Effects of algal meal in prepared feed on growth, body composition and survival of the Common carp (*Cyprinus carpio* var. *communis)*

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Ishrat Bashir
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Thesis

Submitted to

Faculty of Fisheries
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in partial fulfilment of requirement for the award of the degree of

Master of Fisheries Science
(Fish Nutrition and Feed Technology)

2021

Dedicated

To my

beloved Mother

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Fisheries, Rangil, Ganderbal

Certificate – I

This is to certify that the thesis entitled, **"Effects of algal meal in prepared feed on growth, body composition and survival of the Common carp (*Cyprinus carpio* var. *communis)*"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Fisheries Science (Fish Nutrition and Feed Technology)**, to the **Faculty of Fisheries, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** is a record of bonafide research work carried out by **Ms. Ishrat Bashir (Reg. No. MSFY/2018/90)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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Certificate – II

We, the members of the Advisory Committee of **Ms. Ishrat Bashir (Reg. No. MSFY/2018/90)**, a candidate for the degree of **Master of Fisheries Science (Fish Nutrition and Feed Technology)** have gone through the manuscript of the thesis entitled, **"Effects of algal meal in prepared feed on growth, body composition and survival of the Common carp (*Cyprinus carpio* var. *communis)*"** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of the degree.

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Certificate – III

This is to certify that the thesis entitled, **"Effects of algal meal in prepared feed on growth, body composition and survival of the Common carp (*Cyprinus carpio* var. *communis*"** submitted by **Ms. Ishrat Bashir (Reg. No. MSFY/2018/90)** to the **Faculty of Fisheries, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** in partial fulfilment of the requirements for the award of the degree of **Master of Fisheries Science (Fish Nutrition and Feed Technology)** was examined and approved by the Advisory Committee and External Examiner on …………….

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ABSTRACT

The present study was conducted to evaluate the effect of algal meal on growth, body composition and survival of Common Carp (*Cyprinus Carpio var. communis*) through dietary supplementation. In order to check this hypothesis, algal bloom were collected from Dal lake of Srinagar and after identification algal mixture of *Spirogyra,Oedogonium* and *Ulothri*x were found in the mixture. Proximate analysis of the algae was conducted and the results revealed that the Spirogyra contains 25% crude protein, *Oedogonium* contains 14% of crude protein and Uothrix contains 45% of crude protein. Keeping in view the protein content of algae, an algal meal was prepared and incorporated into the diet of Common Carp fingerlings at 35% crude protein level. A sixty day feeding trial was carried out to elucidate the effects of algal meal at different inclusion levels (0.0%, 10.0%, 20.0%, 30.0%, 40.0% and 50.0%) in the diet of *Cyprinus Carpio* fingerlings to study response on growth. The results of present study revealed that percentage weight gain, specific growth rate and feed conversion ratio exhibited significantly higher ($p<0.05$) values in treatment group (T_4) fed with 40% algal meal. Highest ($p<0.05$) weight gain was observed in treatment group (T_4)

compared to control treatment group (T_0). Highest SGR was recorded in treatment group (T_4) compared to control group and other treatment groups (T_2, T_3 and T_5). Lowest FCR was observed in treatment group T_4 whereas highest FCR was observed in control group followed by other treatment groups. Protein efficiency ratio showed significant increase in treatment group (T_3) fed with algal meal at 30% inclusion level compared to control group and other treatment groups(T_1, T_2, T_4 and T_5). The results revealed that there was no significant difference in survival rate in treatment groups fed with algal meal at different inclusion levels. Survival rate was 100% in all treatment groups and in control group. The results obtained in the present study suggest that the dietary inclusion of algal meal at 40% augments the growth, body composition and survival of *Cyprinus Carpio* fingerlings. The findings of the present study also revealed that the fish meal in the diet of common carp may be replaced upto 40% without affecting growth. It will have direct bearing on reduction of cost of production and making feed cost effective.

Key words:

Signature of Student | Signature of Major Advisor

Dated____________ | Dated _________________

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Ishrat Bashir

Place: Rangil, Ganderbal

Dated:

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Chapter 1

INTRODUCTION

"No fish-no dinner…."

"Give a man a fish and you will feed him for a day.

Teach him how to fish and he can feed his family forever"

Old Chinese proverbs

Food security is considered achieved "when all people at all times have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preference for an active healthy life"(UN FAO, 2012). The term nutritious food is important as only a balanced diet can serve the requirements of a body. An important constituent of nutritious food is protein and what better source of protein than fish. In many of the underdeveloped nations of Asia and Africa fish constitutes 50% of total animal protein intake (FA0, 2020). Freshwater and marine fish are often considered to be healthy component of human diet due to relatively high rate of polyunsaturated to saturated fatty acids (PUFA:SFA) compared to other animal source protein. Therefore for improving the quality of the food in underdeveloped and developing regions of the world like India, sustainable development of aquaculture can provide the same. This strategic importance of fisheries sector was recognized way back in 1996 in a conference on Sustainable contribution of fisheries to food sector organized by Food and agricultural organization of United Nations (UN).

Fisheries has always played a very significant socioeconomic role in many countries and communities; as a subsistence produce, fish is a vital resources towards poverty reduction and food security for poorer households (UN FAO, 2018). Fisheries provide not only food but also employment to millions of fish folk in South Asia for example whole of Maldivian population is considered a fishing community. Labour demographics of India indicate that near about half of the workforce lies in the agriculture and allied sectors. Aquaculture represents a substantial percentage of the sector (0.91 percent) of total GDP and 5.23 per cent of agriculture GDP (National fisheries development board). Therefore we can increase per capita income of labour sector by improving the aquaculture.

The consumption of fish has doubled since 1973 and underdeveloped nations have been responsible for 90 per cent of this increase. In 2016 fish produce was at 171 million tones (MT) (FAO). World aquaculture production of aquatic animals is dominated by Asia with 89 per cent share in the last two decades. China leads the way with closely followed up by India, Indonesia, Vietnam, Bangladesh. Over 85.7 per cent of fish production were freshwater fish species (carps, tilapia and catfish) and to a lesser extent diadromous fish species (9.2 pc including salmonids etc) and marine fish species.

Indias aquaculture sector is too vibrant with 11 fold increase in fish production in just six decades i.e from 0.75 million tonnes in 1950-51 to 9.6 million tonnes during 2012-13. This has placed the country only after china in global fish production. The foreign exchange earnings were to a tune of US$ 3.51 Billion (2012-13). Freshwater aquaculture constitutes to over 95 per cent of total aquaculture production. Carp production forms the bulk of freshwater aquaculture. The three Indian carps, namely Catla (*Catla catla*), rohu (*Labeo rohita)* and mrigal (*Cirrhinus mrigala*) form upto 75 per cent of total freshwater fish production, followed by silver carp, grass carp, common carp, catfishes forming the rest of 25 percent. According to estimates only 40 per cent of available area of ponds and tanks has been used putting onus for development of freshwater aquaculture. The culture systems of country are classified as low, medium and high input technologies. In the low input system productivity is increased by using low cost inputs like organic and in organic fertilizers, aquatic weeds etc. In medium input system, supplementary feeding is provided apart from fertilizers for enhancing fish production. In high input system higher feed inputs are used. While the low input system is used for indigenious carps, other systems are used for exotic species. Standard practices in carp culture include: 1) stocking of carp at combined densities. 2) Pond fertilization from organic and inorganic fertilizer 3) provision of supplementary feeds in the form of rice bran and groundnut oilcake in equal ratio. From the above discussion it is clear that feeding cost has major say in development of freshwater carp aquacultutre and hence the need for studies to reduce the feeding cost.

Union Territory of Jammu and Kashmir has tremendous potential for aquaculture due to large number of cold water resources which are ideal for a number of fish species. The UTs production is already beyond 20 thousand tones with Kashmir province contributing 80 per cent of total production. The main fish species cultured in Kashmir province are trout, mirror carp and common carp. Today, the main problem which UT is facing is the supply-demand gap in fish production. While the fish consumption demand stands at 70, 000 tonnes, supply is lagging far behind at 20 thousand tonnes. In order to meet the growing demand of consumers fish culture must be intensified. While doing intensive fish culture, artificial feeding has an important role. As feed is the major input cost of any aquaculture activity there is the need to explore cheap and locally available ingredients. Since the UT of Jammu and Kashmir is mainly culturing trout, there is need of introducing more resilient and fast growing fish species like common carp to meet the fish consumption demand of the UT.

The growth of aquaculture is expected to double in coming 10-15 years with the requirement of fish meal to grow from 32MT TO 50MT. Feed as a part of aquaculture is a variable cost of aquaculture economics, therefore if we can decrease the cost of fish meal, we can make aquaculture as a viable source of income for the population. So, if we can replace the costlier proteins part of fish meal by some cheap and easily available proteins, the variable cost can come down significantly. The replacement must have same nutritional value at competitive cost. This replacement strategy has been studied extensively. Use of plant protein like from grains and oilseeds in place of animal protein is one way. The problem with plant based substitutes is that they lead to changes in the nutritional quality of fish produced. Use of terrestrial protein sources like algae in place of plant protein has been studied extensively with positive outcomes. Two basic factors which make algae both macroalgae (weeds) and microalgae (phytoplanktons) as good candidates for use as replacements in fish meal are: 1) algae are at the base of aquatic food chains that produce the food which fish readily consume. 2) immense biochemical diversity. Algae are found to contain all the essential amino acids required for fish growth, taurine: the non protein sulphonic acid an essential nutrient, polyunsaturated fatty acids (PUFA) another essential growth nutrients. The effects of algal replacement are best seen in larval feeds which is why most of studies are on fingerlings. The choice of the algae for fish feeding studies appear to have been selected largely for convenience because of their low cost and availability. There is both environmental and economical impact of the present study which needs to be evaluated. There is a great variety of algae and fish about which studies have been made indicating a great synergy and compatibility between the algal species as replacement in fish meal and the target fish species. One important feature arrived at from the various studies is that the replacement strategy works best upto a certain statistically arrived percentage, after that the benefits taper off gradually like the famous bell shaped curve. The parameters under study in these studies were growth parameters and physiological parameters. The growth factors considered were weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and protein productive value (PPV). The physiological factors considered were lysozyme activity, complement system and immunoglobulin levels. All of the above said parameters reported an improvement to significant levels upon substitution by algae in fish meal.

