# EFFECTS OF DIETARY SUPPLEMENTATION OF *Sargassum vulgare* ON GROWTH AND HEMATOLOGICAL PARAMETERS OF ORNAMENTAL FISH KOI CARP (*Cyprinus rubrofuscus*)


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**BY**


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# *Certificate*

This is to certify that thesis entitled, **"EFFECTS OF DIETARY SUPPLEMENTATION OF *Sargassum vulgare* ON GROWTH AND HEMATOLOGICAL PARAMETERS OF ORNAMENTAL FISH KOI CARP (*Cyprinus rubrofuscus*)"** submitted to the Faculty of Agriculture Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (MS) IN BIOTECHNOLOGY**, embodies the result of a piece of bona fide research work carried out by **MOHAMMAD IQBAL HOSSAN,** Registration No. **19-10362** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged & style of the thesis have been approved and recommended for submission.

**Date: December,2021**
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***Dedicated To***

***My Beloved Parents***

***Who Always***
***Inspired Me To***
***Continue My Study***

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***Dated: December, 2021*** ***The Author***

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# EFFECTS OF DIETARY SUPPLEMENTATION OF *Sargassum vulgare* ON GROWTH AND HEMATOLOGICAL PARAMETERS OF ORNAMENTAL FISH KOI CARP (*Cyprinus rubrofuscus*)


## ABSTRACT

A research was conducted to assess different formulated diets supplemented with different doses of seaweed (*Sargassum vulgare*) on ornamental fish Koi carp (*Cyprinus rubrofuscus*) in the aquaculture laboratory of Department of Aquaculture, Sher-e-Bangla Agricultural University to evaluate the growth and hematological parameters. Total four treatments were applied including Treatment- 1 (Control, 0% seaweed), Treatment-2 (5% seaweed), Treatment-3 (10% seaweed) and Treatment-4 (15% seaweed) each with three replicates. Four iso-proteinase feed was formulated where average percentage of protein, lipid, fiber and carbohydrate in formulated diet were 27.59%, 6.1%, 7.59 and 48.96%. The initial mean weight of fishes was 14.4±2.12, 14.5±0.75, 13.1±2.4 and 12.8±1.43 g in T-1, T2, T-3 and T4 respectively. The experiment was concluded in 100L glass aquarium with mechanized water filter system for each for 8 weeks and weekly sampling were done. Feed was applied twice a day. Temperature, pH, TDS, $NH_3$, $NO_2^-$ and $NO_3^-$ data were collected daily during the experimental period and there was no significant difference in water parameters among treatments. Mean weight (g) of fish, length (cm) and width (cm) data were collected weekly basis. The mean weight gain was found 7.13±1.84, 8.06±1.58, 6.98±1.84 and 4.72±0.52 in T-1, T-2, T-3 and T-4 respectively. The highest weight gain was found in T-2 (5% seaweed) and the percentage of weight gain 55.86% found in the same treatment. There was no mortality found in T-2 and T-4. Survival rate were found 96.6% in Control. The lowest FCR 1.43±0.26 found in T-2 and SGR also found highest in the same treatment. Hematological parameter showed that blood hemoglobin and glucose level was higher in T-2 than others treatments which were 11.6±0.17gm/dl and 6.33±0.26 mmol/l. Serum protein was found 6gm/dl in T-2 and lowest value was found in control. Spleen Somatic Index (SSI) percentage of experimental fishes was also found higher in T-2 and lowest one was recorded in T-1. Refractive index (RI) of blood serum was found better in T-2 fishes compared to other treatments. It can be concluded that feed supplemented with 5% seaweed (T-2) diet showed better growth performance and hematological parameters compared to other treatments. Feed supplemented with brown seaweed *Sargassum vulgare* as a dietary ingredient can be used for better immunity and growth performance of ornamental fish.

# TABLE OF CONTENTS

# TABLE OF CONTENTS (Contd.)

# TABLE OF CONTENTS (Contd.)

# LIST OF FIGURES

# LIST OF FIGURES (Cond.)

# LIST OF TABLES

## LIST OF PLATES



## LIST OF APPENDICES

# LIST OF ABBREVIATIONS AND ACRONYMS

| Abbreviations | Elaborations |
|---|---|
| **%** | Percentage |
| **&** | And |
| < / > | Less than / Greater than |
| ± | Two Possible Value/ Range |
| **$^0$C** | Degree Celsius |
| **ADG** | Average Daily Weight Gain |
| **cm, cm$^{-1}$** | Centimeter, Per centimeter |
| **DM** | Dry Matter |
| **DO** | Dissolve Oxygen |
| **EDTA** | Ethylene Diamine Tetra Acetic acid |
| ***et al.*** | And others |
| **etc.** | And other similar things |
| **FCR** | Feed Conversion Ratio |
| **G** | Gram |
| **$H_2SO_4$** | Hydrogen sulphate |
| **$KMNO_4$** | Potassium per Manganate |
| **MFW** | Mean final weight |
| **MIW** | Mean initial weight |
| **MWG** | Mean weight gain |
| **mg/l** | Milligram per liter |
| **ml, ml/l** | Milliliter, Milliliter per liter |
| **mmol/l** | Millimole per liter |
| **$NH_3$** | Ammonia |
| **$NO_2^-$** | Nitrite |
| **$NO_3^-$** | Nitrate |
| **pH** | Puissance of Hydrogen |
| **SD** | Standard Deviation |
| **SSI** | Spleen Somatic Index |
| **T** | Temperature |
| **TDS** | Total Dissolved Solid |
| **WG** | Weight Gain |
| **ppm** | Parts per million |

# CHAPTER I
# INTRODUCTION

Fish play a pivotal role in the Bangladeshi diet, providing more than 60% of animal origin food, representing a very important source of micro-nutrients, and possessing an extremely strong cultural attachment. Fish is the second most precious agricultural crop, and its manufacturing contributes to the livelihoods and employment of millions. Bangladesh has extensive and highly diversified fisheries resources. Department of Fisheries (DOF) statistics calculated total fish production of 2.56 million tones, of which aquaculture accounts for 39% (DOF, 2010)

Development of aquaculture has been assisted by growth of input businesses and suppliers, in specific those attached to manufacture of seed and feed. Rapid development of private sector hatcheries and nurseries has followed initial investments in the public sector and has been perhaps the single most decisive factor in the expansion of aquaculture in Bangladesh. At least 98% of seed supplies now derived from private hatcheries. 'Raw' unformulated feeds are widely used in homestead and commercial carp aquaculture (DOF, 2009).

Ornamental fish are those fish which classified as aquatic organisms that are reared as pets in a glass aquarium. They are both from freshwater and marine environments; however, approximately 90% of the species from freshwater are reproduced in confinement while those from marine waters are mostly cached (FAO, 2009). Ornamental fish rearing is very normal throughout the world and is some time observed in many private homes and gardens of the modern countries in the world. About ten percent peoples in the world retain the aquarium in their homes. Among the thousands of fish, only a few hundred species are now being reared as pet fish by a large number of fish hobbyists worldwide. Ornamental fish rearing and their propagation have been an attractive activity for many people, which gives not only satisfaction but also financial profits. It has been calculated that over 1.5 million people are attached in this sector, and over 3.5 million hobbyists add up to trade in the world (Pandey *et al*., 2017).

Koi Carp, also called as Nishikigoi, are very well-known ornamental fish which are sometime reared in a pond or aquarium. They are tinted types of the Chinese Amur

Carp (*Cyprinus rubrofuscus*), and are reared as pets with fascinating decoration. Koi is a normal group name for the color variation of the carp, of which there are many types (some are like pedigree dogs). There are many types of ornamental koi, derived from reproducing that started in the early 19th century in Niigata, Japan (Kock *et al*., 2015). Many varieties are identified by the Japanese, differentiated by coloration, featuring, and scalation. Some of the prime colors are white, black, red, orange, yellow, blue, brown and cream, besides metallic shades look like as gold and silver-white ('platinum') scales. The ancestry of the Koi Carp dates back to 200BC, where the Amur Carp was escorted to Japan as an origin of food at the time of Chinese invasions. With other animals, the Amur Carp encounter a level of natural genetic dissimilarity arising in skin color mutations. The Japanese specifically reproduced fascinating color variants to magnify the colors and features leading to the Koi Carp we know today. This ensued in the Amur Carp creating its way off the food menu and into the extremely sought-after pet trade, with reproducing as we now know it started after the 1800's. These were mostly unrecognized and established exclusively in native Japanese culture until they were revealed at an exhibition held in Tokyo in 1914. From there on, the attractiveness in Koi Carp expended all over Japan and in the end worldwide. Since then, specific reproduction of Koi Carp has taken off globally with many countries utilizing specific Japanese brood stock as parent fish. Countries known for reproducing Koi Carp outside of Japan include Israel, USA, Poland, UK, Malaysia and many more. As a rule of thumb, Japanese fish some time appeared with the largest price tag. Advanced breeders have been experimental for many decades and competitiveness is fierce, given the Koi has turn out to be an iconic ideogram of Japan. With that said some of the forenamed countries are now also reproducing outstanding Koi Carp (Airinkai, 2017)

Ornamental fish feed is normally created to please the aquarium hobby of consumers as color and feature acceptance, feed price and least environmental pollution. Nurturing of spawn, fry and fingerlings or aquarium fish till they reach satisfactory size and their subsequent culture in rear in aquarium or ponds need proper and nutritionally stabilized diet for increasing manufacturing. This has been one of the crucial necessities in the enlargement of aquaculture. Feed formulation is done for utilization essential nutrition. A number of terms and features are established that will be put to specific use as information is dispensed on the nature and standard of different feedstuffs and the information presented on the nutrient requirements of fish. Specific understanding of these terms is important to their correct use. One must know that some of these terms have a built-in error that cannot be avoided. This does not remove their functionality in feed formulation. However, one must value the fact that some are convenient approximations of the values and not true values (Hardy, 1978).

The feed supplements are the crucial element of the fish diet. The application of feed supplements in the animal's diet is a major role to increasing development and immunity of the aquatic animals. Feed supplements are elements which are used in trace amounts in fish diet it is also perform as ingredients developer or preserve it. The vital feed supplements are performed as preservatives, binders, feeding stimulants, and food colorants etc. In favor of developing aquaculture in a suitable fashion, the use of supplements must be in correct amount. The requirement of supplements is depending on species and their accessibility. Some authors revealed that the feed supplement to have antimicrobial, anti-oxidative, growth enhancing potentiality as well as it is upgrade the fish immune system (Yada *et al.*, 2021).

Food supplements created from seaweeds are mainly polysaccharides, long chained molecule that is separated from brown and red algae. Their potentiality lies mostly in their capability to emulsify, stabilize and thicken, but that they derive from a natural origin is a crucial benefit. Human do not seem to have the capability to crack them down, so they can be very convenient in low-fat foods. Some coralline algae are now applied as natural sources of calcium and magnesium sulphate. Seaweeds are colonized aquatic habitats used mainly by coastal populations. Many seaweed species are generally utilized in unrefined form, in medicine, human diets, animal feeds and for developments in agricultural soil as fertilizers (Jamal *et al.*, 2017). They are plentiful in potassium, sodium, calcium, magnesium and phosphorus and are a origin of crucial trace elements, such as iron, manganese, copper, zinc, cobalt, selenium and iodine. A bit of known about their bioavailability in nutrients. Seaweeds are uncomplicated organisms, which are able to take dominance of sunlight to transform carbon dioxide into sugars and oxygen, in the time of the photosynthesis process. The most familiar varieties of consumable algae include: *Neopyropia/ Porphyra/Pyropia spp.*, *Undaria pinnatifida*, *Saccharina latissima*, *Palmaria palmata* and *Chondrus* *crispus*, varieties that are related with many health advantages, such as reducing blood pressure, intercepting spills and they are a valuable protein source (Probst *et al*., 2016).

Seaweed biomass is a valuable similar ingredient for livestock. Macro-algae, in normal, show significant differences in proteins, minerals, lipids and fibers. The high mineral quantity of seaweeds is due to their capability to soak up inorganic elements from the nature; they hold a small quantity of lipids, mainly polyunsaturated fatty acids (PUFAs), although they are abundant in polysaccharides. Seaweed has only a little percentage of lipids (1–5%), but the most of those are PUFAs (polyunsaturated fatty acid). Predominantly, brown and red seaweed carry more PUFAs 20:5 n-3 (EPA: Eicosapentaenoic acid) and 20:4 n-5 (arachidonic acid) than green algae (Misurcova et al., 2011). Seaweed has a extremely changeable composition, which depends on the species, time of collection, habitat and on exterior circumstances such as water, temperature, light intensity and nutrient concentration in water (Dawczynski *et al.*, 2007). Algae carry extreme-levels of non-protein nitrogen, such as free nitrates, ensuring in nitrogen-to-protein conversion factors of 5.38, 4.92 and 5.13 for brown, red and green seaweed, respectively (Mišurcová *et al*., 2011). There are numerous consumable seaweeds for human utilization with high protein content, with changeable essential amino acids. They also soak up minerals from seawater and accommodate 10 to 20 times more than the land plants (Gaillard *et al*., 2018). In normal, it is obtained that green and red algae have higher nutritional merit than brown algae due to low protein and high mineral percentages. However, brown algae have a higher and diversified content on bioactive molecules with extreme economical interest. Therefore, algae can produce energy, minerals and proteins to animal feed and have prospective as substitute protein source for ruminants (Angell *et al.*, 2016). Among the marine organisms, seaweed constitutes one of the richest origins of natural antioxidants and antimicrobials. They are also an outstanding source of vitamins such as A, $B_1$, $B_{12}$, C, D and E, riboflavin, niacin, pantothenic acid, folic acid as well as minerals such as Ca, P, Na, K and I (Azenha *et al*., 2019).

Basically seaweeds, any of the red, green or brown marine algae that reproduce along seashores. Seaweeds are normally anchored to the sea bottom or other rigid structures by root like ‘holdfast’ which execute the sole function of bonding and do not release nutrients as do the roots of higher plants. A number of seaweed species are safe to eat, and many are also of commercial significant to humans. Some are utilized as fertilizers or as origin of polysaccharides. The variants of seaweed growing near the high-water mark, where plants are sometimes revealed to air, differ from those growing at bottom levels, where there is tiny or no exposure. In this study we are using *Sargassum vulgare*, a brown seaweed, lithophytic on coral reefs, rocks and stones with a main axis to about 40 cm long, floating upper side by means of gas bladders; at sheltered or revealed places of all tropical and subtropical shores from high tide level to sub-littoral (Rodriguez, 2022).

Small fish farmers, fish rearers, and laboratory workers are therefore left with the choice of purchasing huge amount of expensive feed, which sometime goes to waste. Small amount of fish feed can be formulated very effortlessly in the laboratory, classroom, or at home, with usual elements and simple kitchen or laboratory tools. In this research I used formulated fish diet supplemented with different percent of seaweed for better result.

**Objective**

The overall objective of the study to find out some vital information about the feed formulated with seaweed (*Sargassum vulgare*) on growth and hematological parameters of Koi carp (*Cyprinus rubrofuscus*) in laboratory condition. The specific objectives of the research were:

i. To formulate and analyze the nutritional composition of diets supplemented with seaweed (*Sargassum vulgare*)
ii. To assess the growth performance of Koi carp (*Cyprinus rubrofuscus*) fed with diets supplemented with seaweed.
iii. To assess the hematological parameters of Koi carp fed diets supplemented with different doses of seaweed.

# CHAPTER II
# REVIEW OF LITERATURE

Raising price of commercially available and poor quality of ornamental fish feed turning fish hobbyists to follow the alternative way to continue the fish rearing. Formulated fish feed can be obtained by using different useful ingredients to form an nutritionally valuable feed for proper growing performance of fish. Besides formulated fish feeds show better performance on fish comparing with commercial feeds. For this reason, researchers and scientists give more concentrations in this field recently. Now a days more research activity is going on to find useful ingredients to make more effective feeds for proper growth and for increasing nutritive value in fish.

Nguyen *et al.* (2014) formulated a feed for Koi carp (*Cyrinus carpio*) juveniles and it had the positive responses in growth performance and pigmentation of fish skin with the formulated feed containing 80 mg/kg dietary astaxanthin, 36.02% protein, 7.78% lipid, 4.20 kcal/g GE (gross energy). Particularly, fish fed formulated feed developed with replacement of 60% dietary fish meal by SM, PBM and corn showed better growth performance and feed utilization.

Hasan *et al.* (1985) evaluated the effects of supplementary diet on the growth performance of Nile tilapia (*O. nilotica*). Four supplementary feeds using combination of rice bran, wheat bean mustard oil cake (moc) and fish meal in different proportions were tested. Fishes were stocked at the rate of 15 $m^3$ in all the floating ponds. Fishes were found to survive and grow well in floating ponds having no mortality. The gain in length exhibited by different supplementary feed containing 30% rich bran + 30% wheat bran + 40% fishmeal was significantly ($p<0.05$) superior to rich bran 25% + wheat bran 25%+ moc 25% + moc 25% + fish meal 25% and rice bran 30% + wheat bran 30% + fish meal 25%.

Islam *et al*. (1981) have studied on the growth performance of *Catla catla* fingerlings in cash indigenous ingredients. Cage culture of *Catla catla* was performed with a different artificial feed. The feed contains rich bran, mustard oil cake powder and multivitamin. A direct relationship was observed between growth rate of fishes of fishes and the amount of crude protein and total lipid present in the feeds.

Ahmed *et al*. (2013) carried out a field experiment which was conducted to evaluate the growth performance of monosex tilapia using homemade feed with Peninsula Group fish meal and commercially available feed with local fish meal in earthen mini ponds. Three ponds were supplied with prepared feed and the other three ponds with commercially available fish feed. The Specific Growth Rate (SGR) was recorded 3.09 and 2.97 and the Food Conversion Ratio (FCR) was 1.51 and 1.40 respectively. There was significant ($p<0.05$) variation among the survival rate (%) of fishes which were 75.55 and 90.37% in $T_1$ and $T_2$, respectively. The prepared feed showed better performance with monosex tilapia in compared with commercial fish feed with local fish meal.

