Effect of *Spirulina* meal as feed additive on growth performance and body composition of climbing perch

A dissertation Submitted in Partial Fulfillment of the Requirement for the Degree of Master of Science in Fisheries

Submitted By:

Examination Roll No.: 4204

Session: 2013-2014

Registration No.: Ha-1414

Session: 2008-2009

Department of Fisheries

University of Dhaka

January 31, 2016

DEDICATED

TO

MY BELOVED PARENTS

AND

HONORABLE TEACHERS

CERTIFICATE

This is to certify that this thesis entitled **"Effect of *Spirulina* meal as feed additive on growth performance and body composition of Climbing perch "** submitted by Md. Zahidul Islam has been carried out under my complete supervision. This is further to certify that it is an original work and suitable in partial fulfillment for the degree of MS in Fisheries, University of Dhaka.

(Co-supervisor) (Supervisor)

Md.Alamgir Kabir **Md. Ghulam Mustafa, Ph.D**
Assistant Professor Professor
Department of FisheriesDepartment of Fisheries
University of Dhaka.University of Dhaka.
Dhaka-1000, Bangladesh. Dhaka-1000, Bangladesh.

ACKNOWLEDGEMENTS

First and foremost, I wish to express all of my devotion and reverence to the almighty Allah who has blessed with my life , time and energy and enable me to peruse my higher education in Fisheries and to complete this research work and this manuscript for the degree of Master of science (MS) in Fisheries.

I wish to express my deep sense of gratitude sincere appreciation and indebtness to**Dr.Md. Ghulam Mustafa**, Professor, Department of fisheries, University of Dhaka for his kind suggestion , guidance and supervision to carry out this study.

I am grateful to my co-supervisor **Md. Alamgir Kabir**, Assistant professor, Department of fisheries, University of Dhaka for his kind co-operation, designing and implementing the research work with valuable suggestion and supports.

Mst. Wahida Haque, Chairperson, Department of fisheries, University of Dhaka also deserves special thanks for giving inspiration while prosecuting the thesis work.

Thanks are also been to my adorable parents for their affection, inspiration and forbearance.

I would like to my cordial thanks to all staffs for helping me in laboratory procedure.

Special thanks to Ministry of National Science and Technology for co-operation and financial assistance during the thesis work.

Finally I expresses heartful respect and thanks to my sisters and friends for their active cooperation, moral support, kindness, blessing and endless love which made everything possible to my life.

January 31, 2016 Author

Abstract

An experiment on culture of climbing perch (*Anabas testudineus*) in plastic tanks was conducted to assess the effect of *Spirulina meal* as feed additive on growth performance and body composition of 20 days old koi (*Anabas testudineus*) fry in intensive culture of this fish .The experiment was carried out in Department of Fisheries, University of Dhaka for a duration of 75 days in re-circulating system with 2 treatment in 6 tanks containing 500 L of water each. 20 fry were stocked per tank. The initial weight was 1.57 ± 0.5g for Diet I (Prepared by wheat flour, corn meal, soyabean meal and fish meal) and 1.84±0.05 g for the DietII *(Spirulina* meal). Initial length for both groups was 4.01cm and was feed with two types of feeds.There are three replicas for each group. At the end of the experiment average weight of koi was 15.01g.. The feeds were applied twice a day at the rate of 6% (initially) to 4% (later on) of the body weight of the fry/day. Due to presence of *Spirulina*fish meal, soybean meal, wheat flour and corn meal, Diet II was proved to be the best proteinacous. The results showed that the growth of fry varied significantly ($P<0.05$) with different diets. The higher growth and lower FCR were found in the trial where fishes were fed on *Spirulina* meal containing 56.23% followed byDiet II. Specific growth rate and mean body weight were higher in fish fed with *Spirulina*meal. The poorer growth rate was shown by Diet-I containing 31.12% protein. Change in growth and feed utilization by the koi fish fed on two types of fish feed during the rearing and feeding trial have been assessed by the determination of specific growth rate (SGR%), feed conversion ratio (FCR), protein efficiency ratio (PER), feed efficiency and average daily gain (ADG) etc.There was no significant difference in survival rates among the fry fed with *Spirulina* and formulated diets. The better FCR was found (1.10±0.105) in T_2 (Diet II containing *Spirulina* as a feed additive).This might be due to the attractive appearance, color, taste, flavor and good quality nutrient composition of the experimental diets. Results also showed thataddition of *Spirulina* in the formulated feedhas profound effect on the PER, ADG, SGR of *A. testudineus.*

Results of the current study suggest that supplementation fish feed with 56.23% protein level has significantly positive effect on the growth performance and body composition of *A. testudineus.* The experiment suggest that Diet-II (*Spirulina*meal) can be recommended for the augmentation of growth and feed utilization in intensive culture of *A. testudineus.*

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATE **Page No.**

Title

Plate no.

.

.

LIST OF SYMBOLS AND ABBREVIATIONS

Symbols	**Details**
℃	Degree Celsius
G	Gram
Cm	Centimeter
%	Percentage
ANOVA	Analysis of variance
ADG	Average daily gain
SPSS	Statistical package for the social sciences
SGR	Specific growth rate
FCR	Feed conversion ratio
PER	Protein efficiency ratio
SR	Survival rate
T_1	Treatment 1
T_2	Treatment 2

INTRODUCTION

Bangladesh is an agro-based riverine country enriched with huge fisheries resources. The total area of inland water in general estimated about46.99 lakh hectares. Water area of inland fisheries including rivers, beels, Kaptai lake, flood plains and polder/ enclosure as open water body (capture based) comprise about 39.16 lakh hectares; ponds and ditches, baor and coastal shrimps farm as closed water body (culture based) comprise about 7.82 lakh hectares (DOF,2014).The republic has a 710km long coastline. The nation's exclusive economy zone contains up to 41,040 square nautical miles from the coastline.Thus the nation's total area of water having fish production potential is of very great importance. Fisheries play a vital role in our national economy and contribute 4.37% to the GDP, 23.37% to the agricultural products and 2.01% to the export earnings. In 2012-2013 Bangladesh has earned 50 thousand core Taka by exporting 34.10 lakh metric tons of fish and fishery products to foreign countries. About 17 million people are engaged directly or indirectly in subsistence fishing and activities related to fish sector(DOF,2014).

