Rohita*.

Vhanalakar, S. A. (2008). Partial replacement of fish meal by locally available plant protein for the indian carp *C.Mrigala*.

Wikipedia-C.mrigala. (2022, July 26). Retrieved from Wikipedia: [https://en.wikipedia.org/wiki/Mrigal_carp#:~:text=The%20mrigal%20carp%20\(Cirrhinus%20cirrhosus,its%20IUCN%20rating%20as%20vulnerable.](https://en.wikipedia.org/wiki/Mrigal_carp#:~:text=The%20mrigal%20carp%20(Cirrhinus%20cirrhosus,its%20IUCN%20rating%20as%20vulnerable.)

Zafar, M., & Ahmed, I. (2008). Studies on Partial Replacement of Fish Meal with Oilseeds Meal in the Diet of Major Carps.

PHOTOPLATE



Photo 1: Feed Preparation



Photo 2: Preparation of 12 treatment ponds



Photo 3: Selection of fingerlings

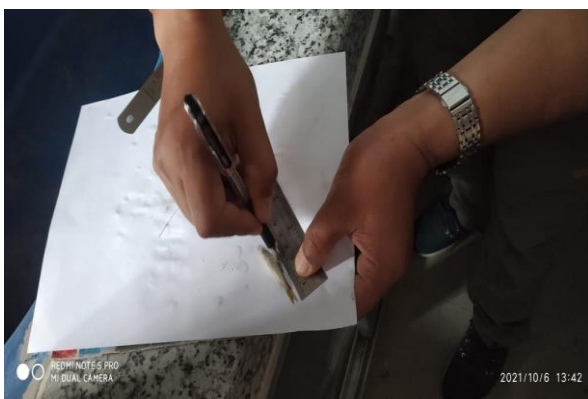


Photo 4: Measurement of length and weight



Photo 5: Introduction of fingerlings in the treatment ponds



Photo 6: Feeding the fish in the pond



Photo 7: Measuring of water parameters of the ponds by using Multiparameter meter



Photo 8: Algal bloom in the pond



Photo 9: Cleaning the pond



Photo 10: Study of fishes that died due to various reasons



Photo 11: Inlet and Outlet of the experimental pond

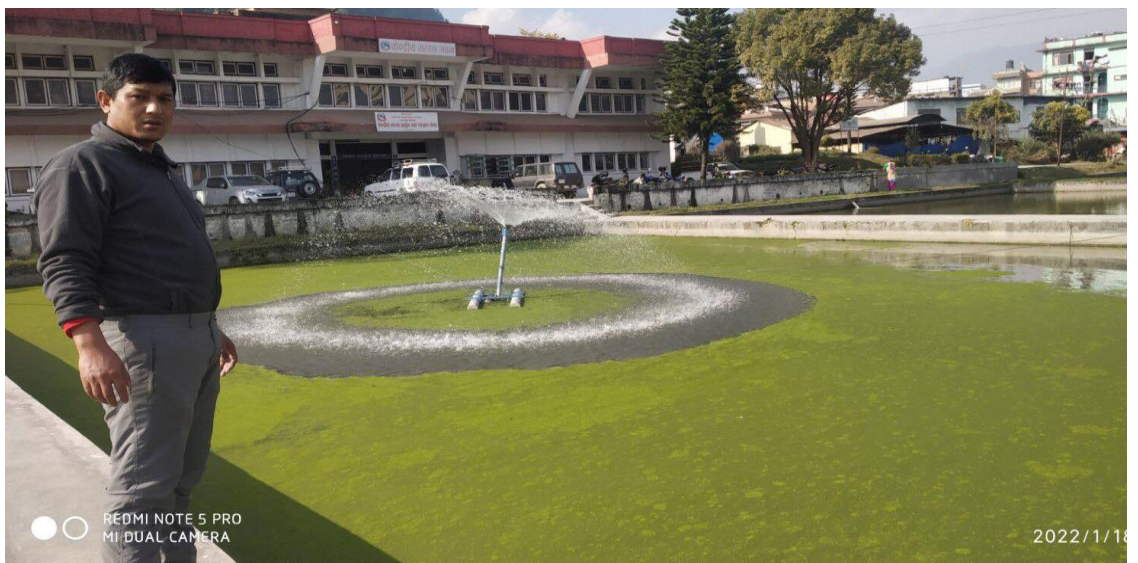


Photo 12: Aeration of the pond



Photo 13: Harvesting of the fishes



Photo 14: Measurement of length and weight after harvesting