James *et al.* (2003) stated that protein requirements varied from around 30% dietary protein for growing omnivorous goldfish (*Carassius auratus*) to 50% for the carnivorous discus (*Symphysodon aequifasciata*). Whereas mineral (phosphorus, iron, magnesium, zinc) requirements have received some attention in the guppy (*Poecilia reticulata*).

Bandyopadhyay *et al.* (2004) revealed that during the growth phase of goldfish (*Carassius auratus*), formulated feed 42.53% animal protein (fishmeal) exhibited better daily net weight gain, weight gain percentage and specific growth rate compared to other formulated feeds which has less protein percentages.

Rehana *et al*. (2015) evaluated the formulation of a commercial feed prepared by Quality Fish Feeds from indigenous raw materials and their effects on growth and maturity of *Cirrhinus mrigala*. An experiment was conducted for 90 days in attached water vessels to evaluate the effect of three dietary protein levels on the growth and body composition of *Cirrhinus mrigala*. Three different iso-caloric feeds which contain approximately 30%, 35% and 40% dietary protein levels were prepared from internal feed ingredients and were fed to *Cirrhinus mrigala* at the rate of 8% of total body weight. The ingredients used for the formulation of feed are fish meal, mustard oil cake, rice bran, starch, vitamin pre mixes. Statistical analysis showed that the feed at a level of 40% protein was significantly different from other feeds and most effective in changing the growth and maturity of *Cirrhinus mrigala*.

Ismail (2019) reviewed summarily describing the significance of microalgae “seaweed” as renewable origin of fish feed. Fish aquaculture prices have including investment and operational prices including feed, energy, labor, fuel, oxygen, water, and medicament costs. The feed cost considering about 40-60% from fish production. Hence, there is a need to lessen the cost of feed with rising the growth performance and increased survival rate of fish. Seaweed is a shield for different ages of marine organisms to encourage growth and feed effectiveness. Moreover, they function as possible option of protein sources for cultured fish because of their high protein concentration and productivity. They have been called as a “super feed”, which has remarkable positive outcome on fish performance due to their nutritive and biological values.

Sameh *et al*. (2016) evaluates the efficiency of macro-algal extracts as antibacterial agent against multidrug-resistant (MDR) bacteria isolated from Nile tilapia (*Oreochromis niloticus*) as well as to enhance the fish growth performance by macro-algae diet application. They used extract of the brown macro-algae *Sargassum vulgare* as an antibacterial source and to enhance the growth performance of conducted feeding experiments on Nile tilapia.

Sometimes fishes directly intake seaweeds. Kiriyai *et al.* (2005) conducted feeding an experiment on *Sargassum fusiforme* in outdoor tanks with six herbivorous fish species that occur in the study area. Three species, *Siganus fuscescens*, *Kyphosus bigibbus*, and *Calotomus japonicus* fed on *Sargassum fusiforme* in notably and left featured chew marks on the thalli: chew marks are regular and smaller jagged scars by *S. fuscescens*, moderately irregular and wider jagged scars by *K. bigibbus*, and inconsistent rugged scars by *C. japonicus*. Of these 3 species, *S. fuscescens* intake 15 to 20 times more on *S. fusifoyme* than the two other species and showed feeding selectivity. On *S. fusifoyme* gatherd from the area where insufficient development typically occurs, 39 to 67% of the specimens featured bite marks made by *Sargassum fuscescens.*

Pratiwy *et al.* (2018) demonstrated that whole thalli of *Sargassum* from an effective supplement for Nile tilapia at least at 4% without negative effects and 8% level inclusion of *Sargassum* gave the best results for growth performance. They used three different 32% iso-proteinous diets which were prepared by replacing rice bran with inclusion of SM of 0% (T1), 4% (T2), and 8% (T3) and fed to replicate trials of individual males, individual females, and mixed sex tilapia. The results showed that the highest values of feed efficiency (FE) and specific growth rate (SGR) in each rearing condition were observed in T3. In addition, the results showed that the inclusion of could produce a different effect on the growth pattern between male and female Nile tilapia.

Costa *et al.* (2013) used Brown Seaweed (*Ascophyllum nodosum*) Meal (BSM) in the feed given to Nile tilapia *Oreochromis niloticus* fingerlings. The completely randomized design experiment lasted 42 days. Nile tilapia fingerlings (n=75) with an average age of 30 days and average weight of 0.43 ± 0.02 g were assigned to 25 plastic tanks and submitted to five treatments in five repetitions. The treatments were increasing levels of BSM (5, 10, 15, and 20 g $kg^{-1}$ of feed) and a control feed (no BSM) and the addition of BSM at the level of 20 g $kg^{-1}$ feed does not result in growth loss and improves the feed conversion ratio and carcass yield in Nile tilapia during the fingerling period.

Rahim (2016) used brown algae (*Sargassum angustifolium*) and red algae *(Laurencia snyderia*) evaluate the effectiveness of marine algae on growth and coloration of ornamental fish, 210 fish (6.5±0.65g) were randomly divided into seven groups (each group contains 30 pieces of fish) and were conserved and fed for 60 days for studying. Control group received only the commercial diet (Biomar) wet with olive oil, other groups, were fed with ethanolic extract of *sargassum* and *laurensia* containing 5, 10 and 15 g/kg dry matter for two months. Growth factors (specific growth rate, feed conversion ratio, protein efficiency rate and condition factor) measured on days 0, 30 and 60 respectively. The results showed that the groups fed with algae, did not show significant changes in growth factors. In order to estimate colors in fish in different groups. In the groups that fed *sargassum*, only the rate of 15 g/kg diet caused a significant increase the amount of pigment in the skin.

Nisha *et al*. (2014) used fingerling of *Carassius auratus* 2.34 g after acclimated to laboratory condition for a period of 15 days were used in various studies. Five types of feed were produced by using algae meal and these feeds contain 35% protein. The fish were divided in to 5 groups. Group I was fed on *Ulva reticulata* free diet. Fish belonging to groups II, III, IV and V were fed on diets with 2, 4, 6 and 8% of *Ulva reticulata* supplementation respectively for 40 days. At the end of the experimental period growth, proximate composition, hematological parameters, carotenoid content were analyzed and suggests that *Ulva reticulata* can be included as an ingredient of gold fish diet up to 8%.

Yangthong *et al*. (2020) observed the growth performance of juvenile spotted scat when fed diets supplemented with *Ulva sp*. Seven iso-nitrogenous and iso-caloric diets with different levels of supplementation with *Ulva sp*. at 0 (control), 5, 10, 15, 20, 25 and 30% were fed to 420 fish, weighing 0.49 ± 0.11 g. The diets were fed to triplicate groups of fish twice a day to apparent satiation for 10 weeks. The results showed that fish fed the diets with 5 and 10% seaweed demonstrated the highest mean WG and SGR ($P < .05$), whereas there were no significant differences in the FCR and PER of fish fed the control diet, and 5 and 10% seaweed ($P > .05$). Fish fed the control diet and that containing 5% *Ulva* sp. showed a lower rate of FI than fish fed diets containing 10–30% ($P < .05$). Moreover, dietary supplementation with seaweed at levels of 5–25% had no significant effect ($P > .05$) FCE although it was higher for those diets than for the fish fed the control diet and that supplemented with 30% *Ulva sp*. ($P < .05$).

Nader *et al*. (2010) showed the effect of green seaweeds (*Ulva sp*.) as feed supplements in fish diet on growth performance, feed efficiency and body composition of red tilapia (*Oreochromis sp*.) 1.15 g initial body weight. Six isocaloric diets (236 kcal metabolizable energy /100g diet) containing 26% crude protein, with different levels of green seaweed *Ulva sp*. (0, 5, 10, 15, 20 and 25% of fish diet) were used. Each diet was used to feed triplicate groups of fish two times a day to apparent satiation for 9 weeks. Obtained results showed that seaweeds (*Ulva sp*.) can supplemented to red tilapia (*Oreochromis sp*.) diet at optimum level of 15% to improve growth performance without any adverse effect on feed efficiency or survival rate.

According to Arguelles *et al.* (2019) *Sargassum vulgare* collected from Batangas, Philippines coast represents a potential source of polyphenols and other bioactive compounds for the production of natural antibiotics and antioxidants. Proximate composition of the dried macro-algae showed that *S. vulgare* contains high carbohydrate, ash and crude fiber content of 34.18±0.32%, 27.09±0.00%, and 22.59±0.21 respectively.

Sudharaka *et al.* (2021) measured the effect of seaweed supplemented diets on the growth of Koi carp. The experimental set up consisted of a control (C) and two experimental treatments (T1 and T2). The control diet (without seaweed) and the two test diets, of which 30% of the weight of control diet was replaced by *Ulva lactuca* and *Sargassum cinereum* were fed to Koi carps in C, T1 and T2 respectively. Proximate analyses were carried out for both ingredients, and the diets were prepared following standard methods. Koi carp in fry stage belonging to the same cohort (weight 2.20±0.08 g, length 3.89±0.04 cm) were assigned to C, T1 and T2 in fiber tanks filled with 150L of water (n=20 per tank) with four replicates each. Fish performance was compared using Body Weight Gain (BWG), Daily Growth Rate (DGR), Relative Growth Rate (RGR), Feed Conversion Ratio (FCR), Feed Efficiency (FE), Fulton's Condition Factor and survival rates. At the end 14th week of the experiment, mean values of the weight, length, BWG, DGR showed a significant difference (p0.05) among treatments. There was no mortality in any groups. They also showed that e *Sargassum* supplemented diet is more beneficial than the *Ulva sp.* supplemented diet for juvenile Koi carp. The results showed the potential use of seaweeds as a dietary supplement or in developing low-cost diets in aquaculture programs.

Nur *et al. (*2020) Formulated fish fid with different percentages of seaweed and showed that seaweed has positive impact on growth and immune system of fish.

Ilham *et al.* (2018) injected *Sargassum sp.* in the body of goldfish and it does not have a significant effect on the number of red blood cells, hemoglobin and differential leukocytes (monocytes and neutrophils), but has a significant effect on leukocyte count, lymphocyte cell count and goldfish survival rate (SR). The highest leukocyte count was in treatment D with a concentration of 15% extract of *Sargassum* sp. with the results of 67.0833 x103 cells / $mm^3$ and treatment A (control) had the lowest yield of 28.350 x103 cells / $mm^3$. The highest lymphocyte cells were treatment D, which was 85.00% and the lowest lymphocyte treatment was treatment A, 78.67%. The lowest survival rate is treatment A (control) 26.67% and significantly different from treatment B concentration of 9% with a result of 80%, while the highest SR results are treatment C concentration of 12% and D concentration of 15% is 100%. Treatment A (control) experienced the most clinical symptoms due to infection from the bacterium *Pseudomonas fluorescens* compared to treatments B, C and D.

Kulkarni (2021) shows the presence of protein, albumin and globulin in the blood serum of fishes such as *Labeo rohita* and *Pangasius bocourti* shows that fishes are healthy and active as they are getting proper nutritional requirement and other organic substances because of proper internal blood transportation physiology by albumin and globulin presence in the blood. He found 5.2 mg/dl and 4.6 gm/dl serum protein in *Labio rohita* and *Pangasius bocourti* in his study.

Thépot *et al.* (2021) experimented the out-come of applying eleven seaweed species and four established fish immunostimulants on the innate immune feedback (cellular and humeral immunity) of the rabbit fish (*Siganus fuscescens*). All supplements added with different seaweeds from the three groups (Chlorophyta, Phaeophyta and Rhodophyta) were included in the fish pellet at 3% (by weight) and had comparably positive effects through the four innate immune parameters they recorded compared to control fish. Diets supplemented with the red seaweed (*Asparagopsis taxiformis*) and the brown seaweed (Dictyota intermedia) showed the best boosts in humeral and cellular innate immune defenses, as well as particular significant increases in hemolytic activities. Diets supplemented with *Ulva fasciata* also showed promising positive outcome on the fish innate immune responses.

Thépot *et al.* (2021) explored different formations of seaweed (whole, methanolic extract and residue of extraction) and dietary insertion levels (1.5%, 3% and 6% of feed) on the growth rate, feed efficiencies and intestinal micro biome of juvenile fish in a 4-week feeding test. The second feeding test evaluate the long-term use (3 months) of *A. taxiformis* supplements on reproduction in *S. fuscescens*, by measuring the proportion of adult fish with sexual organs, their gonad somatic index and their innate immune reaction. The first trial showed the growth development of dietary *A. taxiformis* with up to 49% increase in specific growth rate, and up to 31% reduction in feed conversion ratio, for fish receiving the highest extract inclusion compared with those fed the control diet. Additionally, the hindgut microbiome of the fish fed the seaweed diets had lower abundances of potentially pathogenic *Tenacibaculum* sp. compared with the control fish after 2 weeks. In the second feeding trial, the fish fed the 3% whole seaweed diet had a higher proportion of adult fish and improved innate immune response compared with the control fish but there were no significant effects of seaweed on gonadosomatic index. Overall, the red seaweed *A. taxiformis* shows potential as a novel functional ingredient for farmed rabbitfish to promote their growth, health and sexual maturation.

Nazarudin *et al.* (2020) applied *Sargassum polycystum* as supplement in diets for Asia sea bass fingerlings. Those experimental dietary formulations were supplemented with four graded levels of smashed seaweed (0.0, 1.5, 3.0 and 4.5 %) and fed to sea bass fingerlings (mean initial weight 2.2 g) for almost 55 days. Fish were developed for feed efficiency, growth performance and mortality. The results recorded that survival, feed consumption and efficiency, and growth performance were superior in fish fed the 1.5 and 3.0 % seaweed supplemented feed then the control. Carcass protein and Fe contents, red blood cell (RBC) and white blood cell (WBC) counts were also superior level in the fish fed the *Sargassum* supplemented diets, which were noticeably ($p < 0.05$) higher in the 1.5 and 3.0 % treatments than in the control. Abundance of pure colonies of descendant Gram positive, none-spore forming, cocci and rod-shaped, catalase and oxidase negative bacteria was recorded in the intestines of fish reared with the *sargassum* supplemented feed.

He *et al.* (2021) researched on morphology of the spleen in *Oreochromis niloticus* and stated that spleen weight of *Oreochromis niloticus* varies from .07- 0.25 gm and spleen somatic index varies from 0.03-0.14 depending on fish size, weight and age.

Coeurdacier *et al*. (2011) cultured seabass for 90 days and found 43-50 mg/ml (1mg/ml= 0.1 gm/dl) serum protein in different experimental samples.

Mohammad *et al.* (2022) formulated fish feed applying seaweed (*Hypnea sp.*) that can upgrade the immunity of Nile Tilapia, *Oreochromis niloticus*. Seaweed was supplemented in different concentration with commercial feed to change their effects on their serum bactericidal activities. A 90-days experiment was conducted under five treatments – T1 (5% seaweed), T2 (10% seaweed), T3 (15% seaweed), T4 (20% seaweed) and C (only commercial feed) with a replica for each. Fish blood was collected, serum was separated from blood and serum bactericidal activity was tested. Blood serum of 10% seaweed treated fish showed the highest sensitivity against three bacteria (*Bacillus cereus*, *Salmonella typhimurium*, *Shigella flexneri*) followed by 5% seaweed which showed sensitivity against Bacillus cereus and slightly against *Salmonella typhimurium* and *Shigella flexneri* and 15% seaweed showed slight bactericidal activity against Bacillus cereus only, whereas, no bactericidal activity was observed for control and 20% seaweed treated fishes.

Mazarevica *et al*. (2020) a qualitative relationship that the value of refractive index of red blood cells significantly decreases for the increase of sugar in animal's body compared to a healthy animal.

L. E. Moor *et al.* (2000) showed that, higher RI value indicates good nutritional value in prawn and shrimp. He also showed that blood RI with higher value indicates good immune and health.

# CHAPTER III

# MATERIALS AND METHODS

This chapter deals with the methods, which was maintained to achieve the goal of the present study. It includes the selection of research tools, materials, experimental design and analytical tools. Selection and collection of the feed ingredients, feed formulation and preparation, water parameters measuring, collection of data for morphological and growth parameters, collection of fish sample after experimental period to determine hematological parameters, analytical method and analysis were done to obtain the objectives.

## 3.1 Study Location

The experiment was performed in the newly established laboratory named "Aquaculture Lab" under Department of Aquaculture, Faculty of Fisheries Aquaculture and Marine Science at Sher-e-Bangla Agricultural University which is located in the central part of the capital of Bangladesh. It is one of the most prominent aquaculture lab in Bangladesh. Different fish species culture activities are continuing round the year for the development of aquaculture tactics and fulfilling the increasing demand of human protein. At present, various types of indigenous and exotic species are being cultured in this lab as different experimental purposes like tilapia (*Oreochromis niloticus*), koi (*Anabas testudineus*), thai shol (*Channa striata*), shing (*Heteropneustes fossilis*). Freshwater ornamental fish Koi Carp (*Cyprinus rubrofuscus*) was selected as culture species for the very first time in Bangladesh for assessing its culture suitability and evaluating its survival, growth and other parameters using seaweed *(Sargassum vulgare)* containing formulated feed.