Fish is the most important protein sources in the diet of the Bangladeshi people. Fish supply about 60% of the available protein in the diet and the rest 40% protein comes from livestock and poultry. It indicates the importance of fish in contributing to the level of nutrition of thepeople of Bangladesh (DOF, 2014). In spite of having large fisheries resources, Bangladesh is facing asevere malnutrition problem due to the shortage of animal protein supply in our diet.

The present annual fish intake by an individual is 19.30kg and annual fish demand 37.92 lakh metric tons (DOF,2014). This is due to rapid human population growth and decline of catch from inland open water area. There is a slight prospect for additional yield from open water capture fisheries. Only the culture fisheries seemto be dependable means of the achieving increased yield. In order to meet the need for vast increase in animal protein supplies, animal breeders introduced news high yielding varieties of live stocks, aqua culturist introducing innovative methods of fin fish, shell fish and crustacean culture to make possible animal protein production to keep pace with the increase in population.

*A.testudineus*are generally found in ponds, rivers and swamps. Larvae and young fry fed on phytoplankton and zooplankton, larvae fry and adults feed on crustaceans, worms, moluscs and insects, algae, soft higher plants and organic debris (Potogkam, 1972).

*A.testudineus*has been described a predator, carnivore (Pandey et al.1992).However gastric contents analysis of 204 specimens of *A.* testudineus showed that the stomach contained 19%crustaceans, 3.5% insects, 6% mollusk, 9.5% fishes 47% plant debris and 1.6% semi – digested matter (Nargis and Hossain,1987).Major food item in the gut were found to be more or less consistent irrespectiveto spatial and seasonal distribution in Bangladesh (Nargis and Hossain, 1987) indicating*A.testudineus.*

Air breathing fishes are very popular in the food menu and have become an economically important group of fishes in Bangladesh. Among them Climbing perch (*Anabas testudineus*) commonly known as koi is one of the most popular fishes because of its taste and high market value.It is a potential candidate for aquaculture. This species is considered as a valuable item of diet for sick and convalescents (Mustafa *et al*. 2004). It contains high amount of available iron and copper essentially needed for hemoglobin synthesis. Besides it also contains easily digestible fat of very low melting point and good various essential amino acids (Hossain *et al*. 2012). Protein is the main dietary nutrient affecting performance of fish. Levels of dietary protein and energy, not only influence the body composition, but also digestive enzymes and plasma metabolites in fishes. On the other hand, dietary lipids are important source of energy and essential fatty acids for fish. Moreover, dietary lipids act as carrier of nutrients such as fat-soluble vitamin A, D and K.Besides lipid content of cultured fish is usually higher than that of wild fish.

Feeding *Spirulina* commonly elevated body weight gain, and tended to increase feed efficiency and muscle protein deposition. Furthermore, the use of *Spirulina* as the supplementation of feed resulted in a considerable elevation of biomass production (Mustafa *et al*.1995). Overall the successful completion of the study will help to establish the appropriate and cost effective feed using *Spirulina*as feed additive on growth performance and body composition of *A. testudineus*.

1.1 Nutrients in Fish

Nutrition is a vital feed in fish farming represents 40-50% of the production costs. Fish contributes enormously to the supply of both macro and micronutrients in our diet. It is considered to be the prospective source of proteins and many other micronutrients, such as vitamins and minerals. Fish have some unusual composition features that do not have significant carbohydrates.

For all practical purposes, the caloric values of fishes are based only on theprotein andfat content. The feature is that a few species have their fat predominantly in the form of wax esters instead of triglycerides. These wax esters are believed to be resistant to digestion by human system so that the fat content would contribute considerably to the caloric value of fish (Nettleton, 1985).

Proximate composition of fishes is an essential ecological measure of condition that integrates both feeding condition and habitat quality. Proximate composition can also have significant implications in the study of fish bioenergetics as well as the study of contaminants, given the propensity of many compounds to be related to lipid levels (Lanno *et al.* 1989). Further, certain components such as fat levels have also importance in aquaculture and food technology, where the fish grading, fish quality and value are linked to fat levels in the tissue (Rasmussen, 2001).

The biochemical composition of fish-flesh may vary within the same species of fish depending upon the fishing season, age, sex and habitat (Srivastava, 1985). The variation is also found within the different region's water body (Jacquot, 1961).

1.1.1 Requirement of Water (Moisture)

Water is the major component of all species of fish. Usually water content ranges from 70-80% of the fresh weight, although some deep water species may have some excess of 90%. There are seasonal variations and a slight increase occurs when the fish is starving (Clucas and Ward, 1996).In most bony fish, fat and water content make up to 80% of the fresh weight. In simple terms, the high water content can be held responsible for the perish ability of fish (Clucas and Ward, 1996).

1.1.2Requirement of Carbohydrate

Carbohydrates are the most economical and inexpensive sources of energy for fish diets. Although not essential, carbohydrates are included in aquaculture diets to reduce feed costs and for their binding activity during feed manufacturing. Dietary starches are useful in the extrusion manufacture of floating feeds. Cooking starch during the extrusion process makes it more biologically available to fish. In fish, carbohydrates are stored as glycogen that can be mobilized to satisfy energy demands. They are a major energy source for mammals, but are not used efficiently by fish.