The experimental fish selected in the present study *viz* common carp (*Crypinus carpio*) is easily available and is having well developed breeding technology in the UT of JK. Common carp is high quality edible fish, deeply appreciated by consumers in Jammu and Kashmir with relatively more economic importance. On account of its great adaptability, quick growth, fleshy nature and ease of reproduction makes it suitable species for cultural practices in Kashmir. Common carp bears the low oxygen density and high carbon dioxide density dissolved in water than any other carp making it the most hardy aquaculture fish species.

Keeping in view the importance of Common carp and its culture under controlled conditions, there is a need to standardize its feed with the introduction of cheap and locally available feed ingredients having good nutritional profile. In the present study the algae was selected as a major ingredient having good percentage of protein from algal bloom of Dal lake. The algal mixture was identified as per standard protocol and was found to contain following algae: Spirogyra, Ulothrix and Oedogonium. After identification, appreciable amount of algae were collected and then analysed for its nutritional profile. Nutritional value of the algae were analysed through proximate experimental examination in the laboratory of Fish Nutrition and Biochemistry, Rangil. The nutritional profile analysed was in agreement with values documented in literature. The total content of protein (% dry weight) ranged from 45% (*ulothrix)* to 24% (*spirogyra*), carbohydrate from 20% (*ulothrix*) to 42% (spirogyra) and lipid from 5% (*ulothrix*) to 25% (*spirogyra*).

The present study has an environmental impact as the algae used were from algal bloom of Dal lake which is under constant threat of eutrophication from weed and algal attacks. There is a great scope for community participation as the fishing community around the Dal lake can be apprised about the benefits of the algae in fish feed due to its good nutritional profile and farmers may be trained to formulate and manufacture their own feed. This study holds promise for sustainable development of fishing community around the lake with adequate environmental benefits. The study also has an importance of utilizing algal meal as fish feed ingredient which otherwise pose serious threat to water body. Utilizing algal meal in fish feed as an important protein source, reducing the dependency of aquaculture on fishmeal and other important fish feed ingredients is key for sustainable development for the aquaculture industry of the J & K. One of the major bottlenecks in the expansion of coldwater fish culture in the Kashmir valley is the availability of quality fish feed at low cost. The local fish farmers of the valley are dependent mostly on either outside state for supply of fish feed or on State Fisheries Department, J&K. The State Fisheries Department also procures feed ingredients from outside state which results in high feed cost and increased cost of production and low economic returns to farmers. Recognizing the importance of proper feeding in successful fish production, the present study was aimed at formulation of low cost quality feed using locally available feed ingredients making the feed environmentally friendly as well. The study was also aimed to find the statistically arrived best replacement percentage substitution of algae meal against fish meal so that growth parameters and feed utilization can be maximized.

Chapter 2

REVIEW OF LITERATURE

Use of algae to replace fish meal has been studied extensively from the viewpoint of aquaculture and as an environmental issue. It is described as replacing the fish meal in predetermined proportions with certain algae and observing the correct replacement percentage for higher growth, feed conversion ratio and protein productive value. This is the focus of the present study to replace fixed proportions of fish meal with algal meal and arrive at correct proportions for common carp.

The overall replacement levels that have been studied are 0%(control), 10, 25, 50 & 75%.The replacements seems to work for wide range of fingerlings of different species like carp, tilapia, catfish etc. The replacement also seemed to work for a wide variety of algal species like *spirulina, scendsmus* etc. In general there is a great synergy and compatibility between the algal species as replacement and the target fish species. The growth and physiological factors like body weight gain (BWG), specific growth rate (SGR), protein productive value (PPV), digestibility, immune response, antioxidant defense, body composition all reported improvement to a significant extent.

Appler *et al. (*1982) studied the utilization of filamentous green alga (*Cladophora glomerata*) as a protein source in pelleted feeds for *Sarotherodon niloticus* (Tilapia) fingerlings. The experimental trial was conducted for 8 weeks where in duplicate groups of S. *niioticus* fingerlings were fed with each of five different diets. Diets with 30% crude protein was formulated by adding different proportions of fishmeal and C. *glomerata* meal. The five diets were formulated to supply fishmeal protein*: C. glomerata* meal protein ratios of 30: 0 (diet l), 25: 5 (diet 2), 20: 10 (diet3), 15: 5 (diet 4) and 10: 20 (diet 5) respectively. A sixth diet (diet 6) containing 25% crude protein provided by C. entirely *glomerata* meal was also fed. The highest growth rates and best protein utilization was substantially developed by 1 and 2 diets. Protein digestibility were found to be highest in diets 2 and 3 (94.5 and 93.9% respectively) although the values were not significantly different from other treatments.The weight gain and protein utilization declined as the level of algal protein increased in diets 2 to 6. Algal meals can thus be used a partial dietary protein sources for tilapia.

Mustafa *et al.* (1994) studied the efficiency of algal meal containing three different types of algae, a brown alga *Ascophyllum nodosum*, a red alga *Porphyra yezoensis* and a green alga *Ulva pertusa* in young red sea bream *Pagrus major*, as an important food material for fishes. Oregon moist diet comprising 5% of algae meal were fed for 41 days and the results were observed in terms of growth, feed efficiency and body composition. Algal feeding promoted body weight grain and uplifted feed efficiency and muscle protein deposition. Fish groups fed with algae enriched diets showed substantial increase in liver glycogen and triglyceride build up in muscle. The substancial effects on growth and energy accumulation was observed by feeding *Porphyra*, followed by *Ascophyllum* and *Ulva*. The study indicated the practical effeciency of using algae as a feed additive for the effective use of nutrients in cultured fish.

Nakagawa *et al.* (1997) evaluated the impact of *Ascuphyllum* meal (0, 2.5 and 5%) that was used as a feed additive for red sea bream, *Pagrus major.* The results of this study indicated that muscle protein of fish increased by the inclusion of algal meal in fish diet.

Novoa *et al.* (1998) studied the effect of fish meal replacement by of microalgae *Spirulina maxima* in the diets Tilapia (Oreochromis mossambicus) fry. Animal protein in the fish diet was replaced with algae protein at rations of 20%, 40%, 60%, 80% and 100%.The effect of algal substitution was compared with the control diet with fish meal as a sole protein source. These 6 treatments were tested in triplicate manner. The experiment was conducted for 9 weeks and fish were fed at the rate of 6% of their body weight. The results of this study displayed that growth rate and protein utilization of fish fed with 20% and 40% of *Spirulina* were elevated. Thus *Spirulina* can replace upto 40% of fish meal in tilapia diets.

Wassef *et al. (*2001) conducted a study to evaluate the effects on growth enhancement and muscle structure in the fingerlings of striped mullet (*Mugil cephalus L*), by feeding it with algal meal-based diets. Four different diets (D1±D4) were formulated with 10, 15, 20 and 25% Ulva. A fifth test diet (E) containing 40% dietary yeast enriched with vitamin E was also investigated. The trail was conducted for 15 weeks during which fish were fed with the test diets twice a day at 4% of biomass. The results of this study showed that the best weight gain and feed effeciency were obtained from fish fed with 20% dietary Ulva meal (diet D3), as well as the yeast based diet enriched with vitamin E (diet E). It also showed improvement in quality and firmness of muscles in fish.

Kim *et al.* (2002) conducted a nutritional study to investigate the impacts of dietary *Chlorella* powder supplementation on growth performance, blood characteristics and whole-body composition in juvenile Japanese flounder *Paralichthys olivaceus*. Four different diets were made with varing inclusion levels of 0, 1, 2 and 4% on a dry-weight basis. The results of this study indicated that dietary inclusion of 2% *Chlorella* powder in the commercial diets could uplift growth, feed utilization, serum cholesterol level and whole-body fat contents in juvenile Japanese flounder

Guroy *et al.* (2005) studied the effects of Green macroalgae (Ulva rigida) and brown macroalgae (Cystoseira barbata) meals as a feed additive on growth performance, feed utilization and body composition of nile tilapia, *Oreochromis niloticus* for a period of 12 weeks. The fish were fed to apparent fulness with formulated diets supplemented with various levels of *Ulva* meal (5%, 10%, or 15%) or *Cystoseira* meal (5%, 10%, or 15%).A diet without algae meal was taken as a control diet. The highest values for weight gain were obtained for fish fed with 5% *Cystoseira* diet, control diet and 5% *Ulva* diet. Fish fed with the feed of 15% *Ulva* meal displayed the poorest feed conversion ratio (FCR). Protein and energy utilization reduced in the fish groups fed with algae meals at the highest inclusion level of 15%. Carcass lipid levels declined with increment in the levels of *Ulva* meal, while there was the upliftment in the carcass lipid level with the rise in the levels of *Cystoseira* in the diet. These results indicated that *Ulva rigida* or *Cystoseira barbata* meals could be added in small percentages in tilapia diets.

Wassef *et at (*2005) conducted a nutritional study to examine effects of macroalgae *Pterocladia capillacea* (Rhodophyta) and *Ulva lactuca* (Chlorophyta) as feed additives in gilthead bream *Sparus aurata* L. feed. The trial was conducted for eight weeks in which four test diets having 0, 5, 10 & 15 % Pterocladia (PM) or Ulva meal (UM) were fed to triplicate groups of *S. aurata* fry (0.1+0.05 g). The fish were stocked at 100 fry/aquarium in glass aquaria of (110 L each) filled with sea water. The fish were fed thrice a day to their apparent satisfaction at a mean ambient temperature 21 oC. Results showed that feeding *S. aurata* 10% PM or 5% UM indicated the best growth performance, feed utilization, nutrient retention and survival. *Feeding S. aurata* fry 10%

PM had also greatly uplifted fish stress response after a 5-minutes air exposure test at the end of feeding trial. This study thus suggested the inclusion of dietary *Pterocladia* or *Ulva* meals at 10% or 5%, respectively in *S. aurata* fry diets.