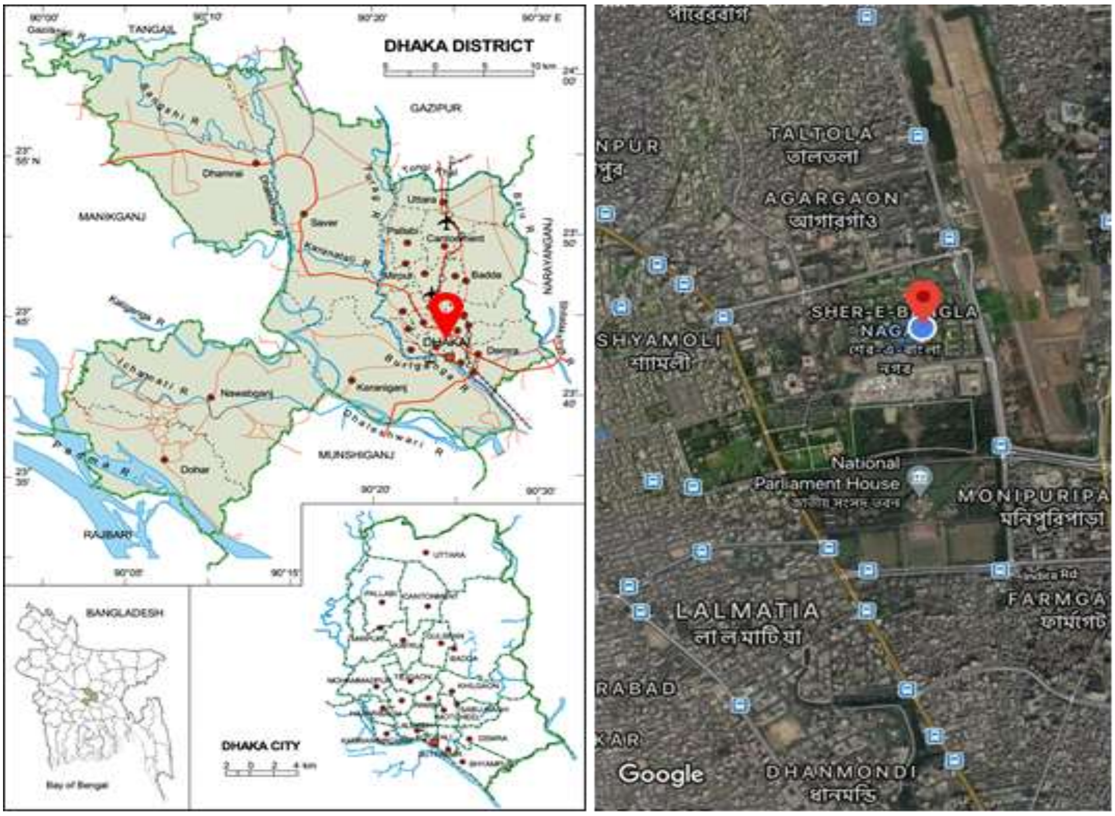


**Figure 01:** Map and Satellite view of the research location.

### 3.2 Duration of Study

The timeframe was set for the study from mid-June to mid-August, 2021 for freshwater Ornamental fish culture in laboratory system according to the researchers and supervisors schedule. Basically, the lab is engaged in different cultures and research works all the year round. The duration was set considering the Koi Carp seed availability and culture opportunity in that lab.

### 3.3 Formulating Dietary feed for Treatment

Different types of feed ingredients were selected such as rice bran, mustard bran, wheat bran, fish meal, meat & bone meal, fish oil, minerals and vitamins premix. Those were purchased from the Chankharpul Fish and Poultry Feed Market, Dhaka. The raw materials of fish meal were collected from the Meghna Ghat, Narayanganj. Seaweed (*Sargassum vulgare*) was collected from the coast of Cox’s Bazar. The feed ingredients were brought to the laboratory of Department of Aquaculture, Faculty of Fisheries Aquaculture and Marine Science at Sher-e-Bangla Agricultural University, Dhaka for further action.

### 3.3.1 Treatment (fish diet)

Four iso-proteinase feed were formulated with different percentages of seaweed (*Sargassum vulgare*). Rice bran percentage was replaced with seaweed powder. The flow diagram of the preparation procedure of formulated fish diet is shown in bellow.

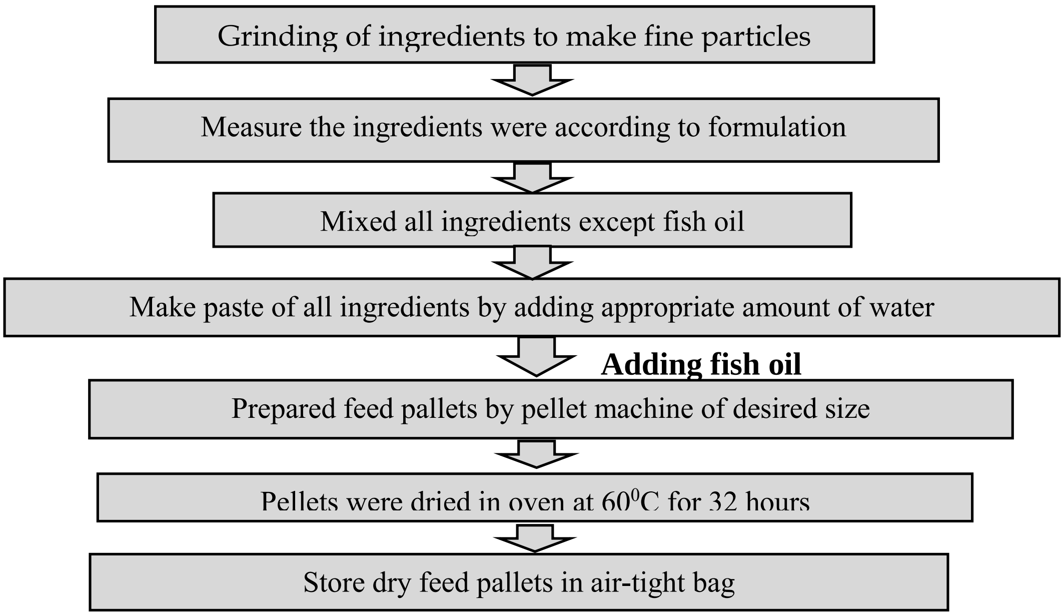


**Figure 2:** Flowchart of protocol for preparation of feed diet

### 3.3.2 Treatment 1: Control (Diet containing 0% seaweed)

Treatment1 was prepared by using the ingredients which were brought from the market (Table1). The selected ingredients were milled and mixed thoroughly. Mixing of ingredients were performed by hand before adding water. Finally, made into pellets using a pellet machine.

The pellets were oven dried then packed in air-tight water impermeable bags and stored in dry and cool place.

**Table 1:** Diet for Treatment 1 (T-1) with different ingredients percentage

| Ingredients | Percentage (%) |
|---|---|
| Wheat bran | 25 |
| Wheat powder | 18 |
| Trash fish (mixed) | 15 |
| Fish meal | 5 |
| Mustered oil cake | 10 |
| *Sargassum Vulgare* | 0 |
| Rice bran | 20 |
| Trace mineral premix | 1 |
| Vitamin premix | 1 |
| Vitamin C | 1 |
| Fish oil | 1 |
| Barley | 3 |
| Total | 100 |

### 3.3.3 Treatment 2: Diet containing 5% seaweed

Treatment 2 was prepared by using 5% of seaweed (Sargassum vulgare) by decreasing rice bran 20% to 15%. The selected ingredients were milled and mixed thoroughly. Mixing of ingredients was performed by hand before adding water. Finally, made into pellets using a pellet machine. The pellets were oven dried then packed in air-tight water impermeable bags and stored in dry and cool place. Due to adding seaweed, the color of diet changed a little bit than the treatment 1 dietary feed.

**Table 2:** Diet 2 for Treatment-2 (T-2) with different ingredients percentage.

| Ingredients | Percentage (%) |
|---|---|
| Wheat bran | 25 |
| Wheat powder | 18 |
| Trash fish (mixed) | 15 |
| Fish meal | 5 |
| Mustered oil cake | 10 |
| *Sargassum Vulgare* | 5 |
| Rice bran | 15 |
| Trace mineral premix | 1 |
| Vitamin premix | 1 |
| Vitamin C | 1 |
| Fish oil | 1 |
| Barley | 3 |
| Total | 100 |

### 3.3.4 Treatment 3: Diet containing 10% seaweed

**Table 3:** Diet 3 for Treatment-30(T-3) with different ingredients percentage.

| Ingredients | Percentage (%) |
|---|---|
| Wheat bran | 25 |
| Wheat powder | 18 |
| Trash fish (mixed) | 15 |
| Fish meal | 5 |
| Mustered oil cake | 10 |
| *Sargassum Vulgare* | 10 |
| Rice bran | 10 |
| Trace mineral premix | 1 |
| Vitamin premix | 1 |
| Vitamin C | 1 |
| Fish oil | 1 |
| Barley | 3 |
| Total | 100 |

### 3.3.5 Treatment 4: Diet containing 15% seaweed

Treatment 4 had the higher value of seaweed which was 15%. The color was also deeper than the other diets and also had lowest percentages of rice bran which was 5%.

**Table 4:** Diet 4 for Treatment-4 (T-4) with different ingredients percentage.

| Ingredients | Percentage (%) |
|---|---|
| Wheat bran | 25 |
| Wheat powder | 18 |
| Trash fish (mixed) | 15 |
| Fish meal | 5 |
| Mustered oil cake | 10 |
| *Sargassum Vulgare* | 15 |
| Rice bran | 5 |
| Trace mineral premix | 1 |
| Vitamin premix | 1 |
| Vitamin C | 1 |
| Fish oil | 1 |
| Barley | 3 |
| Total | 100 |



(a) (b)



(c ) (d)

**Figure 3**: Four iso-proteinas dietary feed with different concentration of seaweed, (a) 0% seaweed, (b) 5% seaweed, (c) 10% seaweed, (d) 15% seaweed.

### 3.4 Proximate Composition of Dietary feed

Protein, Fat (%), Moisture (%), Ash (%), were analyzed for four types of formulated diets.

### 3.4.1 Protein determination

Proteins are complex nitrogenous substance formed by the sub unit of Amino Acid through peptide linkage. Protein occupies a central position in the architecture and functioning of the living matter. All physical and chemical activities in the living cell are catalyzed by the enzymes which are also protein.

The crude protein of the feed was determined by Micro- Kjeldhal method (Pearson, 1999). The basic principle of this method involves the conversion of the nitrogenous protein in to Ammonium Sulphate ($NH_4SO_4$), when boiled with Sulphuric Acid ($H_2SO_4$). Ammonium Sulphate in distillation with Sodium Hydroxide (NaOH) gives Ammonia ($NH_3$) which is absorbed in the boric acid solution containing methyl red. The amount of Nitrogen ($N_2$) absorbed in the boric acid is determined by titration with N/70 $H_2SO_4$ procedure. The process involves the following two steps-

#### 3.4.1.1 Preparation of the digestion solution

Some pieces of ash less filter paper taken and weighed in electronic balance. The experimental feed samples were taken in each filter paper and they are also weighed. A record was kept for the identification of the various types of feed sample. The samples with the ash less filter paper was taken into the washed and dry 50ml Kjeldhal flasks. A mixture of BUCHI digestion unit was prepared by adding 20ml concentrated $H_2SO_4$ (20%) with the traditional digestion mixture (a white powder). These were kept in the digestion unit until the mixture become clear. Thus, a water color digestion solution was prepared.

#### 3.4.1.2 Preparation of sample solution

The digestion solution was then made into 100ml in 100 ml volumetric flasks with distilled water 5 ml of the sample was transferred in a Micro- Kjeldhal distillation apparatus followed by 10 ml of 40%NaOH and 150ml distilled water. 5ml of 2% boric acid was taken into conical flasks and placed into the lower chamber of Micro-Kjeldhal distillation unit. The solution was kept for about 50 minutes. Distillate was collected in excess of 2% boric acid solution with indicator and was titrated by $NH_4SO_4$. After titration the initial green color was changed into the pink color.

#### 3.4.1.3 Calculation of protein percentage (%)

The percentage of nitrogen sample was calculated by the following formula:

$$\% \text{ Protein} = \frac{\text{Milliequivalent of nitrogen}(0.014) \times \text{Titrant value(ml)} \times \text{Strength of HCl}}{\text{Weight of sample(g)}} \times 100$$

### 3.4.2 Lipid (%) determination

The estimation of fat content of experimental feed had been accomplished by Bligh and Dryer method (Bligh and Dryer 1999). The feed sample were dried into the oven and kept for 24 hours to remove the moisture. Oven dried samples were then meshed finely and taken into conical flask. A solvent (Chloroform: Methanol=2:1) was added and kept into air tight condition for 24 hours. Fat content of the sample react with solvent and remain in the solution. After 24 hours the solution of the flask was filtered in another weighed conical flask. Then the flasks were given in a hot water bath to dry up and remove the solvent. After that the flasks were kept in to the oven for an hour to get the actual fat content. Then the flasks were weighed in an electric balance to get the amount of fat content.

$$\text{Total lipid (\%)} = \frac{\text{Weight of lipid (g)}}{\text{Weight of sample (g)}} \times 100$$

### 3.4.3 Moisture (%) determination

Moisture content was determined in triplicate by placing an accurately weighted amount (about 7-8 g) feed sample in a pre-weighted porcelain crucible in a hot air oven (Gallenkamp, HOTBOX, Model OVB-305) at 105°C for 24 h until a constant weight was obtained. The loss of weight was calculated as percent moisture content using this formula:

$$\text{Moisture (\%)} = \frac{\text{Original sample weight (g)} - \text{Dried sample weight (g)}}{\text{Original sample weight (g)}} \times 100$$

### 3.4.4 Ash (%) determination

Ash content of the sample was determined by igniting sample about 4-5g in a Muffle Furnace (Philip Harris Ltd, England), for 6 hours at a temperature of 550° — 800° C.

After cooling, the crucible was weighed again. The ash content was calculated and expressed as percentage of the original sample using the following formula:

$$\text{Ash}(\%) = \frac{\text{Weight of ash(g)}}{\text{Weight of sample(g)}} \times 100$$

### 3.4.5 Carbohydrate estimation

Carbohydrate was determined by difference by following:

Carbohydrate (%) = 100-(P+M+F+A)

P = Percentage of protein, M = Percentage of moisture, F = Percentage of fat, A = Percentage of ash

## 3.5 Pre- stoking Management

Pre-stocking management means some sequential managerial activities before stocking of fish species in tanks or ponds. Broadly it can be said that all the management practices involved in fish culture before stocking.

### 3.5.1 Aquarium Preparation

Twelve aquarium tanks were prepared for treating Koi carp and every treatment has 3 replicates. For disinfecting tanks were treated by $KMnO_4$ (potash) and lime before pouring with water. After filling the tanks with water through purifying aeration was given in all tanks. Salt and lime were for increasing total dissolve solids and enriching the water quality. A water filter was also added to every tank for clearing water and to reduce wastes of Koi Carp (*Cyprinus rubrofuscus*). For proper oxygen supply aerators were also added.

### 3.5.2 Fish Collection

Good fish sample is the precondition of good outcome for any culture. In this research work fish sample was collected from a hatchery source of Faridpur. For getting good results organoleptic tests that means healthy, good-looking, disease-free seeds were selected for collection purposes. Small Juvenile Koi carp was collected for experimental work. Different sizes of juvenile were collected ranged from 8gm to 15gm.

(a) (b)

**Figure 4**: Koi carp sample before stoking transported to aquaculture lab

### 3.5.3 Disinfection of Fish sample

After collecting the sample from any sources, it should be disinfected. Juvenile Koi Carp was disinfected by potassium per manganate (KMnO4) called as potash. Approximately 1gm was applied in the polybag of sample. Then within a minute the fish were given to the research tanks according to the research working guidelines. Before stocking the fish sample in the tank's fishes were properly sorted and equally distributed.

### 3.5.4 Species Stocking Density

Juvenile Koi Carp was equally distributed numerically in all tanks. In this study there were twelve tanks where three of them was used as controlled under treatment 1 and others were used as Treatment 2, 3, 4 respectively. There are 120 pieces juvenile *Cyprinus rubrofuscus* were stocked in twelve tanks as a result every treatment has 30 fish sample.

### 3.6 Post-stocking Management

The phase includes the activities or maintenance from stocking to harvesting defined as post -stocking management. In case of aquarium-based culture system, many activities were similar to traditional system like feeding, sampling, chemical application and harvesting etc. Water was exchanged 30% in 3 days interval. In this work of *Cyprinus rubrofuscus* culture some different activities were performed during the study period.

### 3.6.1 Feeding

Feeding is an important issue for any types of culture. For getting good results proper feeding should have to be maintained. Feeding was properly maintained on daily basis and two times (morning and evening). From the beginning to the last of the study fish diet was given 2 g per every tank every day.

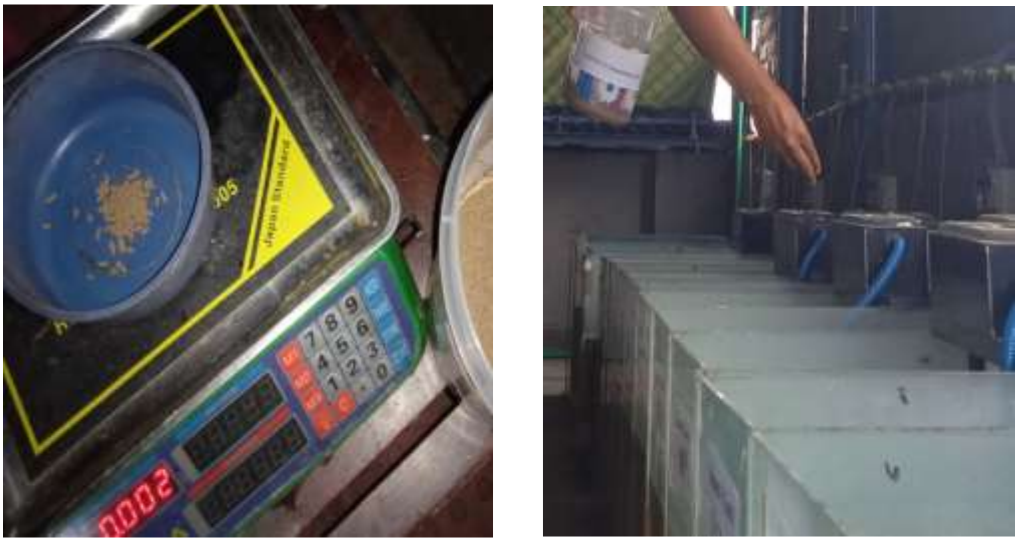


**Figure 5**: Feeding Koi carp every day two time.

### 3.7 Treatment of the Experiment:

Four different treatments were designed to feed the freshwater Koi carp. The treatment combination was given below;

T-1 (Control) = Formulated feed with 0% seaweed

T-2 = Formulated feed with 5% seaweed

T-3 = Formulated feed with 10% seaweed

T-4 = Formulated feed with 15% seaweed

### 3.8 Water Quality Parameters

Fish perform all their bodily functions in water. Because fish are totally dependent upon water to breathe, feed and grow, excrete wastes, maintain a salt balance, and reproduce, understanding the physical and chemical qualities of water is critical to successful aquaculture. To a great extent water determines the success or failure of an aquaculture operation. The following parameters were considered for present study.