1.1.3Requirement ofProtein

Fish supplies not only abundant amount of protein, but also kinds of protein most efficiently used by the body. The cardinal virtue of all fish is their high quality protein. Fish protein is 85-95% digestible and all dietary essential amino acids are present in fish (Nilson, 1946). With few exceptions, most proteins from animal products are complete. All fish provide complete protein having all essential amino acid so that less of it is required by the body to meet its daily protein requirement. Cereal grains are usually low in lysine and sulphur containing amino acids (Methionine and Cysteine) whereas fish protein is an excellent source of these amino acids. In diets based mainly on cereals, a supplement of fish can raise the biological value significantly (Huss 1988). Another feature of fish protein is that it is highly digestible. This means that it is readily digested by the body and easily absorbed. People of all ages from children over a year to older can enjoy fish, because its protein is highly digestible (Nettleton, 1985).

Protein requirements generally are higher for smaller fish. As fish grow larger, their protein requirements usually decrease. Protein requirements also vary with rearing environment, water temperature and water quality, as well as the genetic composition and feeding rates of the fish (Lovell, 1989).

1.1.4Requirement ofLipid (Fat)

Lipids can be utilized to somewhat spare protein in aquaculture feed because of high-energy nutrients. Lipids supply about twice the energy as protein and carbohydrates.

Most fishes are relatively low in total fat and relatively high in its proportion of polyunsaturated fatty acids. This feature gives fish a clear health advantage (Nettleton, 1985).

Fats, especially vegetable oils, contain an essential fatty acid called linolenic acid that the body cannot make for itself. The amount of linolenic acid required in small and is easily obtained from the foods we commonly eat, especially vegetables and fish. It also appears that linolenic acid, a second fatty acid, is probably essential in humans (Holman *et al.* 1982).

It may also be that omega-3 fatty acids in fish oils are necessary for optimum healthy heart (Titus *et al.* 1982, and Oliw *et al.* 1983). Fats are made of different kinds of fatty acids that in turn differ in the amount and arrangement of the carbon and hydrogen atoms they contain. There is still a great to learn about how the body processes different fatty acids, but it seems clear that some fatty acids are more beneficial for health than others (Nettleton, 1985).

In particular, polyunsaturated fatty acids have been shown to be more favorable for healthy blood lipid levels than saturated fats. In many people, achieving a better blood lipid pattern can lower the chances of heart attack or stroke (Grundy*et al.* 1982). Fishes have been used in diets designed to prevent and treat cardiovascular disease, one of the leading causes of mortality in today's world. The best ways to achieve a healthy blood lipid pattern are to eat less fat in total to limit the amount of saturated fats consumed and to keep cholesterol intake below 300 mg per day (Emst, 1985).

Fish liver oil differs in fatty acids and composition from the oil in flesh. Since the liver contains a great deal of fat, however, the liver becomes an important source of polyunsaturated fatty acids (PUFA).

Thus consuming whole fish can greatly the amount of polyunsaturated fatty acids from fish there are two outstanding features about the fat in fish; the total amount is very low in most varieties and fat is rich in polyunsaturated fatty acids having more than four double bonds. The predominant polyunsaturated fatty acids in fish oil are the omega-3 fatty acids having either five or six double bonds. There are about seven ω-3 fatty acids in fish are derived from the phytoplankton in the food chain that fish eat.

The implications from the observations among Greenland Eskimos is that fish oils are protective against heart disease, stroke and possibly diabetes and other diseases as well (Goodnight *et al,* 1982, and Bang *et al.* 1980).

1.1.5 Requirement ofAsh (Minerals)

Fish are the important sources of essential minerals such as zinc, copper, iron, magnesium, sodium, calcium, phosphorus, potassium (Nettleton, 1985; Banu *et al.*1985; Nurullah *et al.* 2003).

Fish provides a well-balanced supply of minerals in a readily usable form (Murry and Burt, 1982). In most species, the total mineral of ash content ranges from 1 to 286. There is a wide variety of minerals in fish flesh and they are usually present in a form, which is readily available (Clucas and Ward, 1996). Fish flesh is regarded as a valuable in particular, but also of iron and copper (Huss, 1988).

Specific nutrition levels play a very important role in fish growth, body composition, feed utilization efficiency and reproduction. Feed is the main factor responsible for affecting growth, spawning success, body composition, and survival of fry including the survival of the brood (De Silva and Perera, 1985).

Proper food selection is important both from nutritional and economical point of view.A food particle should deliver the necessarynutrients and in a form that can easily beconsumed by the fish which will result in moreefficient production and increased profits. Substances incorporated in feed at low level to enhance feed intake, growth and utilization are called as attractants.

The use of an attractant in manufactured aqua feed has received considerable attention in the recent years. Chemicals compounds such as organic bases, betaine, terpenes and sulphur compounds are reported to induce olfactory and gustatory stimuli in fish. Considering the importance of various behavioral components of fish it is logical to assume that by adding attractants to the feed the animal could be attracted towards in a shorter period of time, creating the condition for faster ingestion.

One of the biggest problems facing the utilization of fish nutrition, in many aquaculture operations today, feed accounts more than half of the variable operating cost (NRC 1993).

Therefore, the potential use of unconventional foodstuffs such as algae, for substitution the high cost food stuffs such as fishmeal is very important. Algae have attention as a possible alternative protein source for cultured fish, particular in tropical and subtropical developing countries where algae production rates are high and their higher protein, vitamins and essential fatty acids contents (El-Hindawy *et al.* 2006, and Badawy*et al.* 2008).

Algae can be used directly as live culture or as value added feed supplement. Newly formulated algal diet should satisfy the nutritional requirements of the fishes with high acceptability of the feed.