Dallaire *et al*. (2006) performed a nutritional assay on rainbow trout fry (*Oncorhynchus mykiss*) by using a biomass of photosynthetic micro-organisms. The algal biomass was included in the feed at increasing levels (12.5%, 25% and 50%) to study its effects on survival (%), growth (length and mass) and carcass quality of the fish. The experiment was conducted for eight weeks at 10 °C. The results of this nutritional study depicted that algal biomass may not be used substantially at a high level as substitutes in rainbow trout diets. When algal biomass was incorporated in a proportion higher than 12.5%, the growth performance was significantly reduced.it was thus generalized that a maximum of 12.5% of algal biomass can be incorporated in the feed for rainbow trout fry (*O.*

mykiss) without negative impacts on growth and body content in lipids and energy of fish.

Badwyetal *et al.* *(*2008) investigated the following replacement levels of fish meal with dried microalgae (*Chlorella* spp. and *Scenedesmus* spp) in nile tilapia 0%, 10%, 25%, 50% and 75% (substitution). The parameters examined were fish growth performance, feed efficiency and body composition.The feed was given to 25 Nile tilapia fingerlings in groups for 90 days. The results indicated that parameters were significantly higher for substitution levels of 50% ($P<0.05$) and decreased for further higher substitution levels.bodies of the fish analyzed showed higher crude protein content.

Ergun *et al.* (2008) conducted a study to investigate the effects of dietary lipid levels and supplemental *Ulva* meal on growth performance, feed efficiency, nutrient utilization and body composition of juvenile Nile tilapia*, Oreochromis niloticus*. Four isonitrogenous diets with 0% and 5% Ulva meal were formulated to contain 10% and 20% crude lipid. Triplicate groups of fish were fed thrice a day for 16 week nutritional trial. The results indicate that 5% addition of *Ulva* meal at both dietary lipid levels enhances growth performance, feed efficiency, nutrient utilization and body composition of Nile tilapia.

Anna *et al.* (2009) studied the effects on growth, feed efficiency and carcass composition by the inclusion of red alga *Porphyra dioica* as a fish-feed ingredient for rainbow trout (*Oncorhynchus mykiss*). Isonitrogenous and isolipidic feed was formulated by adding *Porphyra dioica* meal at levels of 5, 10 and 15%. The control diet constituted commercial trout diet without seaweed meal. Fish weight raised on average from 107–261 g when the experimental groups were fed in triplicates for the time period of 12.5 weeks. Weightgain (WG), specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER) and apparent digestibility coefficient of the dry matter (ADCdm) was not influenced significantly by the addition of seaweed meal for any of the diets. Voluntary feed intake (VFI) raised for all seaweed diets realted to the control diet but not significantly (P>0.05). Final weight (FW) was substantially smaller for the 15% *P. dioica* inclusion. Carcass protein content uplifted for all three experimental diets and was substantially higher for the diet with 10% seaweed inclusion. This study also suggested that *P. dioica* can effectively be added in diets for rainbow trout up to 10% without any noticable negative effects on weight gain and growth performance. Addition of *P. Dioica* meal to the feed is also a considerable interest for organic salmon-farming industry.

Dantagnan *et al.* (2009) studied the effect on flesh fatty acid composition by the addition of macroalgae meal (*Macrocystis pyrifera*) as feed component for rainbow trout (*Oncorhynchus mykiss*). Four types of feed were formulated with different macroalgae inclusion levels of 0%, 1.5%, 3% and 6%. The experimental trail was conducted for 124 days in the tanks of 600L capacity that were stocked at the rate of 45 individuals per tank. After the termination of experiment it was evaluated that the macroalgae meal does not increase the quantity of protein and lipid contents of muscles but its addition at the rate of 3% and 6% resulted in upliftment in the levels of polyunsaturated fatty acids (PUFAs) especially EPA, DHA and LIN in the muscles of fish that is ultimately beneficial for human health.

Yildrim *et al. (*2009) evaluated the effects of diet containing two seaweed species, *Ulva lactuca* and *Enteromorpha linza*, on the growth performance, feed utilization and body composition of rainbow trout. Two experimental diets were formulated with the usage of 10% *U. lactuca* meal and 10% *E. linza* meal in feed and control group had no seaweed ingredients. Each experiment was triplicate and each group had fourteen fish specimens with an average weight of 32.96±0.29 g. Fish were fed three times per day for 60 days. The results of the experiment indicated that a diet with 10% of *U. lactuca* and *E. linza* resulted in a poorer growth and feed utilization for rainbow trout when compared to those of control group. Hence, more defined experiments seem to be necessary in order to determine the optimum dietary inclusion level of these seaweeds in rainbow trout diets

Nader *et al.* (2010) evaluated the effects on growth performance, feed utilization and body composition for red tilapia (*Oreochromis sp.*) by the inclusion of green seaweeds (*Ulva sp.*) as a feed supplement in fish diet. Six isocaloric diets were used with different levels of green seaweed *Ulva* sp. (0, 5, 10, 15, 20 and 25% of fish diet). The trial was conducted for 9 weeks in which each diet was fed twice a day to triplicate groups of fish**.** It was estimated that that final body weight, weight gain and specific growth rate (SGR) increased considerably with raising *Ulva* level in fish diet up to 15% Highest feed conversion ratio (FCR) was observed in fish fed with 15 and 10 % *Ulva*. Highest lipid content was observed in the fish fed with the diet containing 10% *Ulva.*The highest carcass protein concentration was observed in fish fed with the diet containing 25% *Ulva*. Thus green seaweeds (*Ulva* sp.*)* could be supplemented to red tilapia **(***Oreochromis sp***.)** diet at requisite level of 15% to improve growth performance without any unfavourable effects on feed efficiency or survival rate.

Xuan *et al.* (2013) studied the potential use of macro-algae *Gracilaria lemaneiformis* in diets containing 5, 10, 15 and 20% (GL) (D1–D4 diet) for the black sea bream, *Acanthopagrus schlegelii*, juvenile to investigate its effects on growth performance, carcass composition, activities of digestive enzymes and transaminases activities. The results of this study indicate that incorporation of the GL in the diet at up to 15% level for juvenile black sea bream is feasible in terms of the growth performance and physiological state.

Nooshin *et al.* (2014) conducted a nutritional experiment to evaluate the impact of replaceing dietary protein sources with different levels of *Sargassum ilicifolium* on growth, survival and body composition of rainbow trout *Oncorhynchus mykiss* for a period of a 60 days. The results of this study displayed that 5 and 7.5% of basal diet can be replaced with sargassum meal with major effects on average weight and total length, feed conversion ratio (FCR), specific growth rate (SGR), weight gain per cent (WG), condition factor (CF) and survival rate (SR) values. It also showed that inclusion of more than 7.5% sargassum meal uplifted growth and FCR.

Abdulrahman *et al.* *(*2014) studied the effect of adding *Spirulina* spp in fish meal on weight gain, feed conversion ratio (FCR), food efficiency ratio in 72 common carp fingerlings. The replacement levels were designated as T1 (with 0% spirulina spp), T2 (1gm *spirulina* per kg of diet), T3 (3gm *spirulina* per kg of diet) and T4 (5gm per kg of diet).the reported parameters were: weight gain of 6.89g, specific growth rate 0.147(lnw_1-lnw_0/T*100), food conversion ratio of 2.14 (control). Food efficiency ratio showed significant improvement in T3 and T4 (62.48 and 62.47).

Mostafa *et al.* (2015) evaluated the impacts of fish meal replacement by *Spirulina platensis* meal on growth, survival, body biochemical composition and reproductive performance in three-spot gourami (*Trichopodus trichopterus*) until the first spawning for 16 weeks. This study indicated that 8.1–9.6 % inculusion level of S. platensis meal in the fish diet improved many reproductive and growth performances in *T. trichopterous*.

Vadher *et al.* *(*2016) conducted a nutritional assay to evaluate the efficacy of seaweed (*Gracilaria sp*.) as feed additives on specific growth rate, weight gain, survival and some biochemical parameters in rohu (*Labeo rohita*) for a period of 45 days. The four isonitrogenous experimental diets with 30% crude protein were formulated by adding *Gracilaria* sp. at 0% (control diet), 10% (T1), 20% (T2) and 30% (T3) inclusion level. *L. rohita* (400 number) fries was randomly distributed into four treatments. The results of this study displayed that the addition of Gracilaria sp. at 10% level can be considered as very interesting in diets for rohu, as no negative consequences on growth performance, nutrient utilization or body composition were observed.

Anisuzamman *et al.* (2017), Studied the effects of different levels of schizochytrium algae in diet of sea cucumber (*Apostichopus japoncus*).the replacement levels in the study were 0%, 2%, 4%, 6%, 8% and 10%. The parameters under consideration of the study were specific growth rate (SGR), Ingestion rate (IR), feces production rate (FPR) and food conversion efficiency (FCE). The trend observed was that as the substitution levels increased there was improvement of growth of sea cucumber. The study observed the optimum level of replacement as 6%.

Uchechukwu *et al.* (2017) studied the utilization of *Chlorella vulgaris* as protein source in the diets of African catfish (*Clarias gariepinus). Chlorella vulgaris* is a rich source of high quality proteins that are abundant in essential amino acids particularly methionine, lysine and alanine. Four different diets were formulated (F1–F4) with 25% (F1), 15% (F2), 5% (F3) and 0% (F4) green algae meal. The experimental trail was conducted for 60 days where post-fingerling African catfish were stocked at 10 fish per tank. The specific growth rate was best for the catfish fed with 25% C. vulgaris diet. The food conversion ratio (FCR) was highest for the 0% algal meal diet F4. Highest average weight gain was observed for fish groups fed with F1 diet. Highest protein efficiency ratio was observed for the F1-fed fish.It was thus evaluated that C. vulgaris is a good protein source for African catfish and can also replace fishmeal in the catfish diets.

Aziz *et al.* (2017), analyzed the effect of substitution of fresh macro algae *Ulva fasciata* and *Enteromorpha flaxusa* in artificial feed on growth performance and feed utilization of rabbitfish (*Suganus rivulatus*).the percentage substitution levels studied by the researchers were 0, 50 and 100 percent. Six treatment plans were devised *viz* T1 (only fish meal), T2 (half fish meal and half *ulva*), T3 (half fish meal and half *enteromorpha*), T4 (*ulva* only), T5 (*enteromorpha* only), T6 (half *ulva* and half *enteromorpha*). The trial continued for 70 days. The T3 was the highest in final weight, weight gain, average daily gain (ADG), relative growth rate (RGR) and specific growth rate (SGR). Thus the optimum dose observed was 50 per cent and that too with *enteromorpha*.