### 3.8.1 Temperature

Temperature is an important issue in case of aquarium-based fish culture. Many other water parameters depend on its balance. Good temperature range helps to grow fish and other microbial community properly. Temperature range was measured everyday morning during the study period. Data was collected by using digital multimeter.

### 3.8.2 pH (Puissance of Hydrogen)

pH is very important for fish culture. pH defines the acid and base concentration of water. High and low pH levels are related with metal concentration, nutrients deficiencies and problems. In this study pH was measured everyday through using digital multimeter. For this, water was collected in mug from treatment tanks and put the pH sensor of multimeter into it. Concentration of pH is not suitable if it ranges in very low (< 4 ppm) or very high (> 9 ppm). In case of Koi Carp culture pH changes can affect indirectly by altering other water parameters.

**Figure 6**: Water pH measuring digital machine

### 3.8.3 Ammonia (NH3)

Ammonia is the most important harmful factor for successful fish culture. In aquarium base fish culture nitrogenous compound is a crucial factor for fish survival rate. Generally, ammonia is formed by the excreta of culture species, deposition of extra feed on the tank bottom level. The most important thing is ammonia is controlled in aquarium culture because of using filter in the tank water.


**Figure 7**: Ammonia testing reagent

### 3.8.4 Dissolve Oxygen (DO)

For aquatic species dissolve oxygen is the most important factor for their survival. Fish breathe oxygen just as we do, so dissolved oxygen (DO) is a critical environmental indicator in aquaculture. Depleted DO is the leading cause of fish kills, and it is known that low-oxygen conditions are their worst enemy. In case of fish culture at least 5.0 ppm DO level should be maintained. In this research work an oxygen motor was used for maintaining DO level. In every tank, there were four air stone used for proper oxygen supply. DO level data was measured by Life Sonic's DO kit at daily basis.

**Figure 8**: DO testing by using reagents

### 3.8.5 Total Dissolve Solid (TDS)

Total dissolve solid means the concentration of mineral, salt and solid substances in water. In case of aquarium-based culture, TDS remains lower than traditional culture. The importance of TDS in water for freshwater Koi Carp is inevitable as it helps Koi Carps body formation. In the culture period it was maintained properly and measured the level by using HANNA DiST 2 TDS tester.


**Figure 9**: Measuring DTS with digital meter

### 3.8.6 Nitrate

Nitrate is produced in aquarium by the biological filter. Beneficial bacteria in the biological filter convert toxic ammonia and nitrite into nitrate. Maintaining a low level nitrate improves the health of fish. Extreme level of nitrate stimulate algal blooms.


**Figure 10**: Nitrate measuring reagent

### 3.8.7 Nitrite

Nitrite is produced in aquarium by the biological filter. Beneficial bacteria in the biolo0gical filter convert ammonia into nitrite then converts nitrite into nitrate. Nitrite in the aquarium is toxic, it will prevent fish from carrying on normal respiration and quick lead to fish death. For koi Carp culture aquarium nitrite need to maintain bellow 0.25ppm.


**Figure 11**: Nitrite testing reagent

### 3.9 Sampling Schedule

Sampling is a process used in statistical analysis in which a predetermined number of observations are taken from a larger population. This tool describes the samples to take in order to quantify a system, process, issue or problem. Sampling saves time and the data can be collected and analyzed more quickly with a sample than a complete count of the entire population. In the study sampling was done properly at 5 days of interval. Random sampling was done manually in every time with the help of scoop net.


(a) (b)

**Figure 12**: Sampling of Koi carp

### 3.10 Final Sample collection

Harvest means the number or weight of fish caught and retained from a given area over a given period of time. Harvesting is done due to analyze the different parameters of any species. In this research work this procedure was accomplished at 8weeks and Koi Carp were harvested after 1 days of research timeframe. This process was done manually.The main purpose of harvesting was to assess the performance of overall growth, survival rate in this timeframe and also assess the hematological parameters.

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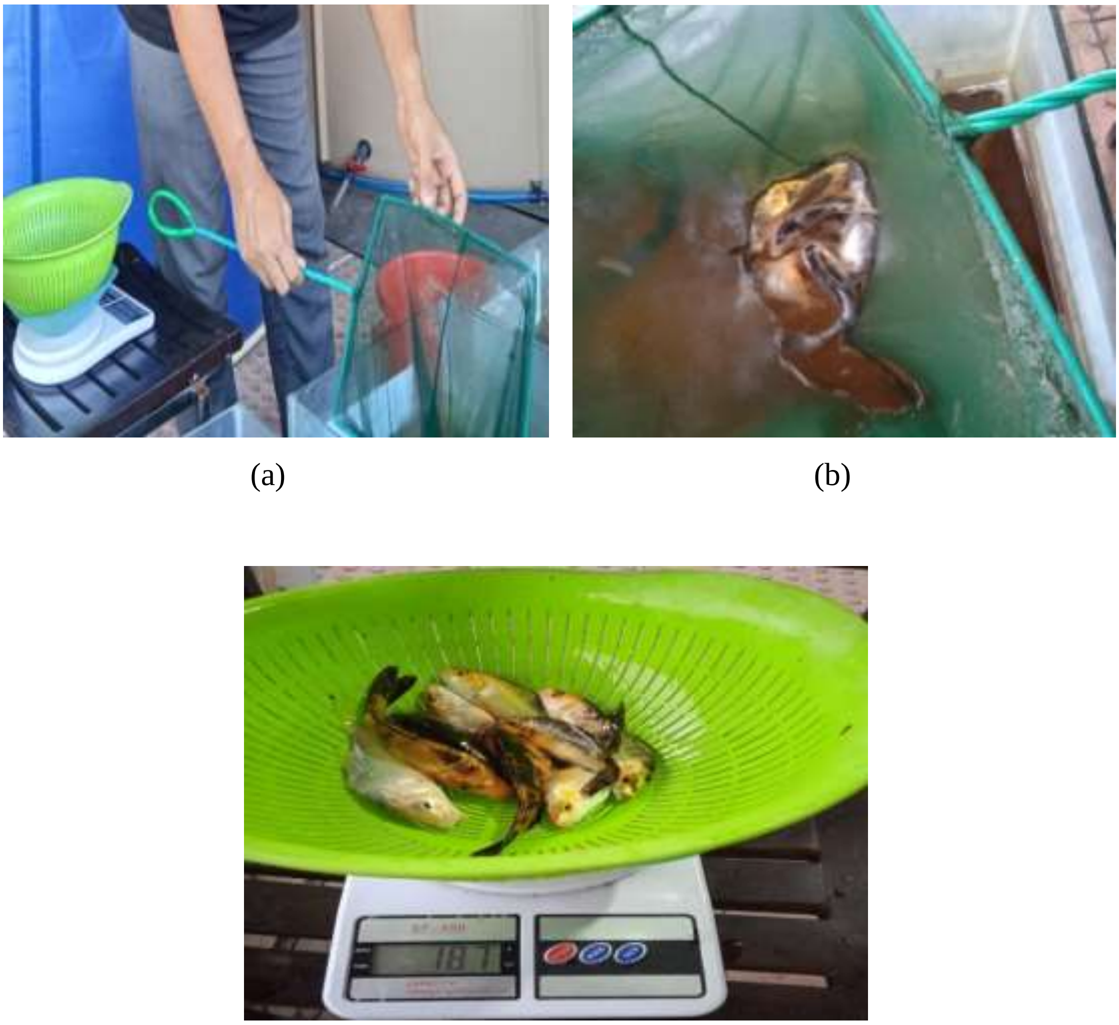


(a) (b)

(c)

**Figure 13**: Collecting koi carp for final data collection, (a) and (b) catching fish, (c) taking weight of fish.

### 3.11 Growth Parameters

Growth is an integrated physiological response encompassing external environmental conditions (food quality and quantity, temperature, water quality) and internal physiological status (health, stress, reproductive state). Growth parameters defines the assessment of subsequent growth and development and risk of disease from the starting level of any species culture considering different factors. The parameters are length, weight, size and shape etc.

**i) Weight Gain**

Weight gain was calculated by using this following formula;

Weight gain = Final weight- initial weight

**ii) Percentage of Weight Gain**

Percentage of weight gain was calculated by using this following formula;

$$\text{\% of Weight gain} = \frac{\text{Mean final weight} - \text{Mean initial weight}}{\text{Mean initial weight}} \times 100$$

**iii) Survival Rate**

Survival rate was assessed by using this following formula;

$$\text{Survival rate} = \frac{\text{No. of total live koi carp}}{\text{No. of total koi carp stocked}} \times 100$$

**iv) Average Daily Gain (ADG)**

Average daily weight gain was calculated by using this following formula;

$$\text{ADG} = \frac{\text{Final Koi carp weight} - \text{Initial Koi carp weight}}{\text{No. of days}}$$

**v) Specific Growth Rate (SGR)**

Specific growth rate was calculated by using this following formula;

$$\text{SGR (\% per day)} = \frac{\text{Final weight} - \text{Intial weight}}{\text{Time of harvest} - \text{time of stock}}$$

**vi) Feed Conversion Ratio (FCR)**

$$\text{FCR} = \frac{\text{Total feed consumed}}{\text{Live weight gain}}$$

**3.12 Blood parameters:**

Blood parameters include red and white blood cell, hemoglobin concentration, glucose level of blood, blood serum which is widely used clinical indicators of health and diseases. These traits are tightly regulated in healthy individuals and under genetic control.

### 3.12.1 Blood collection

Before collecting blood sample, fish need to immobilize for handling. Many researchers used clove oil for their study to immobilize fish for handling, sorting, tagging, artificial reproduction procedure and surgery and suppress sensory system during invasive procedure. So, in this research I used 2 drop of clove oil in 200ml normal water to immobilize Koi carp.

(a)

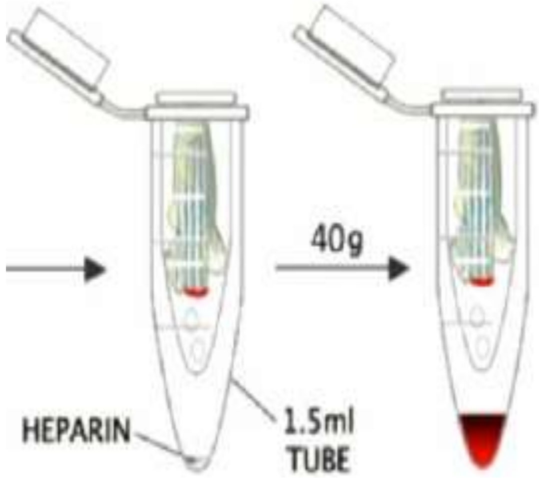

(b)

(c )

(d)

**Figure 14**: Immobilizing Koi carp using Clove oil. (a) Fish sample for blood collection. (b) 200ml of water in a pot for mixing clove oil. (c) Adding clove oil in the water. (d) Immobilized fish due to the effect of clove oil.

After using anesthesia weight and length of fish was taken and blood sample was collected by cutting the tail because these koi carp are small in size, as a result collecting blood through injection is hard.





(a) (b)

(c)

**Figure 15**: Blood collection of Koi carp. (a) cutting fish tail, (b) drop of heparin used for prohibiting blood clotting, (c) collected blood in a test tube.

### 3.12.2 Hemoglobin

Hemoglobin is composed by polypeptide chains, known as globin, each having a prosthetic group called heme, identical in every fish species studied to date. On the other hand, globin’s differ from species to species and among isoforms. Remarkably, globin seem to occur in all organisms and tissues, exhibiting a diversity of quaternary structures and a large number of functions apart from oxygen transportation and storage, as illustrated by cytoglobins and neuroglobins. For in this research hemoglobin of Koi carp measured with Easy Touch GCHb meter.

**Figure 16**: Hemoglobin measuring meter.

### 3.12.3 Blood glucose

Glucose plays a very important role in the body function of fish. It provides energy to fish body during lack of feed. In this research blood glucose of Koi carp measured with Viva Chek Eco meter.


**Figure 17:** Glucose measuring meter.

### 3.12.4 Serum Protein

RBM Multi-Analyte (MAP) Technology can be applied to serum or plasma samples. There are several procedures for collecting serum sample from blood.

**Materials**

1) Serum separator tube
2) Transfer pipette
3) Cryovials (suggest using 1.0 mL or 1.8 mL)
4) Disposable gloves
5) Standard centrifuge

**Safety Implications**

Need to observe proper precautions for bio-hazardous materials.

**Blood Serum Collection Procedure:**

Following procedure was used to collect blood serum for experimental purpose. Every step was performed carefully to obtain better result.

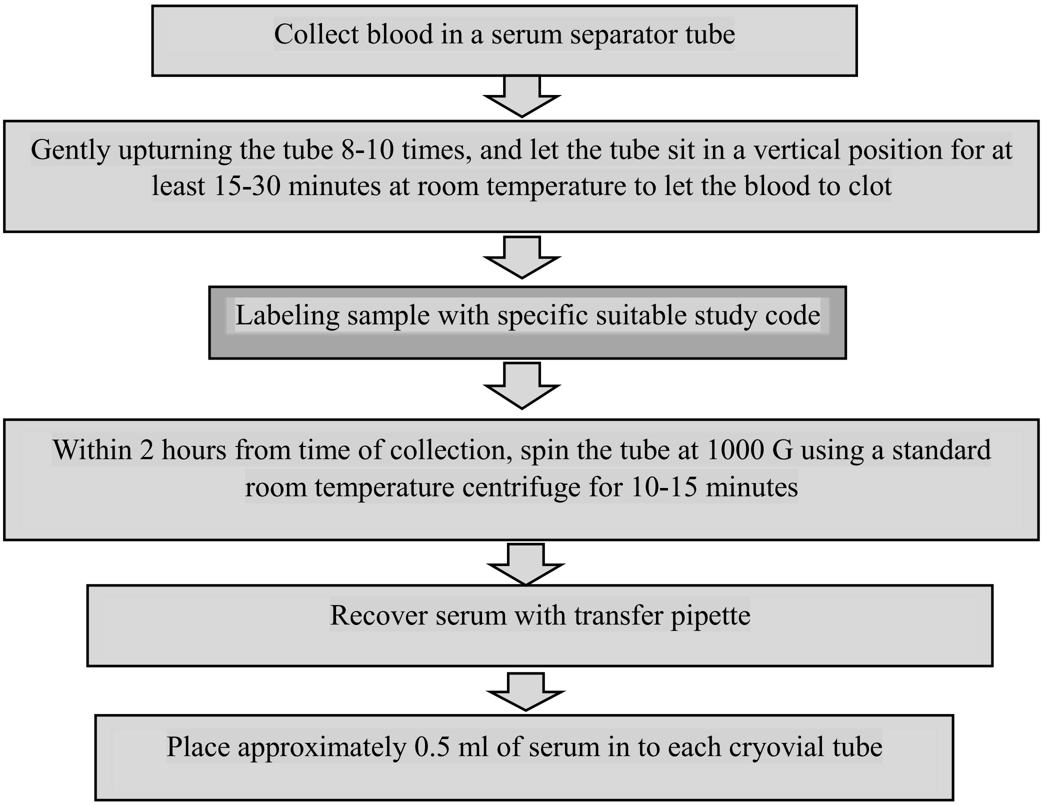



**Figure 18**: Flowchart of protocol for blood serum collection

After collecting serum Refractometer was used for measuring serum protein. Refractometer is instrument to measure substances dissolved in water and some oils. The refractometer performs using the theory of light refraction through liquids. As light moves from air into a liquid it slows down. This phenomenon is what gives a "bent" look to objects that are partially submerged in water. To put it normally, the additional dissolved solids water contains, the leisurely light travels through it, and the more noticeable the "bending" effect on light. Refractometers follow this principle to measure the number of dissolved solids in liquids by movement of light through a sample and showing the refracted angle on a scale.

The scale most frequently used is referred to as the Brix scale. The Brix scale is defined as: the number of grams of pure cane sugar dissolved in 100 grams of pure water (grams sugar/100 grams $H_20$). Other scales have been developed to measure salt, serum proteins (albumen) and urine specific gravity. The Model PU-ATC temperature compensated (ATC) serum protein/urine refractometer provides a three-scale reading of 0-12g/100mL serum protein, 1.000 to 1.050 urine specific gravity, and 1.3330 to 1.3600 refractive index. A high degree of accuracy can be assured with the ATC feature. The unit maintains its calibration with the built-in ATC function.

If the unit has to be recalibrated or checked for accuracy, a drop or two of distilled water at 20ºC (room temperature 68ºF) is placed on the prism, then the secondary cover is closed. When held up to the light, the scale should read 1.333 R.I. If it does not, the operator turns the adjustment screw until the critical line reads 1.333 R.I.

This allows for fast and accurate determinations for protein concentrations of a blood serum, plasma, or protein serum (e.g. albumin solutions). Urine-specific gravity readings can be obtained, as well as direct readings in refractive index, which provide the operator measurements of concentrations in a variety of solutions. The eight-ounce unit is approximately 6 1/2" long.