1.2 Significance of Algae as feed in aquaculture

Algae have been proved to be one of the most important food sources and feed additives in the commercial rearing of many aquatic animals, especially fishes and penaeid prawn larvae (Borowitzka, 1997;Belay *et al*. 1996 and Khatoon *et al.* 2010).
Macro and microalgae have been supplemented in diets for different cultured fish species and have been reportedto have positive effects on growth performance, feed utilization, lipid metabolism, carcass quality, stress tolerance anddisease resistance (Mustafa and Nakagawa 1995; Nakagawaand Montgomery 2007; Güroy *et al* 2011).

Macro and micro-algae, such as *Ascophyllum, Laminaria, Undaria, Porphyra, Ulva, Spirulina and Chlorella*, as feed additives have been reported toimprove growth, feed utilization, lipid metabolism,body composition, stress responses, liver functionand disease resistance in ayu, *Plecoglossus altivelis.*

Microalgal speciesare of great value because of their high bioactive materialscontent, including polyunsaturated fatty acids, β-caroteneand other pigments (antioxidants) (Cohen and Vonshak 1991;Mahajan and Kamat 1995; Bhatt and Madyastha 2000; Reddy*et al.* 2000), sulfated polysaccharides (anti-virals) and sterols(antimicrobials) (Ötleş and Pire 2001).

1.3Importance of*Spirulina* as a feed additive

Spirulina has been one of the most widely used micro-algal species in aquafeeds due to its high contents of protein, vitamins, essential amino acids, minerals, essential fatty acids and antioxidant pigments such as carotenoids (Nakagawa and Montgomery 2007). Also, its immune modulator activity has been shown in animal experiments, which demonstrated its enhancement of phagocytic and natural killer activities (Qureshi and Ali 1996).

Nowadays, *Spirulina* can be used to establish immune-potentiating functions in carp (Watanuki*et al*. 2006; Tongsiri *et al.* 2010).Several studies have been conducted to investigate the effects of *Spirulina*on growth, nutrient utilization and immune responses of various fish species, including rainbow trout *Oncorhynchus mykiss* (Matty and Smith 1978), red sea bream *Pagrus major* (Mustafa *et al.* 1997),common carp *Cyprinus carpio* (Nandeesha *et al*. 1998), tilapia *Oreochromis niloticus* (Takeuchi *et al.* 2002), Mekong giant catfish *Pangasianodon gigas* (Tongsiri *et al.*2010), and African sharptooth catfish *Clarias gariepinus* (Promya and Chitmanat 2011).

Mu *et al.*(2000) and Nandeesha*et al.*(2001) indicated that *Spirulina* could be used as an effective partially or completely replacement for fishmeal in formulated aqua feeds.
Spirulina is a unique high quality natural diet with enriched optimum protein for fish and shrimp which has proven as a best supplementary feeding in aquaculture.

Spirulina is blue green algae similar to a spiral of long thin threads under genus *Arthrospira,* the phylum Oscillatoriaceae. *Spirulina* is called blue green algae (Cynobacteria) because of presence of both green (chlorophyII) and blue (phycocyanin) pigments in its cellular structure. The two common species are more important for its nutritious value, *Spirulina maxima* and *Spirulina plantensis.*

1.3.1 Nutritional food value of *Spirulina*

The use of *Spirulina* as supplementary feed in various sectors of aquaculture resulting fast growth factors, enhancing theimmunity systems andpigmentation. It is considered as an excellent food, lacking toxicity and having corrective properties against the pathogenic micro organisms.

It lacks cellulose cell walls and therefore do not requires chemical or processing in order to become digestible. The digestibility is about 83 – 84 %.

Spirulina is regarded as a rich source of protein, vitamins, essential mineral, amino acids, EFFA like gamma LNA and antioxidant pigments like carotenoids.

Spirulina contains :

Factor	Percentage (%)
Protein	60-70
Carbohydrytes	15-25
Fats(Lipids)	6-8
Mineral (Ash)	7-13
Fiber	8-10
Moisture	3-7

1.3.2 Biochemical composition of *Spirulina*

Protein & Amino acids

Spirulina provides 60-70% protein along withcarotenes,linolenic acids, phenolic acids, tocopherols, and for which represents an important staple in diets. The essential amino acids are present around 47 % of total protein weight. The spectrum of amino acid represent that the biological value of proteins in *Spirulina* is very high.

Amino acid and Biological function of Fishes & Shrimps:

1. **Isoleucine:** Required for optimal growth, nitrogen equilibrium in the body .Used to synthesize other non-essential amino acids.
2. **Leucine**: Increases muscular energy levels.
3. **Lysine**: Building block of blood antibodies strengthens circulatory system and maintains normal growth of cells.
4. **Methionine**: Vital lipotropic (fat and lipid metabolizing) amino acid that maintains liver health. An anti-stress factor.
5. **Phenylalanine:** Stimulates metabolic rate.
6. **Threonine**: Improves intestinal competence and digestive assimilation.

7 **Tryptophane:** Increases utilization of B vitamins, improves nerve health.

8 **Valine** : Stimulates muscle coordination.

Cabohydrates

Spirulina contains about 16 -20 % carbohydrates in the form of Glucose, fructose, sucrose, mannose,rhamnose, xylose and galactose. It provides the appropriate and important food stuff for aquatic culture animals with problems of poor intenstinal absorption. Carbohydrates occur in sufficient quantities of mesoinositol phosphate which is the excellent source of organic phosphorus and inositol. A high moleculatory weight polysaccharide is believed to have effect on DNA repair mechanisms, immune-stimulatory and immune-regulatory properties.

Nucleic acids (NA)

S*pirulina* contains 2.2% - 3.5% of RNA and 0.6 %-1% DNA, which represents less than 5% of these acids,based on dry weight.