Mishra *et al.* (2017) studied the effects of algal based diets on growth and physiological parameters of Indian major carp, rohu (labeo rohita). The study used two pronged approach for the study, first they observed the effect of replacement of algae in fish meal (40% of the total composition) and then they used only algal meal mixture. The algae used in the study were *Anabaena cylindrical* (AN), *Nostoc salbasa* (NS), *Spirulina platensis* (SP) and *Westleopsis prolifica* (WS). Average fish weight used in the study was 2g+-0.1g and the feeding went on for 8 weeks. The study concluded that algal feed as replacement fared quite well as compared to all algal meal mixture. And among all diets WS was found to have good growth (protein content) and physiologic (fatty acid profile) factors.

Morshedi *et al.* (2017) conducted a nutritional study of 40-days to evaluate the ability of red algal (*Gracilaria pulvinata*) meal used as a protein source in formulated diets for barramundi (*Lates calcarifer*). The results of this study displayed that *Gracilaria* meal can be included to about 3% in fish diet, without any evident negative effects on the growth performance, body composition and health parameters in *L. calcarifer*.

Gong *et al.* (2017) conducted a study to investigate the apparent digestibility coefficient (ADC) of defatted biomass derived from microalgae *Nannochloropsis* sp. and *Desmodesmus* sp. when fed to Atlantic salmon postsmolts in seawater. It was evaluated that the microalga *Nannochloropsis* sp. has higher digestibility than *Desmodesmus* sp. and the process of extrusion can be used as a means to improve digestibility of certain nutrients.

Yongjin *et al.* (2018) studied the effect of microalga *Isochrysis galbana* in feed for *Trachinotus ovatus* on growth performance and fatty acid composition of fish fillet and liver. Five isonitrogenous and isolipidic feed diets were formulated by replacing portion of fish oil with microalgae *Isochrysis galbana*. These diets were fed to triplicate tanks of *Trachinotus ovatus* for 80 days. This study displayed that a moderate inclusion (about 4.5–5.0 wt%, equivalent to the replacement of 24–26 wt% fish oil) of biomass in fish diet uplifts fish growth performance, lipid deposition and enhances total n-3 fatty acids, DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) contents in neutral and polar lipids (PLs) of fish muscle and liver of *T. ovatus*. Thus *T. ovatus* can be used to solve the problem of depleting fish oil resource in a cost-effective manner.

Sein *et al. (*2018) also analyzed the effect of algal meal substitution in fish meal for growth parameters of Indian major carp, *Labeo rohita* fingerlings as in the above study. The only change was the use of *wolffia sp*. as replacement. The experimenters concluded that the growth of replaced meal fish was higher as compared to control ones. The parameters under study were weight gain, protein efficiency ratio, food conversion ratio and specific growth rate. Nutritional quality of *Labeo rohita* improved considerably.

Velazquez *et al. (*2018) evaluated the impacts of partial fish meal and fish oil replacement by algal meals in diets of red drum *Sciaenops ocellatus*. The results of this study indicate that up to 50% of dietary fish meal, soy protein concentrate and fish oil can be replaced by *Arthrospira* sp. (28.87%) and *S. limacinum (*6.72%) meals without showing any negative impacts on fish performance. Further *S. limacinum* meal can be used as the primary source of lipid for this species.

Simanjuntak *et al. (*2018) conducted a nutritional study to evaluate the effects of blue green algae *Spirulina platensis* supplementation in gurami fish, *Osphronemus gourami* on growth, hematological, body composition and biochemical blood parameters. The algae was incorporated at increasing rate of 0, 2, 3, 4 and 6 g/kg of diet and the trial was performed for 56 days. The results of this study showed that *S. platensis* inclusion can uplift growth, hematological and biochemical blood parameters with best results being obtained when supplemented at the rate of 6 g/kg of diet.

Zeinab *et al. (*2018) also studied replacement effects on nile tilapia but replacement entities were spirulin (*Arthrospira platensis*) and nannochloropsis

(*Nannochloropsis gaditana*). The substituted feed was designated as T1 (0% algae), T2 (3%spirulina), T3 (5%spirulina), T4 (7% spirulina), T5 (3% nannochloropsis), T6 (5% nannochloropsis), T7 (7% nannochloropsis). Fish were given the feed in groups of 15. The period of study was 95 days. For T6 higher final body weight, average weight gain and average daily gain were recorded.T4 was observed to have high protein efficiency ratio and protein productive value.so T4 and T6 were greatly improved growth performance parameters of tilapia fish.

Roohani *et al. (*2018) performed a nutritional assay to study the impacts of spirulina **(***Spirulina platensis*) as a complementary ingredient to reduce dietary fish meal on the growth performance, whole-body composition, fatty acid and amino acid profiles and pigmentation of Caspian brown trout (*Salmo trutta caspius*) juveniles. The results of this study suggested that 52.8 g/kg of spirulina (8% Fish meal sparing treatment) can be beneficial in terms of uplifting Caspian brown trout growth, carcass composition and colour.

Raji *et al. (*2019) studied the replacement of fish diet with *Spirulina platensis*(SP) and chlorella vulgaris(CL) individually for African catfish (*Clarias gariepinus*) fingerlings. The substitution levels studied were 0%, 12.5%, 25%, 50% and 75%. The meal was then fed to triplicate groups of 10 fingerlings for 56 days. The parameters analyzed were weight gain, specific growth rate, protein efficiency ratio & feed conversion ratio. The statistical analysis (regression analysis) revealed that replacement levels were best for growth at 68.5% and 69.4% for SP & CL respectively. After this the benefits tapered off. Carcass of fish showed greater protein in SP 12.5%. Lipid levels increased with increasing SP & CL levels.CL was found to be more viable for growth and feed utilization than SP.

Fukada *et al.* (2019) conducted a nutritional study on algal meal as a new dietary docosahexaenoic acid (DHA) source that could completely replace dietary fish oil (FO) in fish diets. Under this study fish oil was replaced with plant oil mixtures and algal meal in juvenile yellowtail *Seriola quinqueradiata* to examine its effects on growth performance and fatty acid composition. Algal meal was incorporated at the different levels of 0% (AM0), 1% (AM1), 2% (AM2), 3% (AM3) and 4% (AM4). The trial was conducted for 8-weeks. The results of this study displayed that algal meal (AM2) can be used as an efficient DHA source in yellowtail aquaculture, that can reduce the use of FO in fish diets.

Katerina *et al. (*2020) conducted a nutritional trail for the replacement of fish oil in modern salmon diet by microalgal *Schizochytrium limacinum* biomass for improving its growth and filet quality. It was evaluated that *Schizochytrium limacinum* biomass is a good alternative source of marine omega 3 fatty acids for the fish that improves its growth and filet pigmentation. The results indicate that *S. limacinum* biomass can be used in the feed for salmon during the whole production cycle from parr to slaughter without any ill effects on growth or fish quality.

Sein *et al. (*2020) examined the effect of *Wolffia* sp. as supplemental feed on growth performance and the body composition of Indian major carp, *Labeo rohita* fingerlings. The results of this study indicated that feed supplemented with *Wolffia* sp. was beneficial for *L. rohita* fingerlings as it improved its growth and body composition thus improving the nutritional quality of *L. rohita* fingerlings.

Magouz *et al. (*2020) also had their work done on tilapia (*Oreochromis niloticus*) but genetically improved one. The replacement algae used was *Azolla*. The replacement levels used were 10%, 20%and 30% and the trial was run for 90 days. Feed conversion ratio changed marked in fish fed with 30% *azolla.* Physiologic parameters like lysozyme activit, blood phagocytic index etc did not show any major improvement. So *azolla* meal was found to be very beneficial for feed utilization in tilapia.

Raji *et al. (*2020) in other study linked to one above studied the growth and apparent digestibility coefficient (ADC) of amino acids in African catfish.the trial period of study was 42 days. The diets with replacement by spirulina (*Arthospira*) and chlorella showed higher ADC for all amino acids.

Kiadaliri *et al. (*2020) observed the following physiologic effects of part meal substitution by red algae (*Laurencia caspica*) in rainbow trout (*Oncorhynchus mykiss*) infected with *Aeromonas hydrophila*. The different levels of substitution were as 0, 5, 10, 15 and 20g algal extract per kg of basal diet. A total of 750 fish were available for the study in groups of 50. The study indicated that algal substitution improved lysozyme activity, complement system and immunoglobulin levels. Study recommended to use 1.5% of algal extract in rainbow trout feeds.

Zhou *et al. (*2020) observed the physiologic and growth parameters of crucian carp (*carrasius auratus*) upon substitution of their meal by *Enteromorpha prolifera* polysaccharide (EPP). The study comprised of five groups of fish with body weight of 51.24g ± 4.08g. The replacement levels of feed were 20, 40, 60 and 80g per kg EPP. Study period was 60 days. The 40g level of replacement was found to have a marked effect on body weight gain, feed conversion ratio, specific growth rate, crude protein content, digestive enzyme functions, lysozyme activity and many other physiologic factors. This study recommended using EPP as dietary feed in crucian carp.

Chapter 3

MATERIAL AND METHODS

3.1 Collection and Biochemical analysis of Algae

The algae to be used as replacement in fish meal were collected from algal bloom of Dal Lake, Srinagar. The algae were brought to Department of Botany, University of Kashmir for identification. After identification the algae were thoroughly washed to remove dirt. The algae were then dried at room temperature for one week. After drying algae were ground into powder in a laboratory grinder and sieved into fine powder and stored in plastic containers at room temperature until use. The proximate analysis were carried out in the laboratory of Fish Nutrition and Biochemistry, Faculty of Fisheries, Rangil. Proximate analysis of percentage moisture, dry matter, protein, fiber and oil were carried out using methods described by AOAC. Then algal mix of all the algae was prepared to replace part of fishmeal to be used as feed for a trial period of 60 days.