For veterinarian and human use, this refractometer provides a quick and accurate indication of vital fluid levels. The triple scale that gives Urine Specific Gravity(SG), Total Serum Protein(SP) and Refractive Index (RI) and ATC which automatically adjust itself to correct temperature discrepancies during use.

In this study, serum refractometer was used to fine out some vital parameters of koi carp fish blood to obtain the experimental goal.

### 3.12.5 Refractive index

The refractive index is the measure of bending of a light ray when passing from one medium to another. It can also be defined as the ratio of the velocity of a light ray in an empty space to the velocity of light in a substance, $n = c/v$. In this research refractive index was measured with Refractometer.

(a)

(b)

(c)

(d)

(e)

(f)

(g)

(h)

**Figure 19**: Serum collection. (a) collected blood in test tube, (b) test tubes were put in stand for forming blood clot, (c) centrifuging the blood, (d) blood serum, (e ) Refractometer, (f) one drop of serum on Refractometer prism, (g) observation, (h) serum protein level.

### 3.12.6 Spleen Somatic Index of Koi carp

The spleen is the longest lymphatic organ in the body. Covered by a connective tissue capsule, which extends inward to divide the organ into lobules, the spleen consists of two types of tissue called white pulp and red pulp. The white pulp is lymphatic tissue consisting mainly of lymphocytes around arteries.

**Collecting procedure:**

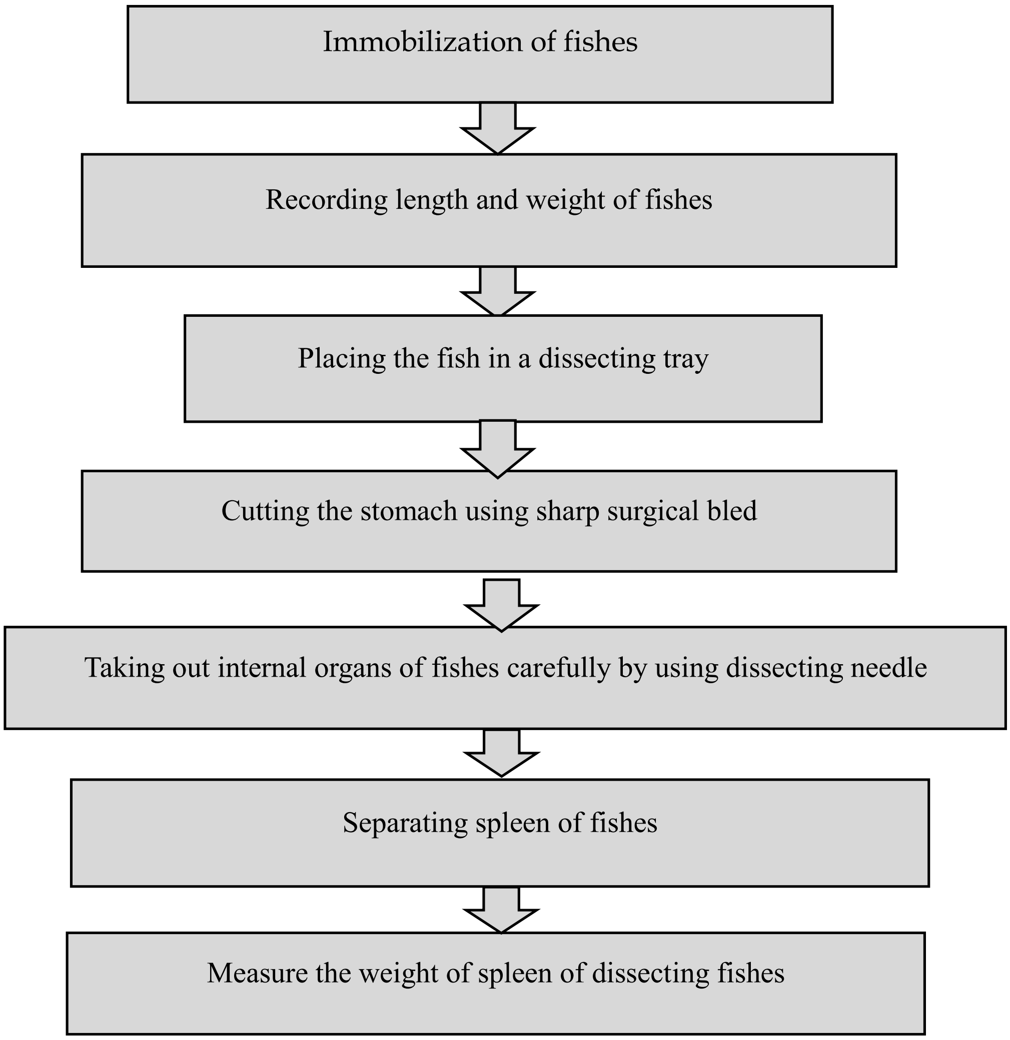



**Figure 20:** Flowchart of protocol for collecting spleen from fish stomach

After collecting spleen, the percentage of spleen needs to be calculated by using a formula showing bellow.

$$\textbf{SSI \%} = \frac{spleen\ weight}{Body\ weight\ of\ fish} \times 100$$

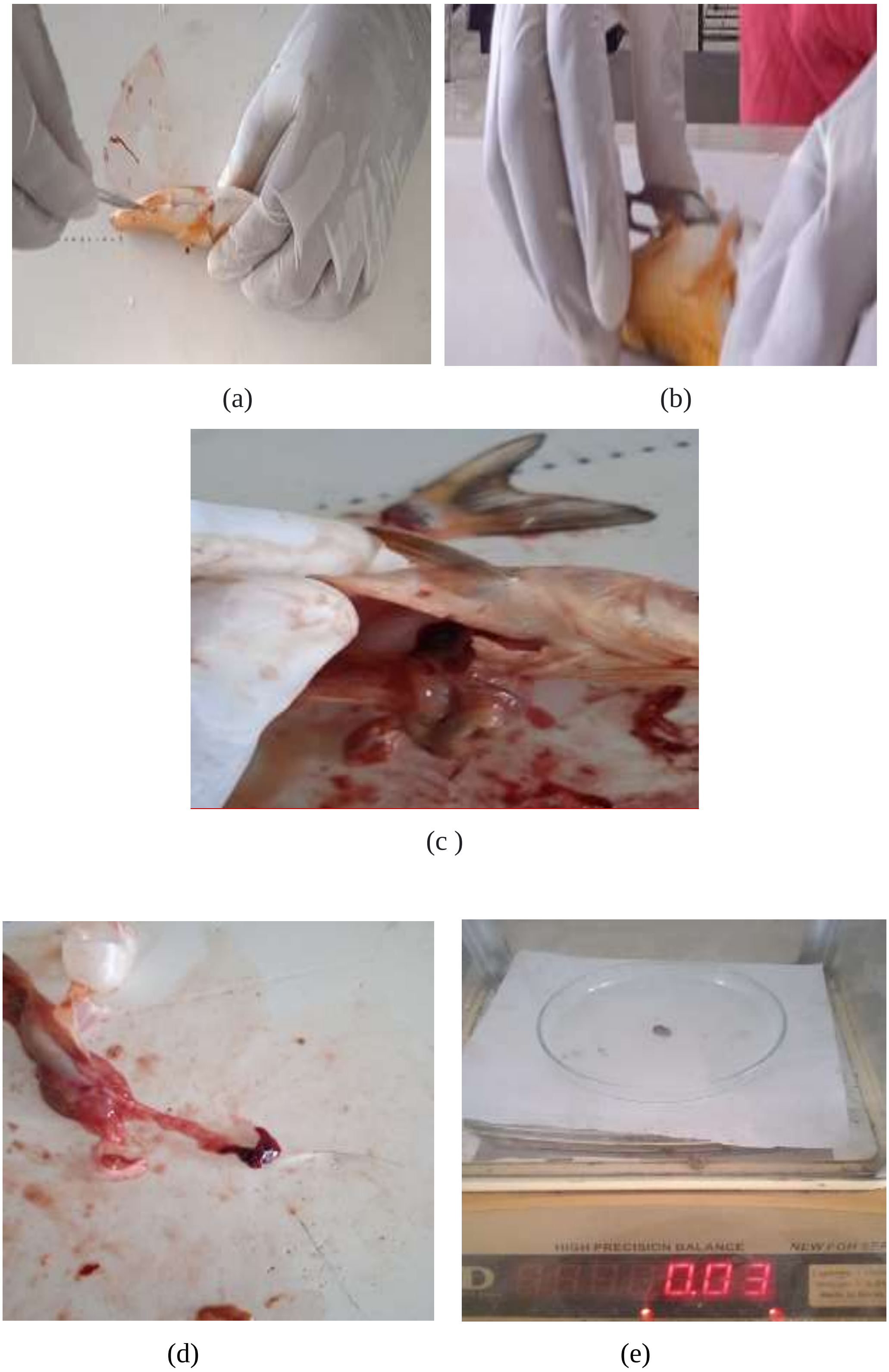



**Figure 21**: Fish spleen sampling, (a) and (b) cutting fish stomach with sharp surgical bled, (c) take out the internal organs of fish, (d) spleen separated from other organs, (e) weight taking of spleen.

### 3.13 Statistical Analysis

Statistical analysis was done for the purpose of getting better outcome of the research result. That was accomplished by using MS Excel, and SPSS (Statistical Package for Social Sciences). All non-repeatedly measured variables (prawn growth parameters) were analyzed by one-way ANOVA Tukey HSD programmed using SPSS software at 5% level of significance.

# CHAPTER IV

## RESULTS AND DISCUSSION

This chapter confined the results on the investigation accomplished during the research timeframe to fulfill the mentioned objectives earlier. Growth performance, different environmental and physiochemical parameters involved in aquarium culture of Koi Carp are presented. The experimental findings are given below;

### 4.1 Water Quality Parameters

Water quality maintenance and monitoring in aquaculture are the essential practices focusing on the achievement of the developing cycles. Temperature, dissolved oxygen (DO), pH (puissance of hydrogen), ammonia concentration ($NH_3$), salinity (for salt water species), TDS (total dissolve solids) are some examples of parameters that ought to be continuously checked and monitored, especially in Aquarium Culture System (ACS). The comprehension and understanding of water quality parameters and its interactions in ACS are crucial to the correct development and maintenance of the production cycle. Water quality is significant in fish cultivating as low-quality water can impact the wellbeing and development of the fish.

**Table 5:** Water quality parameters (Mean±SD) of different treatments tanks.

| **Water Parameters** | **T1 (Control, 0% Seaweed)** | **T2 (5% Seaweed)** | **T3 (10% Seaweed)** | **T4 (15% seaweed)** |
|---|---|---|---|---|
| **Temperature (° C)** | 27.33±0.34 | 27.80±0.27 | 27.46±41 | 27.55±0.04 |
| **pH** | 8.27±0.01 | 8.33±0.06 | 8.27±0.02 | 8.25±0.01 |
| **TDS (ppm)** | 199±5.7 | 217.33±1.73 | 233.33±16.74 | 279±9.223 |
| **Ammonia (mg/l)** | 0.5 | 0.5 | 0.5 | 0.5 |
| **Dissolved Oxygen (mg/l)** | 7.71±0.72 | 7.04±0.52 | 7.61±0.15 | 7.65±0.51 |
| **Nitrate** | 0 | 0 | 0 | 0 |
| **Nitrite** (mg/l) | 0.05 | 0.05 | 0.03 | 0.03 |

In the present study, it was found that mean temperature range was more or less similar in every treatment ranged from 27.3 to 29.5 $^{0}$C (Table 07). The mean pH and $NH_3$ in different treatments were almost similar during the experiment that defines the positivity of good water quality for Koi Carp culture. Range of mean dissolve oxygen (DO) was also more or less similar in every treatment ranged from 7.04 to 7.71 mg/l (Table 07).

### 4.2 Proximate Composition of Feed

After analysis it was revealed that formulated feed has nutritional value including 27.46-27.75% crude protein, 48.8-49.09% carbohydrate, 6.33% ash,11.02% moisture and 6.1% crude fat (Table 10). Pratiwy *et al.* (2018) demonstrated that whole thalli of *Sargassum* form an effective supplement for Nile tilapia at least at 4% without negative effects and 8% level inclusion of *Sargassum* gave the best results for growth performance they use 32% protein. Nader *et al*. (2010) used 26% protein in seaweed supplemented fish diet and found best result using 15% seaweed. Basically maximum researcher use 5-10% fat in formulated diets.

**Table 6**: Proximate Composition (% on DM basis) of formulated feed

| Features | T-1 | T-2 | T-3 | T-4 |
|---|---|---|---|---|
| **Moisture %** | 11.78±0.12 | 11.21±0.08 | 11.56 ±0.37 | 11.85±0.25 |
| **Carbohydrate %** | 48.7±.051 | 48.32±0.25 | 48.13±0.21 | 47.69±0.46 |
| **Crude Protein %** | 27.1±0.45 | 27.58±0.35 | 27.46 ±0.45 | 27.82±0.37 |
| **Ash %** | 6.23±0.31 | 6.58±0.17 | 6.33 ±0.28 | 6.21±0.24 |
| **Crude fat %** | 6.19±0.22 | 6.31±0.15 | 6.10 ±0.11 | 6.43±0.08 |

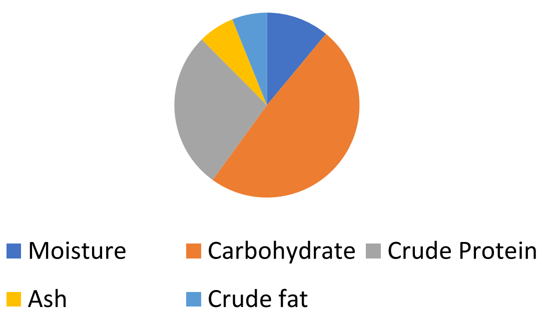



**Figure 22:** Proximate composition of dietary feed prepared for the experimental trial.

### 4.3 Morphological Parameters of Koi Carp

Morphological parameters assessed at birth help predict subsequent growth and development. It also indicates the physical development of species which helps to obtain desired objective for experimental work. In this study data was recorded 7 days interval for assessing the progress of koi carp growth and probability of disease infestation.

#### 4.3.1 Mean Length (cm) of Koi Carp

Mean length is an important parameter that indicates the morphological development of species. It has direct relationship with the growth rate of any organism. During the study period mean length of koi carp were recorded after completing sampling at every 7 days of interval.

**Table 07:** Mean length (cm) of cultured Koi carp in experimental period (days)

| Treatments | Mean length± SD (cm)/ week | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **0** | **1st** | **2nd** | **3rd** | **4th** | **5th** | **6th** | **7th** | **8th** |
| **T1** | 6.12±0.53 | 6.35±1.14 | 6.7±0.66 | 7.00±.72 | 7.26±0.07 | 7.66±0.49 | 8.24±0.49 | 9.04±0.39 | 9.28±0.32 |
| **T2** | 6.07±0.06 | 6.48±0.04 | 6.97±0.03 | 7.6±0.24 | 8.14±0.11 | 8.78±0.11 | 9.31±0.27 | 9.99±0.41 | 10.57±0.58 |
| **T3** | 5.70±0.97 | 5.8±0.89 | 6.21±0.76 | 6.92±0.51 | 7.75±0.17 | 8.36±0.25 | 9.05±0.17 | 9.64±0.24 | 9.81±0.32 |
| **T4** | 5.63±0.28 | 5.86±0.28 | 6.15±0.3 | 6.35±0.34 | 6.78±0.31 | 7.34±0.44 | 8.1±0.44 | 8.78±0.25 | 8.98±0.25 |

From table 06, it is clearly stated that the initial mean length of koi carp varies from 5.63 cm to 6.12cm, stocked for conducting the experiment. After one week mean length was seen higher (6.48 cm) in T-2 (5% seaweed) tanks and that continued up to 2$^{nd}$ week though sampling was done on picking individual basis. After that, on the 3$^{rd}$ week Treatment-2 (5% seaweed) gave better results comparatively than other treatments. It was observed that after 5 weeks mean length was higher in Treatment-2 (5% seaweed) than others. After accomplishing harvest, the highest mean (10.57 cm) was got in T-2 (5% seaweed) tanks. After 8 weeks of experiment the highest mean length of koi carp also found in T-2.

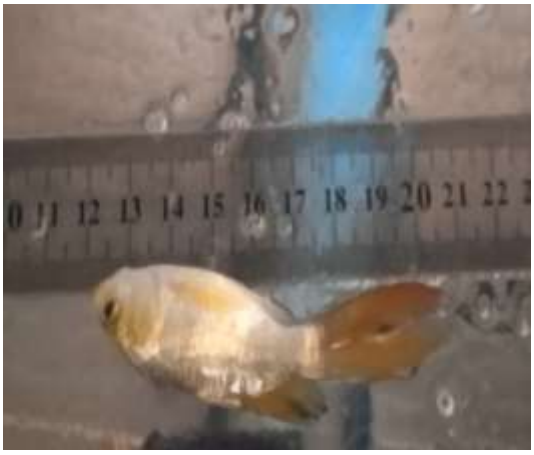



**Plate 1**: koi carp sampling


**Plate 2**: 14th day highest value


**Plate 3** : Highest value in 35th day in Treatment-2 (5% seaweed)

### 4.3.2 Mean Weight (g) of Koi Carp

A weighted mean is a kind of average. Instead of each data point contributing equally to the final mean, some data points contribute more “weight” than others. If all the weights are equal, then the weighted mean equals the arithmetic mean. The following mean weight was recorded on the basis of sampling at 7 days interval in experimental duration. Total 8 sampling was done during experiment. Samples were collected individually and weight was measure carefully to get more accuracy for further analysis of the sample.