Essential fatty acids (EFA)

Spirulina has a high amount of polyunsaturated fatty acids (PUFAs) and 1.5–2.0 percent of total lipid. *Spirulina* is rich in γ-linolenic acid (ALA), linoleic acid (LA), stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA).

Vitaminsandβ-carotene

Spirulina contains vitamin B1 (thiamine), B2 (riboflavin), B3 (nicotinamide), B6 (pyridoxine), B9 (folic acid), B12 (cyanocobalamin), vitamin C, vitamin D and vitamin E. The β-carotene, B-group vitamin, vitamin E, iron, potassium and chlorophyll available in the *Spirulina* can promote the metabolism of carbohydrate, fats, protein, alcohol, and the reproduction of skin, muscle and mucosa. *Spirulina* contains large amounts of natural β-carotene and this β-carotene is converted into vitamin A.

Minerals

*Spirulin*a is a rich source of potassium, and also contains calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, zinc, molybdenum, chloride, germanium and boron.

Photosynthetic pigments

Spirulina contains many pigments including chlorophyll a, xanthophyll, betacarotene, echinenone, myxoxanthophyll, zeaxanthin, canthaxanthin, diatoxanthin, 3-hydroxyechinenone, betacryptoxanthin.

1.3.3Nutritional supplementary property

Spirulina can be used as a partial supplementation or complete replacement for protein in aqua feeds. *Spirulina* is a feed supplement for the all fishes, giant freshwater prawns and marine water shrimps and significantly improvement occurs on growth, survival, immunity, viability and feed utilization.
Spirulina is a cheaper feed ingredient with high protein than others of animal origin. *Spirulina* diet is found as most suitable supplementary feeding to reduce the cultivation time and mortality, and increase shell thickness of shrimp carapace. Feeding on *Spirulina* helps to improve disease resistance of fish and an improvement in their survival rate. Fast growth occurs when fed a diet containing *Spirulina* meal.

Chelating of toxic minerals (neutralization of toxic minerals)

Spirulina provides phycocyanin, a source of biliverdin which is among the most potent of all intra-cellular antioxidants.*Spirulina* has a unique quality to detoxify (neutralize) or to chelate toxic minerals and this characteristic is not yet noticed in any other microalgae (Maeda and Sakaguchi 1990; Okamura and Aoyama 1994). *Spirulina* can be used to detoxify arsenic from water and food. It also may be used to chelatize or detoxify or neutralize the poisonous effect of heavy metals (minerals) from water, food and environment.

Immunomodulatory Property

Spirulina is avaluable immune modulator. It is very effective and exhibits anti inflammatory properties, in particularly by inhibiting the release of histamine from mast cell with mediated allergic reactions .It shows antioxidative and free radical scavenging properties.

Spirulina exposure enhances the phagocytic functions of macrophages in aquatic culture animals. It also has antiviral and anticarcinogenic properties. It improves the bacterial gut tract clearance potential of fish/shrimp and *Spirulina* supplements develop the phagocytic cell. The *Spirulina* is safe diet to use in terms of improved immune competence without compromising the performing behaviors of aquatic culture animals. A novel sulphate polysaccharide of *Spirulina* inhibits the replication of several enveloped viruses.

*Spirulin*a is a powerful tonic for the immune system. This enzyme is a major source of super oxide in an animal's body, and is involved in dozens of degenerative processes involved in disease resistance, aging and similar processes in fish, shrimp and other aquatic animals.

The nutrients of *Spirulina* help to fight free radicals, cell-damaging molecules absorbed by the body through pollution, poor diet, injury, or stress. By removing free radicals, the nutrients help the immune system fight cancer and cellular degeneration.

***Spirulina* in building red blood cells and stem cell**

Spirulina is rich in a bright blue polypeptide called Phycocyanin. Phycocyanin affects the stem cells that make up the cellular immune system and red blood cells that oxygenate the body. Phycocyanin stimulating hematopoiesis, (the creation of blood), emulating the affect of the hormone erythropoetin(EPO). Phycocyanin also regulates production of white blood cells, even when bone marrow stem cells are damaged by toxic chemicals or radiation.

***Spirulina* Anti-Viral and Anti-Cancer abilities**

Calcium-*Spirulina* is a unique polymerized sugar molecule extract of *Spirulina* and containing both Sulfur and Calcium.

The treatment of this water soluble extract has better recovery rates when infected with a lethal Herpes virus. This mechanism occurs because Calcium-*Spirulan* does not allow the virus to penetrate the cell membrane to infect the cell. The virus is stuck, unable to replicate. It is eventually eliminated by the body's natural defenses. *Spirulina* can prevent or inhibit cancers in aquatic animals, and fishes. The unique polysaccharides of *Spirulina* enhance cell nucleus enzyme activity and DNA repair synthesis

Antimicrobial Property

Spirulina excretes variable quantities of products from its metabolism such as organic acid, vitamins and phytohormones. Cell extract of *Spirulina* has shown antimicrobial activities against pathogenic bacteria as like *Bacillus spp., Streptococcus spp.,Saccharomyces spp.* etc.

Bio-mineralization activities

Spirulina thrives in high alkaline waters and it incorporates & synthesizes many minerals and derivative compounds into its cell structure. Transformed into natural organic forms by *Spirulina*, minerals become chelated with amino acids and they are more easily assimilated by the body.

Along with adequate calcium and magnesium in the water (especially for marineorganisms), *Spirulina* helps insure proper electrolyte function, calcium levels over calcium and other mineral.

Enhance the Reproduction activity

Research has shown that fresh and saltwater fish and shrimp exhibit superior growth, maturity, energetic behavior, and more elegant coloring when fed *Spirulina.* It is also well documented that *Spirulina* improves spawning, fecundity, fertility and hatching rates. It stimulates the reproductive processes, increases survival rates of younger fish, post larvae and promotes the appetite of fish/prawn to attain full mature.