3.2 Site of the experiment

The experiment was conducted over a period of 60 days from 26 March 2020 to 24 May 2020 at the temperature controlled polyhouse of the Faculty of Fisheries, Rangil. The fishes were reared in plastic tubs after acclimatization.

3.3 Experimental fish

Fishes used for experimental purpose were advanced fingerlings of Common carp, *Cyprinus carpio* with an average weight of 4.5±1.1g. The fishes were procured from National Fish Seed Farm, Manasbal, district Ganderbal during the month of March 2020. The fishes were transported in a big container (500L) with sufficient aeration to the Faculty of Fisheries, Rangil Campus. They were carefully transferred to fiber glass tanks and were left undisturbed the whole night. In order to ameliorate the handling stress the fishes were given a mild salt

Plate 1: Collection of algae from algal bloom of Dal lake

Plate 2: Drying of algae specimens

Plate 3: Weighing of common carp

and $KMnO_4$ treatment on the next day. The stock was acclimatized under aerated condition for few days.

3.4 Experimental design

One hundred and eighty fingerlings (180) of *Cyprinus carpio* were randomly distributed in six distinct experimental groups, in three replicates, following a completely randomized design.

3.5 Experimental set-up

The experiment was conducted for a period of 60 days in the temperature regulated poly house of Faculty of Fisheries, Rangil. The setup consisted of 18 plastic circular tubs (75L capacity) covered with net. The tubs were initially washed and filled with potassium permanganate solution ($4mgL^{-1}$) that was left overnight. The tubs were flushed out the next day and were thoroughly washed with clean water. One hundred and eighty fishes were randomly distributed in the six distinct experimental groups. Each group had three replicates following a completely randomized design. Round the clock aeration was provided. The aeration pipe in each tub was provided with an air stone and a plastic regulator to control the air pressure uniformly in all the tubs.

3.6 Rearing

Ten fishes of uniform size were kept in each tub. Each tub was covered with a net to prevent the animals from jumping out. The fishes were fed with a control diet for few days before the commencement of the experiment. The fishes were given formalin treatment at 5ml/20L of water at regular intervals in order to remove the ectoparasites. The experimental conditions were kept same throughout the experiment. The body weight was measured at intervals of 15 days to assess the growth. The fishes were starved overnight before taking the bodyweight.

Plate 4: Arrangement of tubs and regular siphoning of tubs

3.7 Cleaning and siphoning

The experimental tubs were cleaned manually and siphoning was done every day in order to remove the excess feed pellets and the remaining faecal matter. An equal volume of clean water replaced the siphoned water. This was carried out throughout the experimental period of 60 days.

3.8 Formulation and preparation of experimental diets

Six diets (control and five treatment groups) were formulated containing different concentration of algal mix. Diet formulation and proximate composition of experimental diet were performed in Fish nutrition and Biochemistry Laboratory, Faculty of Fisheries, Rangil. The dry ingredients of each diet were thoroughly mixed and then 100ml of distilled water per kg diet were added and the ingredient were blended using kitchen blender to make paste of each diet. In the experimental diets, algal mix was added at 10%, 20%, 30%, 40%, 50% in $Treatment_1$, $Treatment_2$, $Treatment_3$, $Treatment_4$ and $Treatment_5$ respectively. Pelleting of each diet was carried out by passing the blended mixture through hand pelletizer with (1-mm) diameter mesh. The wet pellets were dried in an oven at 60˚C. The diets were stored in plastic bags in a refrigerator (4˚C) for further use.

3.9 Feeding

Feeding was done at 5% of the body weight throughout the 60 days feeding trial. The daily ration was divided into two equal parts and fed at 10.00 am in the morning and 6.00 pm in the evening.

3.10 Physico-chemical parameters of water

Water quality parameters *viz*. Temperature, pH, dissolved oxygen, total hardness, ammonia, were recorded during the experimental period as per APHA (2012).

Plate 5: Weighing of Ingredients

Plate 6: Pelletization

Plate 7: Final feed

3.10.1 Water Temperature

The water temperature of all the experimental tubs were recorded using digital thermometer. The reading obtained were expressed in °C.

3.10.2 pH

The pH was measured by a digital pH meter for all the experimental tubs.

3.10.3 Dissolved oxygen

The dissolved oxygen content of water was measured by Winkler's titrimetric method. The water samples from each experimental tub were collected in 100ml glass stoppered bottles taking care that no air bubbles remained in the bottle. The water sample were fixed immediately by adding 1ml of Winkler A (Manganous sulphate solution) and 1ml of Winkler B (Alkaline potassium iodide). The bottles were closed by placing stopper and then inverted upside down at least 6 times to allow the brown precipitate to settle. The precipitate was then dissolved by adding 1ml of concentrated H_2SO_4. Then 50ml of this solution was taken in a titration flask and titrated against the titrant (0.025 N solution thiosulphate solution) to pale straw colour. After adding 2 drops of starch as indicator, the colour of the sample changed to blue. The solution was further titrated against 0.025 N sodium thiosulphate solution ($Na_2S_2O_3$) till it turned colourless.

Calculation:

$$\text{Dissolved oxygen (mg/l)} = \frac{8 \times 1000 \times N \times v}{V}$$

v= volume of sample (ml)

V= volume of titrant used (ml)

N = normality of titrant

3.10.4 Carbonate hardness

Carbonate hardness was determined by EDTA titration method employing EDTA and erichrome black.50ml of water sample was taken in a flask to which 1ml of ammonia buffer and 5 drops of Eriochrome black T indicator solution was added. The colour of the sample turned wine-red. The solution was then titrated with EDTA solution, until clear blue colour appeared and the reading was noted.

Calculation:

$$\text{Carbonate hardness (mg/l)} = \frac{\text{Volume of titrant used}}{\text{volume of sample}} \times 1000$$

3.10.5 Ammonia

Un-ionized ammonia concentration was estimated spectrophotometrically at 635nm wavelength by phenate method and compared with standard graph. The concentration was expressed as μgL^{-1}.

3.11 Proximate analysis of diets

3.11.1 Moisture

The moisture content of the diets and animal tissue were determined by taking a known weight of the sample in the petridish and drying it in hot air oven at 100-105^0 C till a constant weight was achieved. The difference in weight of the sample gave the moisture content, which was calculated by using the following formula.

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

3.11.2 Crude protein (CP)

The Nitrogen content of the sample was estimated quantitatively by Kjeltec semi automated method (2200 Kjeltec Auto Distillation, Foss Tecator and Sweden) using titration as the means for determining nitrogen percentage. The crude protein percentage was obtained by multiplying the Nitrogen percentage by a factor of 6.25.

$$\text{Crude Protein (\%)} = N_2\text{ (\%)} \times 6.25$$

3.11.3 Ether extract (EE)

Ether extract was estimated by Soxhlets apparatus using petroleum ether (Boiling point 40-60^0 C) as the solvent. The calculation was made as follows.

$$\text{Ether extract (\%)} = \frac{\text{Weight of the ether extract}}{\text{Weight of the sample}} \times 100$$

3.11.4 Ash

Ash content was estimated by taking a known weight of sample in silica crucible and placing it in a muffle furnace at 600^{0}C for 6 hrs. The calculation was done as follows:

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

3.12 Growth parameters

Samplings were done at intervals of 15 days to assess the body weight of the fishes. Fishes were starved overnight before taking the weight. The weight was taken on an electronic weighing balance. The parameters used in the study to evaluate growth and feed utilization can be summarized as below :

3.12.1 Percentage weight gain

The percentage weight gain was calculated using the following formula:

$$\text{Weight gain (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

3.12.2 Specific growth rate (SGR)

The Specific Growth rate was calculated by the following formula

$$\text{SGR (\%)} = \frac{\text{Log}_e\text{ final weight} - \text{Log}_e\text{ initial weight}}{\text{Number of days}} \times 100$$

3.12.3 Feed conversion ratio (FCR)

The Feed Conversion Ratio was calculated by the following formula

$$FCR = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}} \times 100$$

3.12.4 Protein efficiency ratio (PER)

Protein efficiency ratio was calculated by the following formula.

$$PER = \frac{\text{Net weight gain (wet weight)}}{\text{Protein fed (g)}} \times 100$$

3.12.5 Survival rate

At the end of the experiment, all the experimental tubs were dewatered and the number of the experimental animals in each tub was counted and the survival rate (%) was calculated by the following formula

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

3.13 Statistical Analysis

The data were statistically analyzed by using appropriate statistical tools. To discover where there were significant differences between the levels of the main factor, Least Significant difference was used.

Chapter 4

EXPERIMENTAL FINDINGS

4.1 Proximate analysis of algae

The proximate composition of three different algae namely *Spirogyra spp.*, *Ulothrix spp.* and *Oedogonium spp.,* are presented in Table 1. Crude protein, crude lipid, carbohydrate and crude fibre content in percentage were analyzed. The crude protein content (%) in *Ulothrix spp.* (45) were significantly higher than *Spirogyra spp.* (24.4) and *Oedogonium spp.* (13.5) respectively. Crude lipid content (%) of 20.0 in *Ulothrix spp.* was significantly higher than *Spirogyra spp* (14.8) and *Oedogonium spp.* (19.2) respectively. The carbohydrate content analyzed was showing lowest value of 20.0% for *Ulothrix spp.* among all the samples while as, *Spirogyra spp.* has the highest (42.8%) at a significant level ($p>0.05$). The carbohydrate content analyzed for *Oedogonium spp.* was showing a value of 22.0%. The crude fiber content in (%) was analysed and was found highest in *Spirogyra spp.* (25.8) which is significantly higher than *Oedogonium spp.* (17.7%) and *Ulothrix spp.* (5.0%) respectively. On the basis of proximate composition of three algal species, it was found that their nutritive value is good. In the present study these algal species were given as meal in the feed of Common carp (*Cyprinus carpio var. communis*) to ascertain its growth and survival. The algal meal was given at five different concentrations (10%, 20%, 30%, 40% and 50%) respectively and subsequently fish meal was replaced at same concentration to evaluate effect on growth and survival.