**Table 08**: Mean weight of cultured Koi carp during experimental period (per week).

| Treatments | Mean weight ± SD(g)/ week | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **0** | **1st** | **2nd** | **3rd** | **4th** | **5th** | **6th** | **7th** | **8th** |
| **T-1** | 14.4±2.1 | 15.06±2.34 | 15.76±2.4 | 16.86±.261 | 17.77±3.02 | 18.59±2.94 | 19.63±3.18 | 20.54±3.07 | 21.23±3.26 |
| **T-2** | 14.5±0.75 | 15.73±0.48 | 16.69±0.38 | 17.86±0.77 | 19.00±1.22 | 20.16±1.41 | 21.02±1.51 | 22.43±1.19 | 22.60±1.15 |
| **T-3** | 13.1±2.4 | 14.13±2.24 | 15.53±2.22 | 16.76±2.17 | 17.58±1.78 | 18.52±1.26 | 19.2±1.24 | 19.89±1.01 | 20.28±0.97 |
| **T-4** | 12.8±1.43 | 13.43±1.27 | 14.1±1.39 | 15±1.42 | 15.47±1.16 | 15.63±1.14 | 16.35±1.23 | 16.79±1.21 | 17.02±1.23 |

At the beginning of the research approximately mean 12 to 14 g Koi carp was stocked for culture. In first eight days T-2 (5% seaweed) gave better results (15.73g). It is clearly seen that after 8 weeks of experimental duration growth performance was satisfactory.

### 4.3.3 Average Daily Weight Gain (ADG)

Daily weight gain fluctuation is normal. Generally, weight was fluctuated for different reasons like feeding requirements, environmental factors and disease infestation possibilities. In case of optimum level of water quality factors and fish health condition growth progress depends on feeding rate. Good feeding rate will give better outcome.

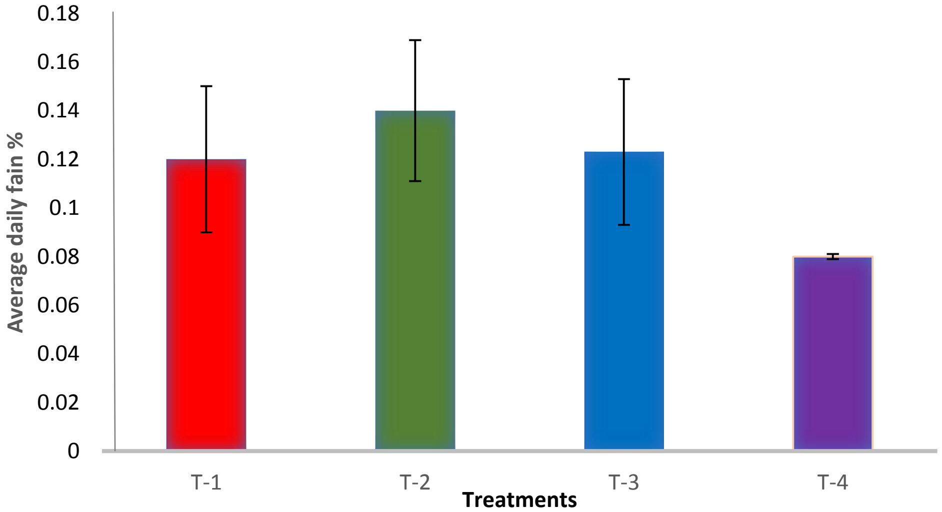



**Figure 23**: Average daily gain of Koi carp during the experimental period

From the figure 20, the highest daily weight gain (0.14g) was found in T-2 (5%seaweed) and T-3 (10% seaweed) has 0.123g value. The lowest ADG fount in T-4 (15% seaweed) where Treatment-1 (Control, 0% seaweed) has a little bit higher ADG value than T-4 (15% seaweed) which is 0.113g. Akter *et al*. (2010) found better ADG value in control condition for measuring the growth performance of *Cyprinus carpio* using Aflatoxin in different concentration and the value of ADG was 0.22g/d.

### 4.4 Growth Parameters of Koi Carp *Cyrinus rubrofuscus*

Growth is an integral biological response including external ecological and internal physiological status. In case of fish, weight gain, feed conversion ratio, survival rate, specific growth rate, protein intake etc. are considered as growth parameters. In this study, freshwater prawn was harvested after three days of study duration. And the following data were recorded from *Cyrinus rubrofuscus* is given below;

**Table 09:** Growth parameters (Mean) of *Cyrinus rubrofuscus* in Aquarium.

| Parameters | T-1 (Control) | T-2 (5% seaweed) | T-3 (10% seaweed) | T-4 (15% seaweed) |
|---|---|---|---|---|
| **MIW** (g) | 14.4±2.1 | 14.5±0.75 | 13.1±2.4 | 12.8±1.43 |
| **MFW** (g) | 21.23±3.26 | 22.60±1.15 | 20.28±0.97 | 17.02±1.23 |
| **MWG** (g) | 6.79±1.39 | 8.06±1.58 | 6.98±1.82 | 4.72±0.52 |
| **WG (%)** | 47.43 | 55.86 | 54.80 | 34.37 |
| **SR (%)** | 96.66±4.71 | 100 | 93.3±9.42 | 100 |
| **SGR (%)** | 12.13±2.48 | 14.41±2.85 | 12.81±3.26 | 8.42±0.93 |
| **FCR** | 1.7±0.33 | 1.43±0.26 | 1.79±0.44 | 2.39±0.24 |
| **ADG** | 0.12±0.03 | 0.14±0.029 | 0.123±0.03 | 0.08±0.001 |

From table 8, it was found that the highest mean weight gain (4.31g) and FCR value was found in T-2 (5% seaweed) and lowest was found in T-4 (15% seaweed).

#### 4.4.1 Mean Final weight

The present study was started with 56 days duration of freshwater Koi Carp culture in aquarium culture system. The research was started with small *Cyrinus rubrofuscus* comprising the initial weight were 14.4±2.1, 14.5±0.75, 13.1±2.4 and 12.8±1.43g and after completing the research final weight were 21.23±3.26, 22.60±1.15, 20.28±0.97

and 17.02±1.23g in T1, T2, T3, T4 treatments respectively (Table 08). The mean final weight was significantly higher in T2 (5% seaweed) comparatively than control and other treatments. Rama Nisha *et al*. (2014) used 8% of seaweed which showed the better result.

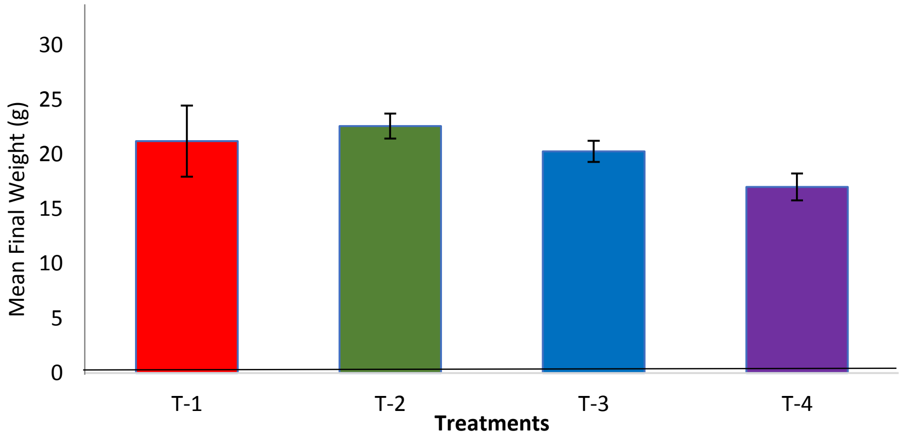



**Figure 24**: Mean final weight of Koi carp during the experimental period

### 4.4.2 Mean Weight Gain

The mean weight gain of cultured Koi carp were 6.79±1.39, 8.06±1.58, 7.18 ±1.82, 4.72±0.52g in T1, T2, T3 and T4 treatments respectively (Table 08). It is clearly said that T-2 (5% seaweed) gave significantly better outcome (8.06 g) than control and other treatments. In case of T-3 (10%
seaweed) the outcome was satisfactory than control. The least outcome (4.72 g) was found in T-4 (15% seaweed). Sudharaka *et al.* (2021) found using less weight gain for *Cyrinus carpio* using 30% seaweed (*Sargassum sp.*) where initial length and weight was 3.91±0.06cm and 2.13±0.13g, final weight and length after 14 weeks was 4.92±0.27cm and 5.36±0.06g.

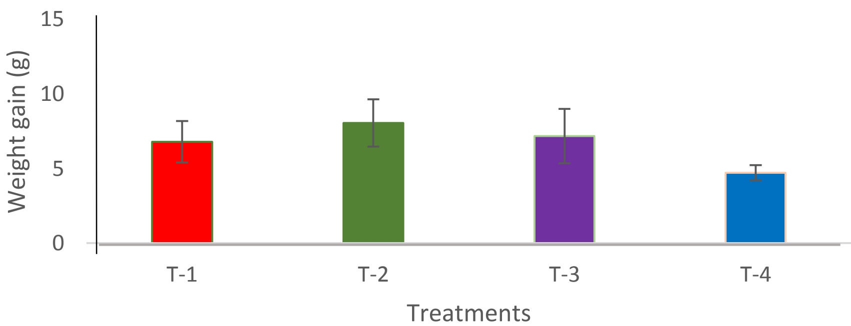



**Figure 25**: Mean weight gain of koi carp in different treatment after the experimental period.

### 4.4.3 Survival Rate (%)

Survival rate is important parameters for fish culture. It defines the live organism's percentage after completing research timeframe. In this research it was found at 96.66±4.71%, 100%, 93.3±9.42% and 100% survival rate in T1, T2, T3 and T4 treatments respectively (Table 08). Survival rate was significantly better in T2 (5% seaweed) and T4 (15% seaweed) than other treatments. Sudharaka *et al.* (2020) found 100% survival rate during the culture of gold fish.

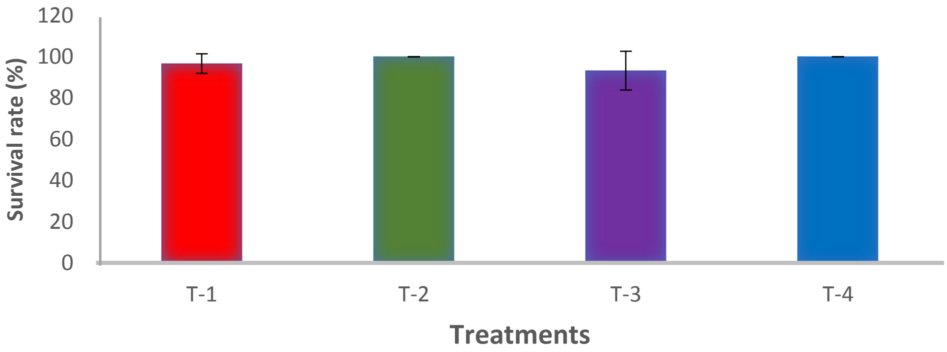



**Figure 26**: Survival Rate (%) of Koi carp in different treatment where T-2 (5% seaweed) and T-4 (15% seaweed) showed no mortality.

### 4.4.4 Feed Conversion Ratio

The FCR values ranges 1.7, 1.43, 1.79 and 2.39 for T-1, T-2, T-3 and T-4 respectively at the end of the study period.

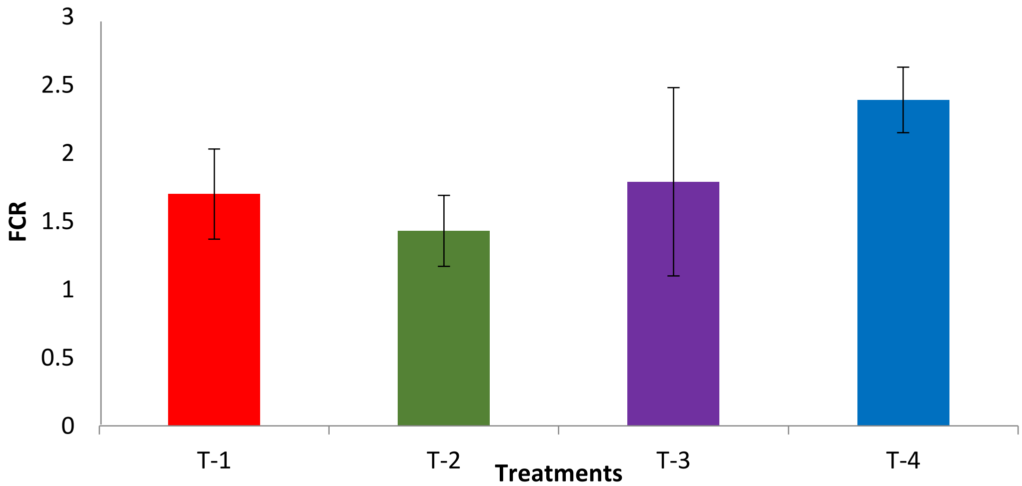



**Figure 27:** Feed Conversion Ratio of *Cyprinus rubrofuscus* in different treatment.

Maiti *et al*. (2017) obtained higher FCR value 2.2 to 3.7 during the culture of *Cyprinus carpio*. In our study, treatment which contains 5% seaweed (T-2) shows the desirable amount of FCR.

### 4.4.5 Specific Growth Rate

The specific growth rate of cultured koi carp were 12.13±2.48%, 14.41±2.85%, 12.81±3.26% and 8.24±0.93% in T-1, T-2, T-3 and T-4 treatments respectively (Table 8). Specific growth rate was significantly higher in T-2 (5%seaweed) than control and other treatments. Bairwa *et al.* (2021) found 8-9 % of SGR value of *Ciprinus carpio* cultured in different colored tank. Swain *et al*. (2018) found 13.2-13.8% SGR of *Ciprinus carpio* in non-aerated culture system.

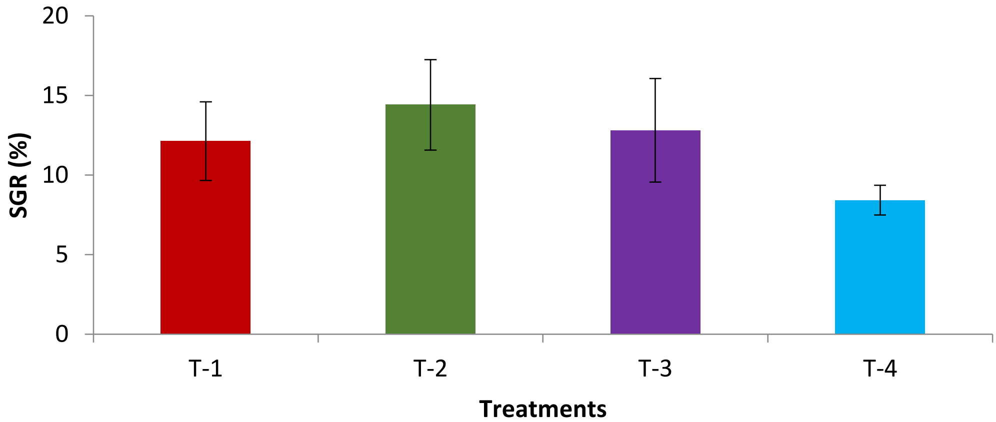



**Figure 28:** Specific Growth rate of Koi carp in Different Treatment where T-4 (15% seaweed) showed the lowest SGR% and highest in T-2 (5% seaweed).

## 4.5 Liner Regression of Length-weight relationship

Regression is a statistical method used to determine the strength and character of the relationship between one dependent variable (usually denoted as Y) and a series of other variables (known as independent variables) which also known as simple regression or ordinary least squares and liner regression is the most common form of this method. Regression captures the correlation between variables observed in a data set, and quantifies whether those correlations are statistically significant or not. In this analysis, mean length of koi carp is dependent variable.

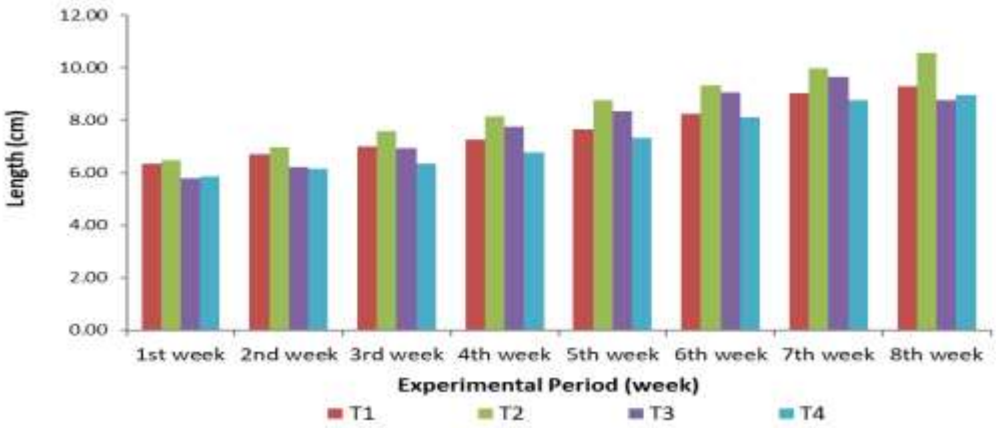



**Figure 29:** Mean length of Koi carp in different treatment during the experimental period.

### 4.5.1 Length-weight relationship in Treatment-1

Regression line predicts the change Y axis when X axis increase by one unit. $R^2$ is a measure of how close each data point fits to the regression line. From the following figure 30, it can be told that 97% mean length-weight was gained is accounted for its regression on experimental duration in T-1 (Control, 0% seaweed diet) experimental aquariums. Positive regression line was found in treatment-1 (Control) and mean length-weight was found almost close according to its predicted time in figure 30.