***Spirulina* as a colourant**

The color appearanceof shrimps and fishes is the most vital characteristicfor choice in case & demand in food market.

*Spirulin*a diet improves the physiological activities for generating the color pigmentations and glazing appearance in various parts of body.Carotenoids are responsible for the development of various colours of crustaceans. A marked increase in carotenoids content of the carapace of black tiger shrimp (*Penaeus monodon*) occurred when *Spirulina*-supplemented diets are given. A practical strategy for the improved pigmentation of cultured *P. monodon*is the incorporation of *Spirulina* diet for one month before harvest.

Astaxanthin is predominant carotenoid associated with the red body colour of the black tiger prawn *Penaeus monodon*. *Spirulina platensis*and *S. pacifica* straincontains the highest levels of β-carotene and zeaxanthin of any natural source .They both are converted to astaxanthin through an oxidative process for the desire red pigment

1.4OBJECTIVES

The overall objective of the present study was to investigate how *Spirulina* affects the growth performance and body composition of climbing perch in laboratory condition.

1. To find out suitable low cost artificial feed using *Spirulina* as feed additive for climbing perch to obtain its maximum survival and growth.
2. To develop culture techniques through determination of effective feeding rate.
3. To know the effectiveness of the formulated feed using*Spirulina* on the quality of climbing perch.
4. To analysis the proximate composition of the experimental species.

MATERIALS AND METHODS

2.1 Description of the experimental site

Experiment was carried out in the aquatic laboratory,Department Of fisheries,University Of Dhaka.The experiments consisted of formulated feed and feed additive (*Spirulina*), preparation of supplemented diet, collection of fry of *A.testudineus,* acclimatization of the fry in the laboratory condition, feeding trial of the fry with the control diet without supplementing feed additives, control diet with 3% *Spirulina*. Before, during and after the feeding trial the fry of *A.testudineus*were collected for analytical purpose as well as measuring the biological parameter required for growth performance, survival rate, feed intake, feed conversion ratio (FCR), protein efficiency ratio(PER),etc.

2.2 Experimental designs

Six plastic tanks were used for the feeding trial of the fry of *A.testudineus* in this experiment.One hundredand twenty fry were stocked with density of twenty fish per tank.Six tanks were assigned in two treatments to one control diet without supplement and another control diets with feed additives.20 days old 120 fry of*A. testudineus* of almost equal sizes were released into the tanks randomly.

Plate no.1 Circulr tanks used for rearing of *A. testudineus*.

2.3 Collection of feed ingredients

Fish meal, Soybean meal, rice bran, wheat bran and vitamin and minerals pre-mix were collected fromthe Fulbaria Fish and Poultry Feed Market, Dhaka.

2.4 Collections of feed additives

Spirulina was collected from Fish Technology laboratory, BCSIR, Dhaka.

Plate no2.*Spirulina* powder

2.5 Preparation of the experimental diets

Two experimental diets were used in this experiment to evaluate the growth performance and body composition on *A.testudineus.*

Diet –I: Formulated feed +3%Ultrabinder lignosulfonate (PMC) as control diet.

Diet-II: Formulated feed+ 3 % *Spirulina*used as a feed additive.

Dietary ingredients of feed additive(*Spirulina*) was ground using a laboratory grinder and then separately blended with formulated feed at fixed ratio into a homogenous doughy matter by adding water, which pelleted by pressing through a 3mm die in a grinding machine. The pellets were then stored in plastic containers at room temperature for further use.

Plate no3. Formulated pelleting feed mixing*Spirulina*

2.6Collection of experimental fish

The fry of *A.testudineus*used in this experiment were obtained from a private hatchery named Satotaaqua Farms situated at Bailor, Trishal,Mymensingh. Live and healthy fish were collected inAugust 2015and carried in oxygenated bags with water. Fry were kept in Circular tanks with adequate aeration and sufficient water. The average weight of each fry was .79-5.98 grams and average length of each species was 3-6.2 cm. then acclimatization for one week. During acclimatization, maintenance feed ration about 5% ofbody weight. After acclimatized with the environment, 120 frywere kept randomly in tanks for experiment.

Classification

Kingdom: Animalia
Phylum: Chordata
Class:Actinopterygii
Order:Perciformes
Family: Anabantidae
Genus:*Anabas*
Species: *A. testudineus*

Common name: Climbing perch, climbing bass, kawai, koi, coi, kai,etc

Plateno. 4 Sample of experimental fish (*A. testudineus)* during study period

2.7Management of the tanks and fry

For maintenance of good health and growth of fry, frequently cleaning of tanks and feeding of fry are necessary. All the tanks were cleaned weekly for removing bacteria accumulated on inner surface of rearing tanks. Feces, waste particles of food and dead bodies of fish were siphoned at regular interval. Moreover, when there was deterioration in water quality 30-40% water was replaced.

2.8 Feeding trial

Each diet was fed to fish by 6% of total fish weight per aquarium per day. The daily frequency was offered in two individual meals, one at 9.00-10.00 am and another at 4.00- 5.00 pm. The feeding trial continued for a period of 75 days. Fish were randomly sampled 6% weighted at 7 days interval to adjust the feed intake following to change of body weight. Feed given are based on the average weight gain of fish in each treatment. The survival rates of the fry were also determined and dead fish were removed from tanks.

2.9 Water quality parameter and measurement

Water quality such as temperature, pH, and dissolved oxygen was monitored in the tanks regularly following different procedures.Tap water was used as the mediate for fish culture.Water exchange was done weekly at 90% in wall tanks. Temperature was measured every day, dissolved oxygen and p^H was measured weekly. The temperature was measured directly in the water column two time everyday (minimum and maximum) after feeding by meter. Dissolved oxygen (DO) was measured directly in the water column of tanks every week by using oxygen meter. pH was measured directly in the water column of tanks every week during experiment by pH meter.