Table 1: Proximate composition of (g/100 g of dry weight) three algal species

Algae	Crude Protein (%)	Crude Lipid (%)	Carbohydrate (%)	Crude Fiber (%)
Ulothrix.	45.0	20.0	20.0	5.0
Spirogyra	24.4	14.8	42.8	25.8
Oedogonium	13.5	19.2	22.0	17.7

4.2 Physico-chemical parameters of water

The physico-chemical parameters of water such as temperature (C^0), pH, dissolved oxygen (mg L^{-1}), free carbon dioxide (mg L^{-1}), total hardness (mg L^{-1}), ammonia (mg L^{-1}), Nitrite-N (mg L^{-1}), Nitrate-N (mg L^{-1}) were recorded and average values of all the treatments are presented in Table-2.

4.2.1 Temperature

The water temperature of the different experimental groups ranged from 16 to 25^0C during the experimental period of 60 days.

4.2.2 pH

There was not much variation in pH values during the experimental period. The pH values were recorded within the range of 7.2 to 8.2.

4.2.3 Dissolved oxygen

The dissolved oxygen concentration of all the experimental tubs was recorded within the range of 6.1 to 7.2mg L^{-1}during the experimental period of 60 days.

4.2.4 Free carbon dioxide

The free carbon dioxide in water was found to be negligible during the experimental period of 60 days.

4.2.5 Carbonate hardness

The carbonate hardness was found to be 135 to 155mgL-^{1} during the experimental period of 60 days.

4.2.6 Total ammonia – N

The total ammonia content of all the experimental tanks was recorded before water exchange. It was found to be in the range of 30 to 70 ug L-^{1}.

4.2.7 Nitrite – N

The nitrite – N content was found to be in the range of 0.1 to 0.3mg L^{-1}.

4.2.8 Nitrate – N

The nitrate – N content was found to be in the range of 40 to 50 mg L^{-1}, throughout the experimental period. The physic-chemical parameters of water are given in table 2.

Table 2: Physico-chemical parameters of water during the experimental period for different experimental groups

Bi Weekly sampling	Treatments	Temperature (°C)	pH (mg/l)	DO (mg/l)	Carbonate hardness (mg/l)	Ammonical nitrogen (µg/l)
		Mean	**Mean**	**Mean**	**Mean**	**Mean**
1st day	Control	16.0	8.2	7.2	140	30
	Treatment 1	16.0	8.1	7..2	140	30
	Treatment 2	16.0	8.0	7.0	140	30
	Treatment 3	16.0	8.0	7.2	140	30
	Treatment 4	16.0	8.1	7.0	140	30
	Treatment 5	16.0	8.0	7.2	140	30
1st 15days	Control	18	7.7	6.8	145	33
	Treatment 1	19	7.7	6.9	148	35
	Treatment 2	18	7.7	6.8	144	34
	Treatment 3	18	7.7	7.0	145	35
	Treatment 4	18	7.7	6.8	143	33
	Treatment 5	18	7.7	6.6	144	34
2nd 15 days	Control	18	7.4	6.6	148	43
	Treatment 1	19	7.3	6.4	149	47
	Treatment 2	17	7.3	6.3	149	48
	Treatment 3	18	7.3	6.3	148	50
	Treatment 4	19	7.3	6.4	148	41
	Treatment 5	20	7.3	6.5	147	41

Table 3: Physico-chemical parameters of water during 3rd 15 days, 4th 15 days experimental period for different experimental groups

of Bi Weekly sampling	Treatments	Temperature (°C)	pH (mg/l)	DO (mg/l)	Total hardness (mg/l)	Ammonical nitrogen (mg/l)
		Mean	**Mean**	**Mean**	**Mean**	**Mean**
3rd 15 days	Control	18	7.3	6.5	149	55
	Treatment 1	21	7.2	6.5	150	56
	Treatment 2	21	7.3	6.4	154	49
	Treatment 3	19	7.2	6.4	152	51
	Treatment 4	19	7.3	6.3	152	51
	Treatment 5	20	7.2	6.4	151	58
4th 15 days	Control	24	7.2	6.3	153	65
	Treatment 1	25	7.3	6.2	153	70
	Treatment 2	23	7.2	6.2	153	70
	Treatment 3	24	7.2	6.1	154	68
	Treatment4	24	7.3	6.3	155	65
	Treatment5	23	7.2	6.1	155	70

4.3 Proximate composition of the diets

The proximate compositions of the different experimental diets are given in Table 4. The per cent dry matter content in the diet ranged from 86% to 88.5 %. The per cent crude protein content was estimated within the range of 20% to 21.82%. The per cent ash content was estimated within the range of 14.91% to 16.65%. The crude fibre % varied from 6.82% to 7.21%. The per cent lipid content was estimated within the range of 10.23% to 12.64%. The ash content (%) were in the range of 14.91% to 16.65% and gross energy were in the range of 406.20Kcal/g to 425.95Kcal/g (Table 4).

Table 4: Proximate composition of the experimental diets (% dry matter (DM) basis) fed to *common carp* fingerlings during experimental period

Chemical composition	Control (0%)	Treatment 1 (10%)	Treatment 2 (20%)	Treatment 3 (30%)	Treatment 4 (40%)	Treatment 5 (50%)
Dry matter (%)	86.0	85.7	88.2	86.5	87.5	88.5
Crude protein (%)	21.82	21.42	20.67	20.00	21.50	21.09
Crude fibre (%)	6.82	7.03	7.07	7.21	6.94	6.99
Ash (%)	14.91	15.79	16.21	16.65	15.01	15.10
Lipid (%)	12.64	11.43	10.82	10.23	11.20	11.22
Gross energy (Kcal/g)	425.95	412.57	406.20	409.53	420.01	422.21

4.4 Growth performance and body indices

The growth performance and body indices of the experimental groups at the end of the 60 days of feeding trial are shown in Table 5.

4.4.1 Body weight gain

The initial body weight of all experimental groups did not differ significantly (Table 5). The growth performance (final body weight, weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio) of Common carp fingerlings fed diets containing different levels of algal meal are shown in Table 5. Highest weight gain was recorded in T_4 group (76.23±0, 04 gm) and the lowest weight gain was observed in the control group (44.54±0.31 gm), which was significantly different ($P< 0.05$).

4.4.2 Specific growth rate (SGR)

The mean of the SGR of the T_4 group (1.41±0.07 %/day) was significantly higher ($P<0.001$) than the all other groups. The lowest SGR value was found in the control group (1.30±0.63 %/day), which was significantly different ($P<0.001$). The SGR of T_1 and T_2 were significantly different from the control group.

Table 5: Growth parameters of different experimental groups fed different experimental diets at the end of the experiment

Parameters	***Algal meal substitution rate***					
	Control (0%)	**Treatment T_1 10%**	**Treatment T_2 (20%)**	**Treatment T_3 (30%)**	**Treatment T_4 (40%)**	**Treatment T_5 (50%)**
Initial wt.	32.36±0.26	33.06±0.24	33.06±0.15	32.26±0.01	32.63±0.37	32.63±0.41
Final wt.	76.90±0.18^{a}	87.13±0.06^{b}	87.13±0.09^{b}	88.76±0.91^{b}	108.86±0.08^{c}	90.96±0.05^{b}
Body wt. gain	44.54±0.31^{a}	54.07±0.03^{b}	54.07±0.06^{b}	56.50±0.07^{b}	76.23±0.04^{c}	58.33±0.07^{b}
%b.Wt.gain	137.63±0.33^{a}	163.55±0.03^{b}	163.55±0.01^{b}	175.13±0.02^{c}	233.61±0.02^{d}	178.76±0.03^{b}
SGR (%/day)	1.30±0.63^{a}	1.11±0.01^{b}	1.10±0.02^{b}	1.01±0.08^{c}	1.41±0.07^{d}	1.11±0.07^{b}
FCR	2.28±0.58^{a}	2.20±0.06^{a}	2.23±0.23^{a}	2.02±0.21^{b}	1.99±0.01^{c}	2.11±0.02^{d}
PER	1.16±0.29^{a}	1.26±0.06^{b}	1.29±0.06^{c}	1.41±0.05^{d}	1.32±0.02^{c}	1.33±0.05^{c}
SR (%)	100±00	100±00	100±00	100±00	100±00	100±00

Data were presented as mean±SE (n=3). Values within the same column having different superscripts are significantly different ($P<0.05$).

SGR-Specific growth rate, FCR-Feed conversion ratio, PER-Protein efficiency ratio.

4.5 Feed conversion ratio (FCR)

The FCR of different experimental groups varied significantly ($P<0.001$). The lowest FCR (1.99 ± 0.01) was recorded in T_4 group. The highest FCR (2.28 ± 0.58) was recorded in the control group. Treatment group T_3 and T_4 are significantly differeny from control group ($P<0.05$).

4.6 Protein efficiency ratio (PER)

Highest PER value was found in groups T_3(1.41 ± 0.05) which are significantly different ($P<0.001$) then the control group. The lowest value was recorded in the control group (1.16 ± 0.29) which was significantly different to other experimental groups.

4.7 Survival rate

No mortality was observed during the experimental period of 8 weeks. Hundred per cent survival rates were observed in all the experimental groups and in the control group. There was no significant difference ($P<0.05$) between treatment groups and control group.