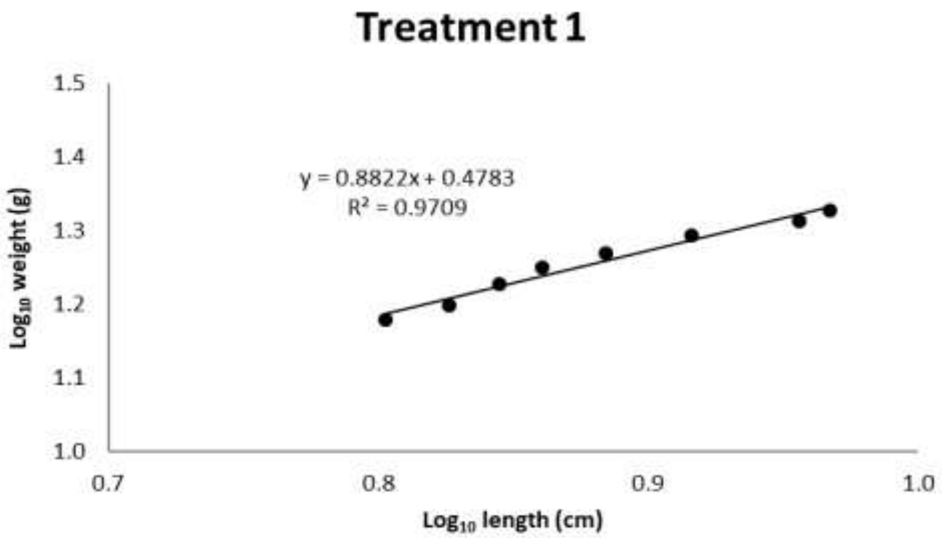



**Figure 30**: Length-weight relationship of koi carp in Treatment-1

### 4.5.2 Length-weight relationship in Treatment-2

From the following figure 31, it can be told that almost 100% mean length-weight was gained is accounted for its regression on experimental duration in T-2 (5% seaweed diet). In treatment-2 the best outcome was recorded during the experiment. Mean length-weight was at satisfactory level compared to other treatments including control. And the regression line means the positivity of the improvement of growth. In the figure 31, it can be told that mean length-weight was very close according to its predicted time and also showed the best outcome.

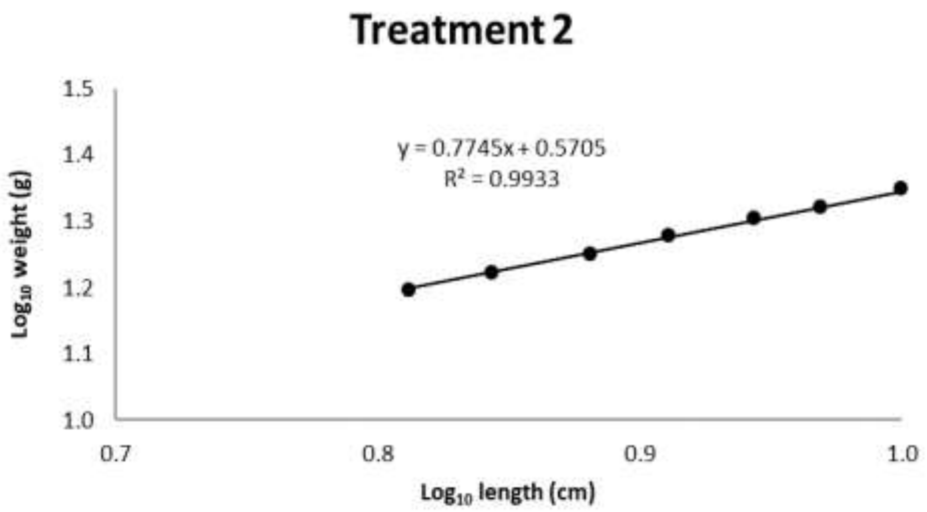



**Figure 31**: Length-weight relationship of Koi carp in Treatment-2

### 4.5.3 Length-weight relationship in Treatment-3

From the following figure 32, it can be told that 94% mean length-weight was gained is accounted for its regression on experimental duration in T-3 (10% seaweed diet). In Treatment-3 positive line was found that means the growth was good level of progress.

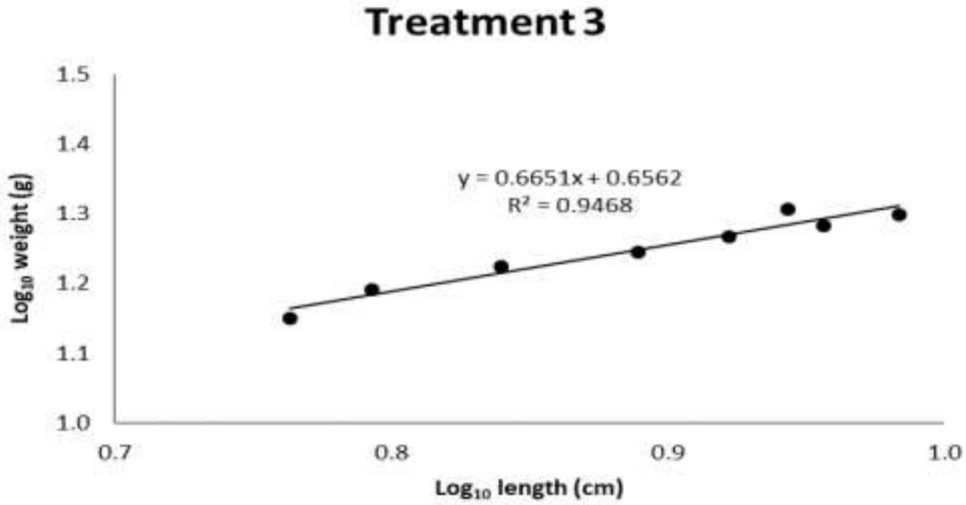



**Figure 32**: Length-weight relationship of Koi carp in Treatment-3

### 4.5.4 Length-weight relationship in Treatment-4

In T-4 (15% seaweed diet), there was a positive line found against the mean weight data and 91% mean length-weight was gained is accounted for its regression on experimental duration.

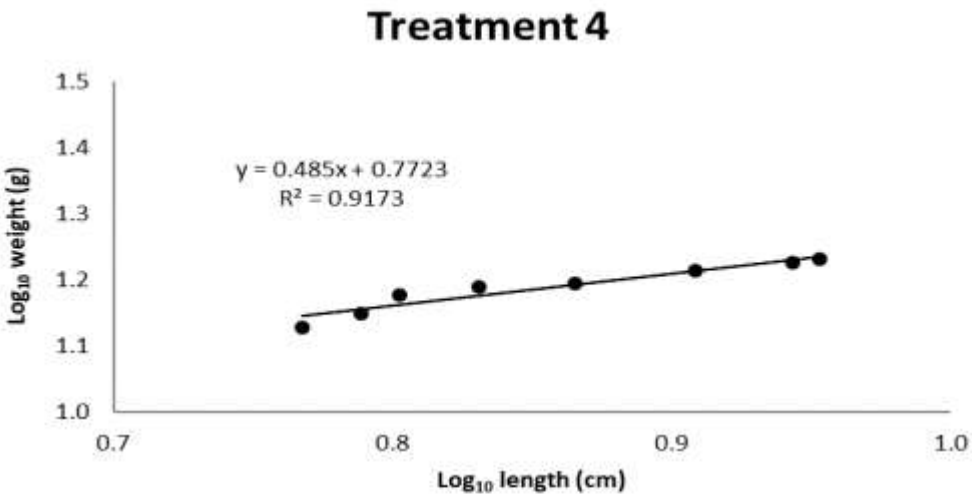



**Figure 33:** Length-weight relationship of Koi carp in Treatment-4

## 4.6 Hematological parameters of Koi carp (*Cyprinus rubrofuscus* )

Hematological parameters are important factor in growth and development of any organisms. Different type of parameters indicates different function for organism's body. Fish blood parameters also indicate its body conditions. Some of the important blood parameters value found in this research of Koi carp is given below.

**Table 10**: Hematological Parameters of Koi carp (*Cyprinus rubrofuscus*)

| Parameters | T-1 (Control, 0% seaweed) | T-2 (5% seaweed) | T-3 (10% seaweed) | T-4 (15% seaweed) |
|---|---|---|---|---|
| Hg (g/dl) | 8.63±0.17 | 11.66±0.17 | 9.26±0.17 | 7.56±0.12 |
| Glucose (mmol/l) | 5.06±0.12 | 6.33±0.26 | 5.53±0.12 | 5.3±0.14 |
| Serum Protein (g/dl) | 3.41±1.08 | 6±0.4 | 3.83±1.17 | 3.5±0.4 |
| Refractive index (RI) | 1.342±0.002 | 1.347±0.008 | 1.343±0.001 | 1.342±0.001 |
| Spleen somatic index | 0.12±0.01 | 0.14±0.01 | 0.13±0.02 | 0.13±.001 |

From Table 9, blood parameters show different value in different treatments. T-2 (5% seaweed) shows the highest value of hemoglobin where T-1 (Control, 0% seaweed) shows the lowest value then other treatments. Glucose concentration also changed due to variation of dietary feed in every treatment. The highest glucose value found in T-2 (5% seaweed) which is 6.33 mmol/l and the lowest in T-1 (Control, 0% seaweed). Serum protein and spleen percentage are also better in other treatments than T-1 (Control, 0% seaweed).

### 4.6.1 Hemoglobin

Hemoglobin is particularly important in fish adaptation as they constitute an interface between the organism and the environment. Fish particularly face a very variable environment and temporal and spatial alterations in oxygen availability, in contrast to terrestrial animals. Structurally, maximum fish hemoglobins are tetrameric; however, those from particular specific species such as lamprey and hagfish dissociate, being monomeric when oxygenated and oligomeric when deoxygenated. In this study, the higher value of hemoglobin found in T-2 (5% seaweed) which is better than the Control (T-1) and T-4 (15% seaweed) shows the lowest value. Ilham *et al* (2018) found 6.46 g/dl in treatment of gold fish with *Sargassum sp.* In this study, hemoglobin value is in quite satisfactory level in T-2 (5% seaweed).

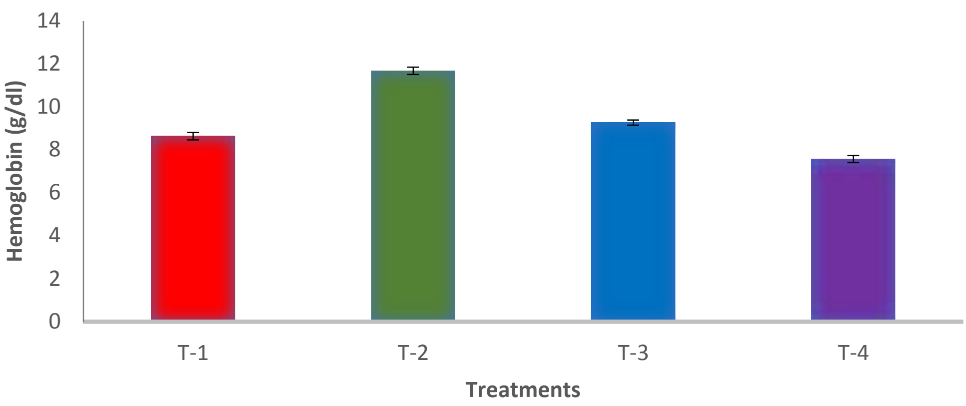



**Figure 34**: Blood hemoglobin is much higher in seaweed treatments compared to control. However due to low feed intake in higher percentage of seaweed inclusion that the value was found low.

### 4.6.2 Glucose

Glucose is an excellent source of energy for any living things. Body glucose provides energy to organisms for movement or other works. Fish body glucose plays very important role in its survival and movement. In this study, T-2 (5% seaweed) shows the higher value of body glucose which is 6.33 mmol/l and the lowest value is found in T-1 (Control, 0% seaweed) which is 5.06 mmol/l. Abdullah *et al.* (2001) found blood glucose values for *Cyprinus carpio* juveniles after exposure for (96) hours to different concentrations of copper in summer season. They used 1-3 mg/l copper concentration and one control treatment and found 29.47 gm/dl in control treatment and highest value found in treatment 4 which was 34.84 g/dl (1 mg/dl= 0.0555 mmol/l). In this study, we found higher value of glucose.

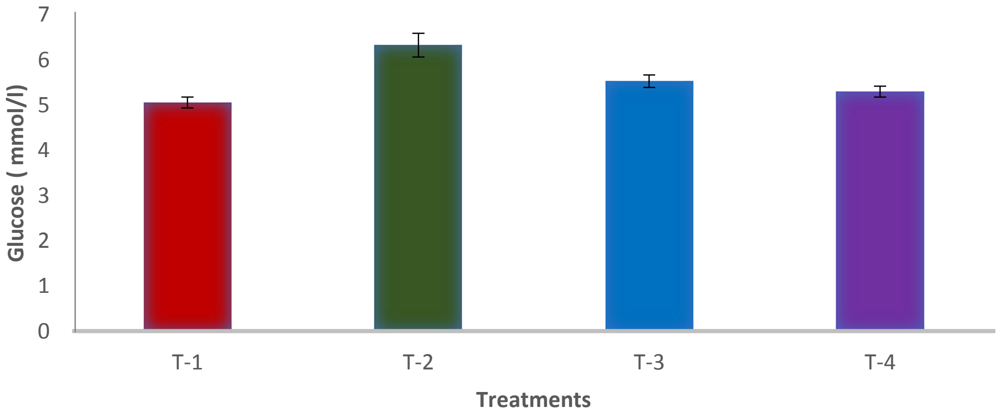



**Figure 35**: Average glucose percentage showed higher in seaweed treatments compared to control

### 4.6.3 Refractive index

Refractive index of an optical medium is a dimensionless number that gives the indication of the light bending ability of that medium. The refractive index determines how much the path of light is bent, or refracted, when entering a material. RI value changes with the value of serum protein level . In this study, the refractive indexes of Koi carp blood serum are 1.342 RI, 1.347 RI, 1.343 RI and 1.342 RI in T-1, T-2, T-3 and T-4 respectively, where T-2 (5% seaweed) blood serum shows the higher refractive index value then other treatments. T-1 (Control, 0% seaweed) and T4 (15% seaweed) shows the similar value of RI. L. E. Moor *et al.* (2000) showed that higher refractive index value indicates better nutrient and immune system in aquatic organism.

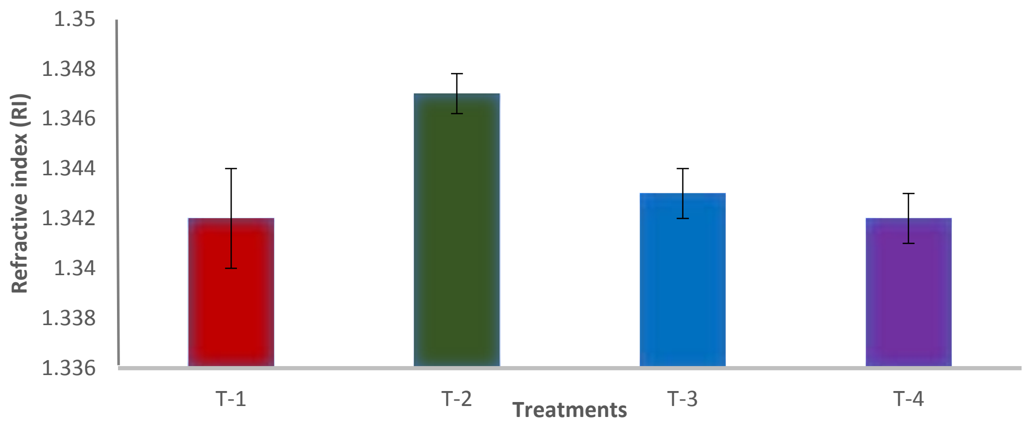



**Figure 36**: RI value was significantly high in 5% seaweed (T-2) diet. That indicates the highest immunity of fishes in this treatment.

### 4.6.4 Spleen Somatic Index of Koi carp

The spleen is the predominant erythropoietic tissue in many fish species including elasmobranchs (sharks, rays), holocephalans (rabbitfish: *Chimaera*), and a few teleosts (*Perca*, *Scorpaena*). It is an important organ in the immune function of fish, and it is also important for hematogenesis and antibody and granulocyte production. In this study the higher SSI found in T-2 (5% seaweed) which was 0.14% of the total body weight is and lowest value found in T-1 (Control, 0% seaweed) which 0.12% of total body weight. Yang He *et al.* (2021) found different SSI percentage in fish body depending on their size, weight and age. He found 0.03-.014 % of spleen in *Oreochromis niloticus*.

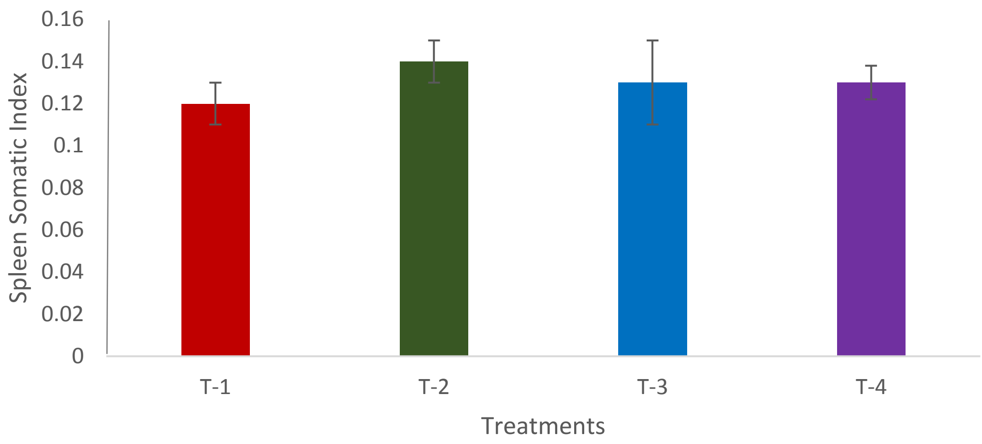



**Figure 37**: 5% seaweed (T-2) diets showed significantly higher SSI (%) compared to Control.

### 4.6.5 Serum protein

Serum protein is widely used to monitor stress situations in fish and can be used as an indicator of poor rearing conditions in fish. In this study serum protein varies in different treatment which is 3.41 g/dl, 6 g/dl, 3.8 g/dl and 3.5 g/dl in T-1, T-2, T-3 and T-4 respectively where T-1 (0% seaweed) shows the lowest value of serum protein. J.-L. Coeurdacier *et al.* (2011) cultured sea-bass for 90 days and found 43-50 mg/ml (1mg/ml= 0.1 g/dl) serum protein in different experimental samples. Kulkarni (2021) found 5.2 g/dl and 4.6 mg/dl serum protein in *Labio rohita* and *Pangusius becourti*. In this study T-2 shows the highest serum protein value which is 6 gm/dl.