2.10 Fish growth performance

Data on growth of fish were gathered. Fish were weighed to the gram using an electronic balance. Fish lengths were measured in centimeters by using measuring scale. All fish growth parameters were calculated on performance such as mean final fish weight,

daily weight gain (g/f/d), percentage of weight gain (%) and specific growth rate, SGR (%/day) etc of fish.

2.10.1 Average daily gain (ADG)

Average daily gain means the increase of body weight per day. It was calculated by the following formula:

$$\text{ADG (g/f/d)} = \frac{\text{Mean final fish weight - Mean initial fish weight}}{\text{Time } (T_2\text{-}T_1) \text{ (days)}}$$

2.10.2 Specific growth rate (SGR %)

Specific growth rate (SGR) was calculated as the percentage increase in weight per animal per day:

$$\text{SGR (\% day}^{-1}\text{)} = \frac{\ln(\text{final fish body weight}) - \ln(\text{initial fish body weight})}{\text{Time } (T_2\text{-}T_1) \text{ (days)}}$$

2.10.3 Condition factor (K)

This is the factor through which condition of the fish is expressed in numerical terms i.e. degree of plumpness or fatness is usually estimated as the condition factor. It was calculated by the following formula:

$$K = \frac{W}{L^3} \times 100$$

Where,

K is the Condition Factor or Coefficient of Condition; referred to as the "K factor"

W is the body weight of the fish in grams (g)

L is the body length of the fish in centimeters (cm)

2.11 Feed utilization

Fish were fed at 6% of body weight. Feed conversion ratio (FCR), protein efficiency ratio (PER) in *A.testudineus* fed Diet I (control diet) and Diet II(*Spirulina*) were determined at the end of the experiment as follow:

2.11.1 Food conversion ratio (FCR)

This is the numerical value used to measure the gross utilization of food for growth in fish. It is also, a measure of efficiency or suitability of a feed. This ratio shows the amount of feed required to achieve a unit weight increase in the product. Food conversion ratio (FCR) is calculated from the number of kilos of feed that are used to produce one kilo of whole fish. Food conversion ratio (FCR) for *A.testudineus* fed control diet (Diet I) and DietII were determined at the end of the experiment as follow:

$$\text{Food conversion ratio (FCR)} = \frac{\text{Feed supplied (g)(dry weight basis)}}{\text{Total weight gained by fish (g)}}$$

2.11.2 Protein efficiency ratio (PER)

Protein efficiency ratio was determined by the following formula:

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Wet weight gain}}{\text{Dry protein intake}}$$

2.11.3 Survival rate (%)

At the termination of experiment, fish were counted and survival rate to calculate as follows:

$$\text{Survival rate (\%)} = \frac{\text{Final no. of fish survived}}{\text{No. of actual fish stocked}}$$

2.11.4 Feed efficiency (%)

Feed efficiency was determined by the following formula:

$$\text{Feed efficiency (\%)} = \frac{\text{Weight gained in wet weight}}{\text{Feed intake in dry weight}} \times 100$$

2.12 Biochemical Analyses of Fish

Sample preparation

After 75 days of rearing the samples were collected, measured and weighted. Then the samples were taken for laboratory analysis to estimate the whole body percentages of moisture, protein, fat and ash. The samples were then weighted and minced in a chemical tissue grinder. Required amount of samples in duplicate were taken for the determination of moisture. Rest of the minced samples was collected as completely as possible. Wet weight was recorded and dried in an oven at 100º C. Weight of the dry sample was recorded. The dry sample was then taken in a mechanical grinder. Proximate analysis was accomplished in dry sample and the values were later readjusted for weight wet.

2.12.1 Estimation of moisture content

a) Principle

It is the weight loss due to the evaporation of water under certain temperature.

b) Apparatus

1. Oven (100-105º C)
2. Moisture dishes, porcelain crucible, glass Petri dish of watch glass.
3. Metal tray (1-2)
4. Electric balance.

c) Procedure

Moisture was determined according to the AOAC (1984) method. At first, weight of the moisture dishes was made constant and 8.0-10.0g fresh sample was taken in moisture dishes. The moisture dishes were then placed in an oven at 100-105º C for 5-8 hours. Then the moisture dishes containing sample were weighed in an electric balance and heated in an oven until constant weight was found each time. The moisture dishes were cooled in a metal tray before weighing.

Then the moisture was determined from the following formula:

$$\text{Moisture (\%)} = \frac{\text{Initial weight (g)} - \text{final weight (g)}}{\text{Weight of the sample}}$$

Where

Initial weight = raw sample weight + moisture dish weight (before heating)

Final weight = dry sample weight + moisture dish weight (after heating)

Dry matter (%) = 100 – moisture content

Estimation of Moisture Factor

$$\text{Moisture factor} = \frac{100 - \text{Moisture}}{100}$$

2.12.2 Estimation of protein content

1. Principle

Protein content can be measured by estimating the nitrogen content of the materialand then multiplying the nitrogen value by 6.25. This is referred to as crude protein content, since the non-protein nitrogen (NPN) present in the material was taking into consideration in the present investigation.