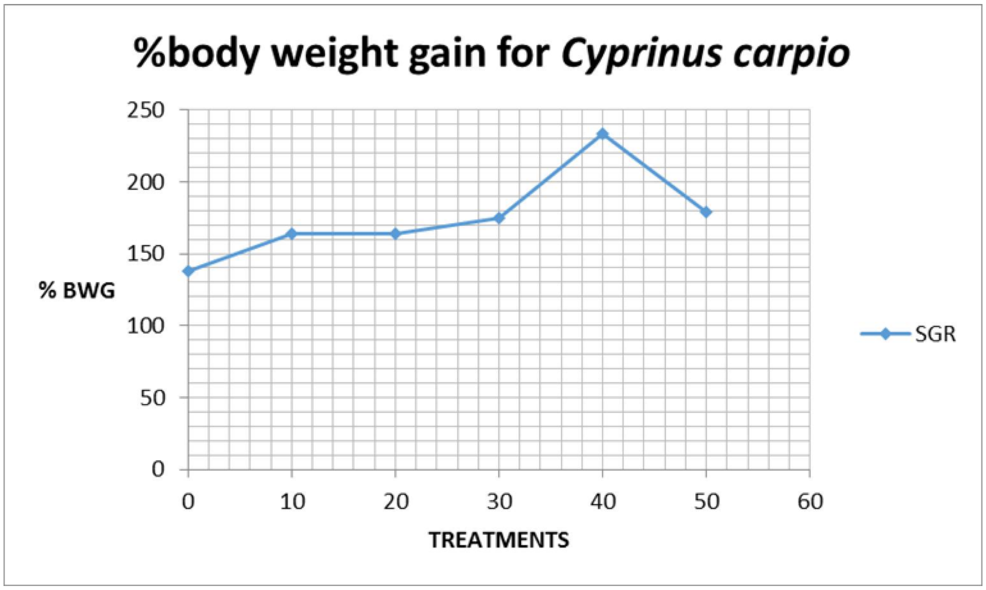

Fig. 1: **Weight gain percentage of *Cyprinus carpio* fingerlings fed with different experimental diets**

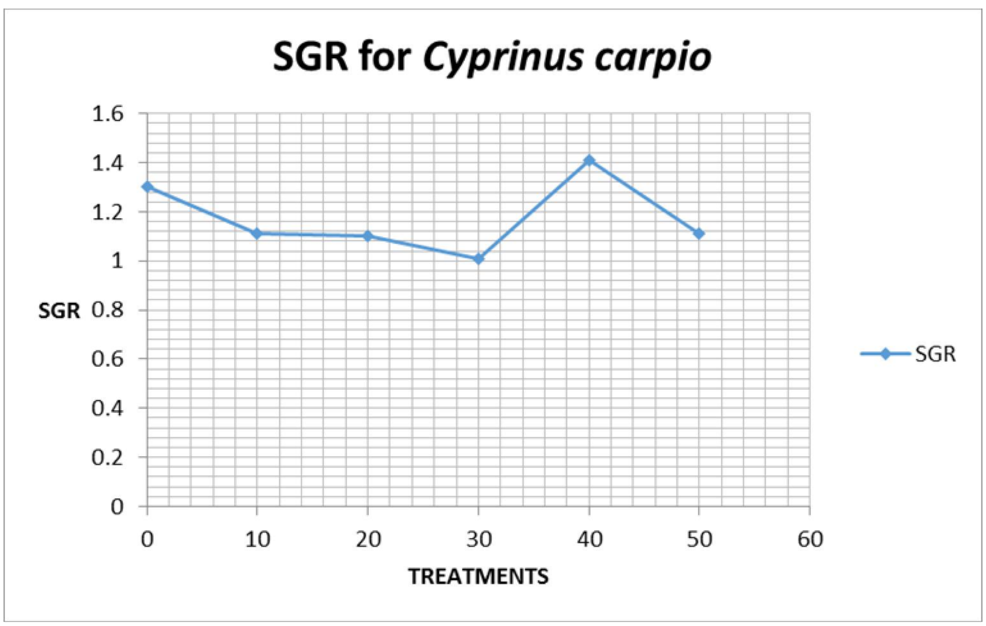

Fig. 2: **Specific Growth Rate of *Cyprinus carpio* fingerlings fed with different experimental diets**

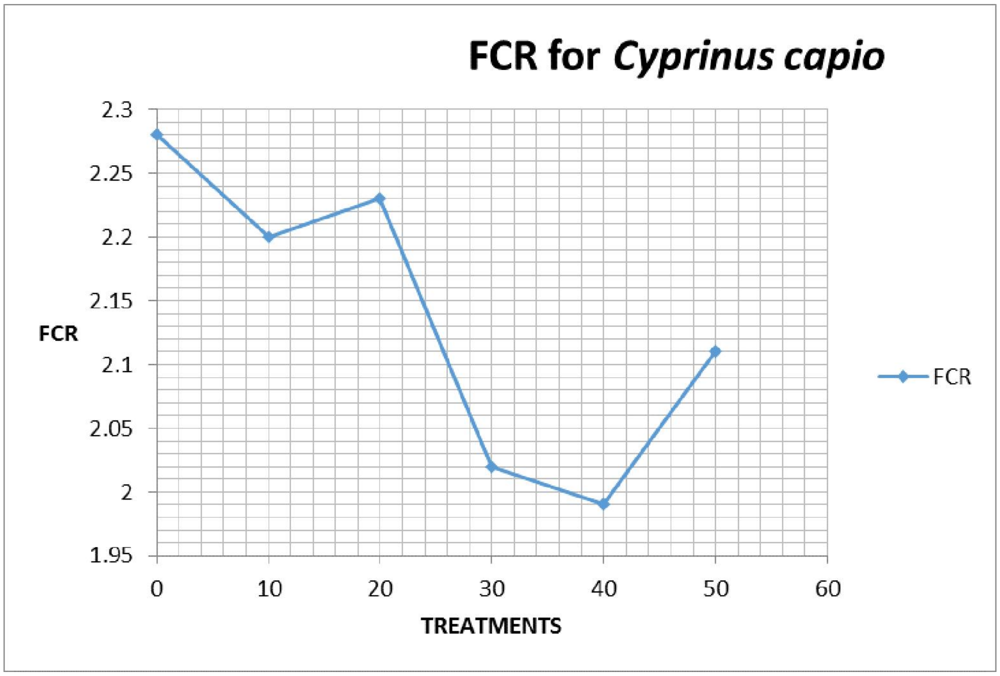

Fig. 3: Feed Conversion Ratio of Cyprinus carpio fingerlings fed with different experimental diets

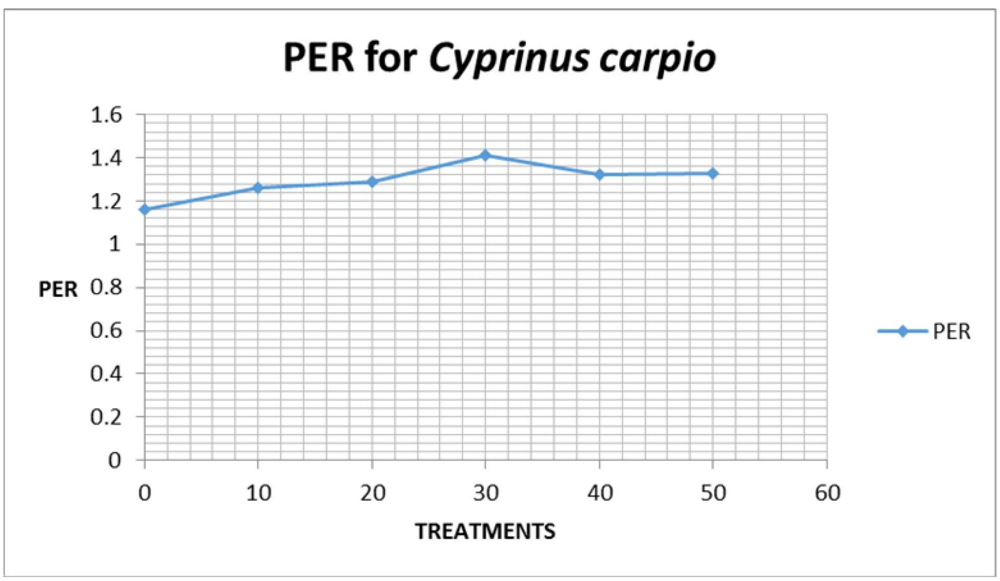

Fig. 4: Protein Efficiency Ratio of common carp fingerlings fed with different experimental diets

Chapter 5

DISCUSSION

The present study was conducted to replace fishmeal with algal meal upto certain maximum level. The present study revealed that algal meal has improved growth and survival of common carp. The results of this study showed that algal meal had a statistically significant impact on the growth performance of *Cyprinus Carpio*, with weight gain (WG), percentage weight gain (WG%) and specific growth rate(SGR) all increasing significantly ($p < 0.05$) at incorporation levels 10%, 20%, 30% and 40%(Table 5) of algal meal in the diet compared to the algae-free diet. Feed conversion ratio (FCR) showed the expected downward trend upto incorporation level of 40% (Fig. 3). Moreover, the positive impact of algal meal on the growth indicators of Common Carp increased as the proportion of algal meal in the diet increased upto 40%.The fishes in the treatment group T_4 exhibited a significantly higher growth performance indices than other treatment groups i.e, $T_{0,}$ T_1, T_2, T_3 and T_4 respectively. In the treatment group T_5 a regressive impact was seen in the growth parameters of fish with the graphs showing the characteristic bell shaped curve (Fig. 1). These results showed that algal meal could be incorporated into common carp diets to replace fishmeal upto 40% level beyond which it showed declining trend in the growth performance instead. Similar results were reported by other researchers for other fish species and other algae types. There is a whole body of literature confirming present hypothesis that algal replacement levels work best upto a certain optimum replacement levels. Also in the present study, no palatability problem was encountered with any of the treatment groups during the experiment. In general there is a great synergy and compatibility between the algal species as replacement and the target fish species.

The reason for the improvement in the growth parameters in the present study with the inclusion of algal meal in the diets of *Cyprinus Carpio* may be due to the high protein content of algae (Table 1) and as for the regressive effects seen beyond the optimum replacement proportions, could be due to the high Nitrogen-free extracts and its possible effects on the digestibility of protein and dry matter. These nitrogen free extracts have been widely studied for their deleterious effects by Xu *et al.* (2011).