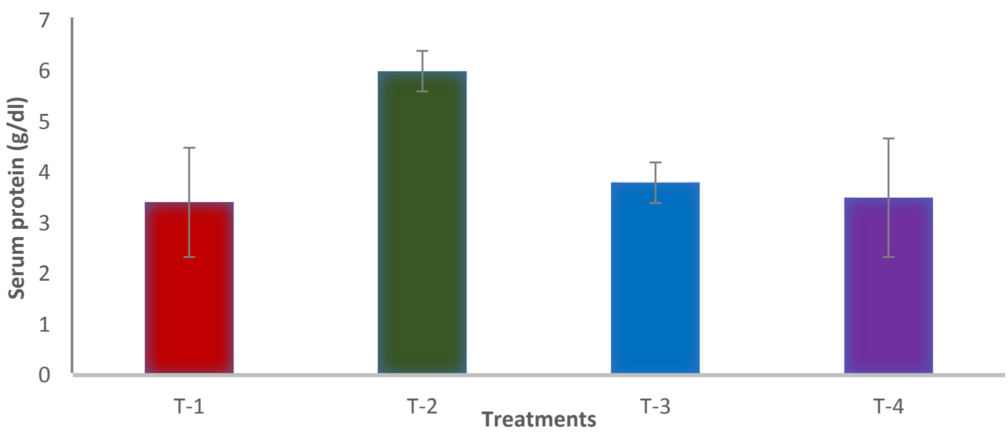



**Figure 38**: Serum protein was significantly higher in 5% seaweed (T-2) diet compared to Control.

# CHAPTER V
# SUMMARY AND CONCLUSION

Ornamental fish trade has a great opportunity in domestically as well as in the world market. But regrettably, in Bangladesh the business of ornamental fish is cramped to its own region till now. We have enormous resources, satisfactory environment and a claimable market in our country. A little awareness may escort enormous profit in fisheries sector. Maximum of the fishes of ornamental fish introduced from foreign country and it charges a lot of money every year importing ornamental fish to encounter the demand of the country. We have a proficient assortment of chromatic indigenous fish species that may be utilized as ornamental purposes and can reclaim these money and apparently can be esteemed as a very possible mean of export earnings. If we reproduced them appropriately and export them, then we can gain a lot of foreign exchange for our country.

In our country, the value of aquarium fish is much higher than culture fish which is consumed as food. The segment between the space needed for aquarium fishes and culture fishes is very significant. Aquarium fishes require a small amount of area to conserve, reproduction and the management cost is cheap compared to the price where culture fish species require a huge space, rearing costs, and comparing to the price. In example, the value of Thai pangasius (*Pangasius hypophthalmus*) is 90-120 BDT/kg but the similar species are familiar as tiger sharks rear in the aquarium an 80-360 BDT per couple which is greatly higher price than its cultural value. Some ornamental fish pair's price is more than 50000-80000 BDT. Now days, a pair of mature Koi carp is found at more than 12000 BDT in our country.

Freshwater ornamental fish culture is carried out by small and marginal farmers who are employing a low input level. The research was performed in "Aquaculture Lab" at Sher-e-Bangla Agricultural University. The experiment was accomplished to assess the growth performance potentiality and hematological development of Koi carp (*Cyprinus rubrofuscus*) and evaluate the nutritional value by using seaweed in diet after completing the rearing through mechanized aquaculture system.

Four iso-proteinas diet were prepared for each group of fishes where every treatment contains three replications. Treatment-1 was used as control (0% seaweed diet) treatment. In Treatment-2, 3 and 4 stocking density was similar as control tanks. The average protein percentage was approximately 27.59%, fat 6.1%, carbohydrate 48.96% and ash 6.33% but different percentages of seaweed were used with dietary feed like 5%, 10% and 15% respectively.

Important thing of aquarium culture system is maintaining the water quality parameters properly. The crucial parameters of water quality like Temperature, DO, pH and ammonia ranged from 27.33±0.34 to 27.80±0.27 $^0$C, 7.04±0.52 to 7.71±0.72 mg/l, 8.27±0.01 to 8.33±0.06, 0.5 mg/l respectively among the treatments were considered to optimum level. The remaining parameters of water quality like TDS, Nitrite were also in optimum level and ranged from 199±5.7 to 279±9.223mg/l and 0.03 to 0.05 mg/l. Except TDS, there was no significant variation in water quality parameters among the treatments.

The highest mean final weight, mean length and mean weight gain were found in T-2 (5% seaweed diet) which was 22.60±1.15 g, 10.57±0.58cm and 8.06±1.58 g. It was observed that, fishes consumed 5% seaweed diet (T-2) much higher compared to higher inclusion of seaweed. In case of survival rate, the highest rate was observed in T-2 (5% seaweed diet) and T-4 (15% seaweed diet) treatments and lowest was found in T-3 (10% seaweed diet)). Treatment-2 (5% seaweed diet) gave the best specific growth rate (14.41±2.85%). The lowest value for ADG was found at 0.08±0.001g and SGR at 8.42±0.93% in T-4 (15% seaweed diet). Liner regression of length- weight relationship also showed that T-2 (5% seaweed diet) represents 100% efficiency of the treatment where T-4 (15% seaweed diet) showed the lowest result.

FCR is an important factor for fish culture. It helps to estimate the quantity of feed that will be required in the growing cycle. The highest FCR was calculated 1.43±0.26 in T-2 (5% seaweed diet) and lowest was in T-4 (15% seaweed diet). It indicating that higher percentage of seaweed didn’t affect Koi carp but lower FCR was recorded in the highest percentage of seaweed diet. It must be due to off flavor of seaweed that didn’t attract Koi carp to consume feed.

Hematological parameters analysis showed the best result in 5% seaweed diet (T-2). The best Hemoglobin amount was found 11.66±0.17 g/dl found in T-2 (5% seaweed diet) and the lowest value 7.56±0.12 g/dl in T-4 (seaweed diet). T-3 (10% seaweed diet) showed better hemoglobin value than Control (T-1, 0% seaweed). Body glucose is an important source of energy which provides energy during stress condition of fish. In this study the highest value of glucose found in T-2 (5% seaweed diet) which was 6.33±0.26 mmol/l and T-1 (Control, 0% seaweed) showed the lowest value of 5.06±0.12 mmol/dl.

Blood serum protein level was comparatively found better in that T-1 than other treatments. The highest serum protein 6±0.4 g/dl found in T-2 (5% seaweed diet). Refractive index was also found better in T-2 (5% seaweed diet) than other treatment where T-1 (control, 0% seaweed) and T-4 (15% seaweed diet) showed the same value of refractive index. Hematological parameters showed that seaweed supplemented diet is better than Control and 5% seaweed (T-2) diet showed best result in the experiment. Spleen is an important organ in the immune function of fish, and it is also important for hematogenesis and antibody and granulocyte production. in this study T-1 showed the lowest percentage of SSI (0.12%) than other treatments. T-2 (5% seaweed diet) showed 0.14% of SSI where T-3 and T-4 showed 0.13% of SSI.

At last, it can be concluded that Koi carp (*Cyprinus rubrofuscus*) culture could be performed in aquarium aquaculture system for meeting the development of ornamental fish. Application of formulated dietary feed with 5% seaweed (T-2) provided the best outcome in case of growth performance and hematological parameters. There was no significant difference in water parameter among the treatments. FCR was found better in seaweed diets but higher FCR was recorded in the highest percentage of seaweed (T-4, 15% seaweed) diet. It must be due to its off flavor that didn't attract koi carp to consume feed. As a result, lowest growth rate was also recorded in the highest inclusion of seaweed treatment (T-4, 15% seaweed). However, 5 seaweed (T-2) showed higher DWG, SGR (%), MWG, survival rate (%) and SGR compared to Control (T-1). Hematological parameters also showed that immune system was better in the 5% inclusion of seaweed in diet. It can be told that

Koi carp and other fish species can be cultured by feeding formulated dietary feed supplemented with seaweed. The results of the research may become an instruction for upcoming researcher and a recommendation for farmer who are interested in this process in Bangladesh and other countries in the world as well.

# CHAPTER VI

# RECOMMENDATIONS

The fish growth parameters and hematological parameters showed by 5% seaweed supplemented diet was satisfactory. Good quality with high food value is necessary for profitable aquaculture although it is very scarce in the market. To fulfill the quality feed, the present study tried to formulate a high quality but at low-cost fish diet n laboratory condition. At the same time the feed is evaluated with brown seaweed (*Sargassum vulgare*) and applied on *Cyprinus rubrofuscus* and found the positive result. The process of formulation of quality feed in a continuous process and after a long-time work, It can be formulate more fish feed with low cost which will help in fish production. The further works may be done, for example,

- Feed can be formulated using different ingredients i.e., Blood Meal containing high protein level and so on.
- Recommended doses of seaweed is 5% for better performance.
- Formulation of feed for other fish may be done maintaining maximum efficiency and minimum cost.
- Using other species of seaweed can also influence the growth performance of fish species.
- Seaweed formulated diets can be tested other species.
- Can be tested other species of seaweed as fish feed ingredient.

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# APPENDICES

**Appendix I:** Different feed ingredients. (a) Wheat bran, (b) wheat powder, (c) Trash fish, (d) Fish meal, (e) Mustard oil cake, (f) Seaweed (*Saragassum vulgare*), (g) Dried seaweed, (h) Rice bran, (i) Trace mineral premix, (j) Vitamin premix, (k) Fish oil, (l) Barley.

(a)

(b)

(c)

(d)

(e)

(f)

(g)

(h)



(i) (j)

(k) (l)

**Appendix II:** Weight of cultured *Cyrinus rubrofuscus* in glass aquarium at 7 days (1 week) interval

| Treat - ments | Rep. | Weight (g) at Sampling Schedule (per 1 weeks) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Initial value** | **1st week** | **2nd week** | **3rd week** | **4th week** | **5th week** | **6th week** | **7th week** | **8th week** |
| T-1 (Control) | 1 | 16.2 | 17.5 | 18.2 | 19.7 | 21.25 | 21.7 | 22.91 | 23.78 | 27.87 |
| T-1 (Control) | 2 | 11.6 | 11.9 | 12.5 | 13.4 | 13.87 | 14.64 | 15.32 | 16.41 | 16.94 |
| T-1 (Control) | 3 | 15.5 | 15.8 | 16.6 | 17.5 | 18.2 | 19.43 | 20.66 | 21.43 | 21.88 |
| T-2 | 1 | 14 | 15.2 | 16.1 | 16.9 | 17.66 | 18.56 | 19.71 | 20.27 | 21.56 |
| T-2 | 2 | 14 | 15.9 | 17.4 | 18.8 | 20.62 | 22.01 | 23.15 | 24.08 | 24.21 |
| T-2 | 3 | 15.6 | 16.1 | 17.3 | 17.9 | 18.73 | 19.92 | 20.24 | 21.94 | 22.02 |
| T-3 | 1 | 16 | 16.7 | 17.3 | 18.1 | 18.99 | 19.16 | 19.54 | 19.98 | 20.64 |
| T-3 | 2 | 10.1 | 11.2 | 12.4 | 13.7 | 15.07 | 16.75 | 17.53 | 18.61 | 18.95 |
| T-3 | 3 | 13.2 | 14.5 | 16.9 | 18.5 | 18.7 | 19.65 | 20.53 | 21.09 | 21.25 |
| T-4 | 1 | 15.5 | 14.9 | 15.7 | 16.2 | 16.5 | 16.68 | 17.47 | 18.03 | 18.26 |
| T-4 | 2 | 11 | 11.8 | 12.3 | 13 | 13.8 | 14.02 | 14.63 | 15.14 | 15.33 |
| T-4 | 3 | 13.1 | 13.6 | 14.3 | 15.8 | 16.11 | 16.41 | 16.95 | 17.20 | 17.47 |

**Appendix III:** length of cultured *Cyrinus rubrofuscus* in glass aquarium at 7 days (1 week) interval

| Treat-ments | Rep. | length (cm) at Sampling Schedule (per 1 weeks) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial value | 1st week | 2nd week | 3rd week | 4th week | 5th week | 6th week | 7th week | 8th week |
| T-1 (Control) | 1 | 6.75 | 7.06 | 7.54 | 7.98 | 8.21 | 8.33 | 8.7 | 9.11 | 9.32 |
| T-1 (Control) | 2 | 5.45 | 5.68 | 5.91 | 6.23 | 6.54 | 7.15 | 7.56 | 8.56 | 8.87 |
| T-1 (Control) | 3 | 6.16 | 6.31 | 6.65 | 6.81 | 7.03 | 7.51 | 8.48 | 9.49 | 9.65 |
| T-2 | 1 | 6.01 | 6.46 | 6.92 | 7.97 | 8.28 | 8.73 | 9.25 | 9.86 | 10.63 |
| T-2 | 2 | 605 | 6.54 | 7.01 | 7.55 | 8.06 | 8.94 | 9.68 | 10.55 | 11.25 |
| T-2 | 3 | 6.17 | 6.44 | 6.98 | 7.41 | 8.01 | 8.68 | 9.01 | 9.57 | 9.83 |
| T-3 | 1 | 6.95 | 7.03 | 7.25 | 7.61 | 7.93 | 8.65 | 9.28 | 9.98 | 10.26 |
| T-3 | 2 | 4.56 | 4.92 | 5.45 | 6.38 | 7.51 | 8.03 | 8.87 | 9.43 | 9.52 |
| T-3 | 3 | 5.61 | 5.45 | 5.93 | 6.78 | 7.81 | 8.41 | 9.01 | 9.51 | 9.65 |
| T-4 | 1 | 6.03 | 6.32 | 6.59 | 6.82 | 7.23 | 7.97 | 8.71 | 9.03 | 9.11 |
| T-4 | 2 | 5.48 | 5.71 | 5.96 | 6.24 | 6.58 | 7.05 | 7.66 | 8.43 | 8.63 |
| T-4 | 3 | 5.38 | 5.57 | 5.91 | 6.01 | 6.55 | 7.01 | 7.95 | 8.90 | 9.2 |

**Appendix IV:** Growth parameters of Koi carp

| Treat-ments | Rep. | Features | | | | | |
|---|---|---|---|---|---|---|---|
| | | Weight Gain (gm) | Weight Gain (%) | SGR (%) | ADG (gm) | Survival rate (%) | FCR |
| T-1 (Control) | 1 | 8.67 | 47.15 | 15.48 | 0.17 | 90 | 1.28 |
| T-1 (Control) | 2 | 5.34 | | 9.53 | 0.09 | 100 | 2.09 |
| T-1 (Control) | 3 | 6.38 | | 11.39 | 0.11 | 100 | 1.75 |
| T-2 | 1 | 7.56 | 55.86 | 13.5 | 0.13 | 100 | 1.48 |
| T-2 | 2 | 10.21 | | 18.28 | 0.18 | 100 | 1.09 |
| T-2 | 3 | 6.42 | | 11.46 | 0.11 | 100 | 1.74 |
| T-3 | 1 | 4.64 | 54.80 | 8.28 | 0.08 | 100 | 2.41 |
| T-3 | 2 | 8.85 | | 15.8 | 0.15 | 80 | 1.58 |
| T-3 | 3 | 8.05 | | 14.37 | 0.14 | 100 | 1.39 |
| T-4 | 1 | 5.46 | 34.37 | 9.75 | 0.09 | 100 | 2.05 |
| T-4 | 2 | 4.33 | | 7.73 | 0.08 | 100 | 2.58 |
| T-4 | 3 | 4.37 | | 7.80 | 0.07 | 100 | 2.56 |

**Appendix V:** Hematological parameters in different treatment of Koi carp

| Treat-ments | Rep. | Features | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Hg (gm/dl) | Glucose (mmol/l) | Refractive Index (RI) | Serum protein (gm/dl) | Spleen weight (gm) | Fish body weight (gm) | Spleen somatic index (%) |
| T-1 (Control) | 1 | 8.8 | 4.9 | 1.345 | 5 | 0.02 | 18 | 0.11 |
| T-1 (Control) | 2 | 8.7 | 5.1 | 1.340 | 2.5 | 0.02 | 15 | 0.13 |
| T-1 (Control) | 3 | 8.4 | 5.2 | 1.342 | 3 | 0.03 | 22 | 0.13 |
| T-2 | 1 | 11.9 | 6.7 | 1.347 | 6 | 0.05 | 36 | 0.14 |
| T-2 | 2 | 11.5 | 6.1 | 1.346 | 5.5 | 0.04 | 28 | 0.14 |
| T-2 | 3 | 11.6 | 6.2 | 1.348 | 6.5 | 0.04 | 25 | 0.16 |
| T-3 | 1 | 7.6 | 5.4 | 1.342 | 3.5 | 0.03 | 20 | 0.15 |
| T-3 | 2 | 7.4 | 5.1 | 1.344 | 4 | 0.02 | 19 | 0.1 |
| T-3 | 3 | 7.7 | 5.4 | 1.341 | 3 | 0.04 | 27 | 0.14 |
| T-4 | 1 | 9.1 | 5.5 | 1.345 | 5.5 | 0.04 | 32 | 0.12 |
| T-4 | 2 | 9.5 | 5.4 | 1.343 | 3 | 0.03 | 22 | 0.13 |
| T-4 | 3 | 9.2 | 5.7 | 1.342 | 3 | 0.04 | 27 | 0.14 |