5.1 Physico- chemical parameters of water

All the physico-chemical parameters of water such as temperature, pH, dissolved oxygen, hardness and ammonia were observed to be within the optimum range of requirements for fish as suggested by many authors (Boyd and Tucker, 1998). In the present study, water quality was maintained at congenial level for the rearing of common carp fingerlings. In the present study, the physico-chemical parameters were monitored weekly. All the water quality parameters were found to vary non-significantly and were within the acceptable ranges during the experimental period. Water temperature plays a significant role in metabolism and growth. *Cyprinus carpio* is an eurythermal fish and can thrive well at temperature range of 10 to 37.8^{o}C. The water temperature varied from 16 to 25^{o}C during the experimental period which is optimum for culture of common carp. As per Jhingran and Pullin (1985) temperature ranging from 10 to 37.8˚C is tolerable by Indian Major Carps. Thus, in the present investigation the temperature was within the desired range for better growth. The water pH recorded in all the treatments during the experiment was almost uniform and ranged between 7.2 to 8.2, which is within the acceptable range of (6.7-8.6) as suggested by (Meade, 1985). Swingle (1957) reported a pH range of 7.5 to 8.5 suitable for optimum growth of fish. Therefore, pH recorded during the present study was within the optimal range for better growth of fish. The Dissolved oxygen level varies with a large number of factors such as water temperature, metabolic rate, biomass density etc. The dissolved oxygen level in waters in a culture system must be maintained above levels considered stressful to fish as directly affects the growth of fish. The Deficiency of dissolved oxygen in water results in large scale mortality of fishes. Jhingran (1982) reported that dissolved oxygen concentration of 5 to 7 ppm is good for survival and growth of fish. In the present study the DO content remain within the desirable range of 6.1 to 7.2 which is within the optimum range of more than 5mg/l for carps (Adhikari, 2006). The suggested value of ammonia nitrogen in water suitable for pond fisheries is <0.2 mg L^{-1} (Bhatnagar and singh 2010). High concentration of ammonia causes an increase in Ph and ammonia concentration in the blood of fish which can damage the gills, the red blood cells and affect osmoregulation. In the present study the concentration of ammonia ranges from (30 µg/l to 70µg/l) which supports the optimum range. The first sign of ammonia toxicity include a slight restlessness and increased respiration, the fish congregate close to water surface. Total hardness during experimental study was observed to be in the range of 140 to 155mg/l. According to Bhatnagar *et al.* (2004) hardness values less than 20ppm causes stress. An optimum level for fish culture is 75-150 ppm and >300ppm is lethal to fish as it increases pH, which results in non- availability of nutrients. However, some euryhaline species may have tolerance limits to hardness. Hence in the present study hardness values were within the favorable range for better growth and survival of carp fingerlings.

5.2 Growth parameters

Body weight gain (BWG) and specific growth rate (SGR) are the basic fundamental parameters that have been analysed in the present study. In the present study highest weight gain was recorded in the treatment group T_4 (76.23±0.04 gm) (Table 5) and the lowest weight gain was observed in the control group T_0 (44.54±0.31 gm) (Table 5), which was significantly different ($P < 0.05$).The mean of the SGR of the treatment group fed with 40% algal meal (1.41±0.07 %/day) (Fig. 2) was significantly higher ($p<0.001$) than the all other groups. The lowest SGR value was found in the control group T_0 (1.30±0.63 %/day), which was significantly different ($p<0.001$). This is in agreement with the results of Wassef *et al.* (2001) who reported that striped mullet (*Mugil cephalus L*), fed with Ulva meal at 20% inclusion level showed significant weight gain and feed efficiency when ulva meal was included in the feed at different inclusion levels i.e, 10, 15, 20 and 25%. Similarly Badwy *et al.* (2008) investigated the replacement levels of fish meal with dried microalgae (*Chlorella* spp. and *Scenedesmus* spp) in Nile tilapia 0%, 10%, 25%, 50% and 75% inclusion levels. The results indicated that growth parameters *viz* body weight gain (BWG) and specific growth rate (SGR) were significantly higher for substitution levels of 50%. Further confirming our proposition Nader *et al.* (2010) evaluated the effects on growth performance, feed utilization and body composition for red tilapia (*Oreochromis sp.*) by the inclusion of green seaweeds (*Ulva sp.*) as a feed supplement in fish diet, he reported that final body weight, weight gain and specific growth rate (SGR) increased considerably with raising *Ulva* level in fish diet up to 15% when ulva meal was included in the fish feed at different inclusion levels i.e, 10, 15, 20 and 25%. The results of the above studies are in agreement with the present study which showed that both BWG and SGR increased upon replacement of fishmeal by algalmeal but only upto 40% inclusion level beyond which the benefits taper off as can be seen from Table 5 and Fig. 1, Fig. 2.

Feed conversion ratio (FCR) is another important growth parameter widely studied by researchers. It defines the input (feed) per unit of output (body mass). In the present study the lowest FCR (1.99 ± 0.01) was recorded in the treatment group T_4 (Table 5). The highest FCR (2.28±0.58) was recorded in the control group. In line with our hypothesis Guroy *et al.* (2005) studied the effects of Green macroalgae (*Ulva rigida*) and brown macroalgae (*Cystoseira barbata*) meals as a feed additive on growth performance, feed utilization and body composition of Nile tilapia, *Oreochromis niloticus* for a period of 12 weeks and reported that fish fed with the feed of 15% *Ulva* meal displayed the lowest feed conversion ratio (FCR) when supplemented with various levels of *Ulva* meal (5%, 10%, or 15%) or *Cystoseira* meal (5%, 10%, or 15%). Further Uchechukwu *et al.* (2017) studied the utilization of *Chlorella vulgaris* as protein source in the diets of African catfish (*Clarias gariepinus)* and reported that African catfish fed with 0% green algal meal showed highest FCR when algal meal was included at 0%, 5%, 15%

and 25% inclusion levels. The results of above studies are in agreement with the results of present study wherein FCR decreases to a certain optimum replacement concentration point (upto 40%) beyond which it increases as can be seen from Table 5 and Fig 3.

Protein efficiency ratio (PER) defined as the ratio of weight gain to the food intake is the another parameter analysed in the present study. Highest PER value was found in the treatment group T_4 (1.41±0.05) (Table 5) which was significantly different ($p<0.001$) from the control group T_0. In slight deviation from our present results Anna *et al.* (2009) studied the inclusion of red alga *Porphyra dioica* as a fish-feed ingredient for rainbow trout (*Oncorhynchus mykiss*) and reported that by adding *Porphyra dioica* meal at levels of 5, 10 and 15%, protein efficiency ratio (PER) was not influenced significantly by the addition of seaweed meal for any of the diets. But in the present study PER was affected by replacement of fish meal with algal meal with highest PER obtained for 30% replacement level as can be seen in Table 5 and Fig 4.

Survival rate i.e, the live fish at the end of experimental period is another parameter that has been studied in fishmeal replacement studies. In the present study no mortality was observed during the experimental period of 8 week as no significant difference between treatment groups and control group was observed (table 5). This is in agreement with the results of Betul *et al.*, who reported less than 10% of fish mortality while studying *Cystoseira barbata* as a feed supplement for Nile tilapia. Dallaire (2006) also reported 100% survival rate in all treatment groups in an experimental study of rainbow trout fry (*Oncorhynchus mykiss*) with supplemented diet of algal biomass.

Confounding factors like water temperature, pH, dissolved oxygen, hardness and ammonia were monitored and kept under optimum ranges. In the present study, water quality was maintained at congenial level for the rearing of common carp fingerlings. The physico-chemical parameters were monitored weekly so that these factors would not affect the rearing of fingerlings which helped the present study in finding an association between the replacement percentages of algae and growth indices.

It has been shown that the algae in general could be incorporated into the diet of *Cyprinus Carpio* upto an inclusion level of 40%. Future studies are needed, however, to optimize the level of algal meal in diets of *Cyprinus Carpio* to improve growth performance. It would also be interesting to assess the cost benefit analysis to assess whether these inclusion levels could be cost effective at a commercial scale.

Chapter 6

SUMMARY AND CONCLUSION

The percentage contribution of aquaculture to fishery products has grown steadily from 4% in 1970 to 36% in 2006 (UNFAO). Aquaculture relies mainly on fishmeal and fish oil for feed purposes. However these products are subjected to price volatility in international and national markets, thereby handicapping the growth of aquaculture. The present study through its novel approach of replacing fishmeal by algal meal in fixed proportions in diets of *Cyprinus Carpio* fingerlings tries to reduce this hindrance.

The present study entitled "Effects of algalmeal in prepared feed on growth, body composition and survival of Common carp *(Cyprinus Carpio var. communis)*." was conducted at Rangil Campus of Faculty of Fisheries, SKUAST-K. Algal bloom was collected from the Dal lake and analysed bio chemically. Three different species of algae *viz*. *Spirogyra, Oedogonium* and *Ulothrix* were identified. Proximate analysis of each of the algae was then carried and the results obtained were as: *Spirogyra* (25% protein and 5% fibre), *Oedogonium* (14% protein and 18% fibre) and *Ulothrix* (45% protein and 5% fibre). The algae was then dried, grounded and properly mixed. The fishmeal was then replaced in proportion of 10%, 20%, 30%, 40% and 50% and control diet was formulated without addition of algae.

The fish selected for the experiment was Common carp (*Cyprinus carpio*) because of its quick growth, tasty flesh, hardy nature and adaptability to cold waters and the ease with which it can be breed in confined waters. Fish were fed on the tested diets at a rate of 5% of body weight for 8 weeks.

The important water quality parameters like temperature, pH, dissolved oxygen and total alkalinity recorded during the experimental period were within the desired range for culture of Common carp. Highest weight gain was recorded in T_4 group with replacement percentage of 40%. The SGR of the T_4 group was significantly higher ($p<0.001$) than the all other groups. The lowest FCR was recorded in the treatment group T_4 group. Highest PER value was found in the treatment group T_3 which are significantly different ($p<0.001$) then the control group.

From the present study it may be concluded that algal meal appears to be a promising and sustainable protein source to replace fishmeal in the feed of *Common carp*. The results demonstrated that algal meal supplemented diets significantly improved the growth performance, body composition and survival of experimental fish *Cyprinus carpio*. Supplementation of algal meal in the feed at 40% inclusion level resulted in better growth performance of the fish in terms of its increased weight gain, low feed conversion ratio, high specific growth rate and protein efficiency ratio. The fish thrives well on feed supplemented with algal meal.This will definitely reduce the cost of feed and in turn the cost of fish production.

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiners Dr. Lateef Ahmad, Assistant Professor, Department of Animal Science, Central University of Kashmir during thesis evaluation and Viva-Voce examination held on 05-04-2021 have been incorporated in the manuscript entitled **"Effects of algal meal in prepared feed on growth, body composition and survival of the Common carp (*Cyprinus carpio* var. *communis*)"** submitted by **Ms. Ishrat Bashir (Reg. No. MSFY/2018/90).**

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