The estimation of nitrogen was made by modified kjeldahl method (1990.988.05 in official method of analysis of the Association of official Analytical Chemists 15thedition page no. 70) which depends on the fact that organic nitrogen, when digested with concentrated Sulphuric Acid (H_2SO_4) in the presence of a catalyst, is converted into ammonium sulphate [$(NH_4)_2SO_4$]. Alkali is added to the sample to convert ammonium (NH_4) to ammonia. The ammonia is steam distilled into a receiver flask containing boric acid and titrated with a standard acid solution. This determines % N that is multiplied by 6.25 to give the value of crude protein.

b) Reagents

1. Digestion Mixture: Mixture of Anhydrous Sodium Sulphate 96%, Copper Sulphate 3.5% and Selenium dioxide 0.5% is called digestion mixture.
2. Concentrated Sulphuric Acid (H_2SO_4): Concentrated Sulphuric Acid (H_2SO_4) was used for titration.
3. Sodium Hydroxide (40%): Forty gram of Sodium Hydroxide was dissolved in distilled water and the volume was made up to 100ml.
4. Receiver solution: Twenty gm of boric acid was added in 750ml de-ionized water in a one liter volumetric flask, heated it on a medium setting until the boric acid was dissolved. Then 220ml of ethanol was added into that flask. An amount of 0.33g bromocresol green and an amount of 0.66g Methyl red were dissolved with 100ml ethanol (C_2H_5OH) in another beaker which is called mixed indictor. An amount of 10ml Mixed indicator (Bromocresol green and Methyl red solution) was then added into that volumetric flask. A few drops of diluted 0.1N NaOH were also added and the total volume was made 1000ml with de-ionized water.
5. N/70 H_2SO_4
6. Phenolphthalein indicator

c) Apparatus

1. Conical flasks
2. Kjeldahl flasks
3. Fume hood digestion chamber
4. Protein distillation chamber
5. Burette with stand
6. Electric balance
7. Pipette

c) Procedure

The kjeldahl method consists of the following steps:

1. Digestion of the sample
2. Distillation
3. Titration

1. Digestion of the Sample

The Sample (0.5-1.0g) was taken weighing paper and measured accurately. This sample was poured into a 500ml clean and dry Kjeldahl flask, to which 1.0g of digestion mixture and 12-15ml of concentrated H_2SO_4 were added. To avoid frothing and bumping, 1-2 glass beads (boiling chips) were placed inside the flask. A blank was carried with all reagents except sample material for the comparison. The flasks were then heated in Fume hood Digestion chamber at 400ºC until the solution became colorless. At the end of the digestion period the flasks were cooled and diluted with 100ml-distilled water. A small piece of litmus paper was placed in the solution was found to be acidic.

2. Distillation

The distilling set of Kjeldahl apparatus was thoroughly washed with distilled water before starting the distillation. In a measuring cylinder 150ml of distilled water and in pipette 5 ml of sample, 10-20 ml of 40% NaOH separately and a few drops of phynephthelene were taken and they were carefully poured down inside the Kjeldahl flask with boiling chips. The mouth of the flask was closed with stopper containing connective tube, which was ultimately connected to the ammonia receiving flask containing 5ml receiver solution. The mixture was boiled at such a rate that water and ammonia distilled over at a steady moderate rate. The heating was not too slow, so that the receiver solution might be sucked into the Kjeldahl flask and not too fast so that the distilling ammonia did not escape the receiver solution without absorption.

3. Titration

The ammonia absorbed in the receiving flask containing receiver solution was titrated with N/70 H_2SO_4. Similarly a reagent blank was distilled and titrated.

Calculation

The percentage of nitrogen in the sample was calculated by the following equation:

$$\% \text{ of Protein} = \frac{(S-B)\times 29\times \text{moisture factor}}{\text{Weight sample}}$$

Where,

S = Titration reading for sample

B = Titration reading for blank

2.12.3 Estimation of fat content

a) Principle

Fat was determined according to the modified method described by Folch *et al.* 1957. Anhydrous chloroform methanol mixture was used to extract the fat from the dry samples.

b) Reagents

Chloroform was mixed with methanol in the ration of 2:1.

c) Apparatus

1. Conical flask
2. Filter paper
3. Oven
4. Electronic air drier
5. Funnel

d) Procedure

At first weight of the blank conical flask was taken. 4-5 grams of dry sample of fish was taken in a conical flask and to it around 20ml of chloroform: methanol (2:1) solution was added. The sample was allowed to stand for overnight and was filtered. Filters papers were washed repeatedly with chloroform: methanol (2:1) solution. The filtrate was taken in a separating funnel and to it 0.58% NaCl solution (20ml) was added. The separating funnel was vigorously shaken for proper mixing and allowed to stand for 4-6 hours. The lower phase washed with sodium chloride solution repeatedly till the lower phase was clear. Finally the lower phase was collected in a conical flask. Total volume of extract was recorded.

Then 5-10 ml of the extract was taken in 25 ml beaker and allowed to air dry and then dried in an oven at 60ºC for the determination of total lipid was determined gravimetrically. Fat content was calculated by followed formula.

Calculation

$$(\%) \text{ of Fat} = \frac{\text{Final weight (g)-Flask weight (g)}}{\text{Sample weight}} \times 100 \times \text{moisture factor}$$

2.12.4 Estimation of ash content

a) Principle

The ash content of a sample is the inorganic residue left over after the organic matter has been burnt away at about 600-700ºC.

b) Material and Equipment

1. Porcelain crucibles
2. Crucible furnace
3. Drier
4. Gas burner
5. Desiccators

c) Procedure:

Two tarred crucible of known weight were taken for each sample and about 1g of macerated sample was taken in each crucible. The sample were first burnt on a flame until it became charred and then in muffle furnace at about 600-700ºC till the residue become white. The crucibles were cooled in desiccators and weighed.

Calculation:

$$(\%) \text{ of Ash} = \frac{\text{Final weight (g) - crucible weight (g)}}{\text{Sample weight (g)}} \times 100 \times \text{moisture factor}$$

2.13 Statistical analysis

Average final fish weights, average daily weight gain (ADG), food conversion value (FCR), protein efficiency ratios (PER), specific growth rate (SGR) and proximate composition of fish were calculated for each dietary treatments at the end of experiment. Analysis of variance (ANOVA) was used to test the significance ($P<0.05$) of all fish growth performance, feed utilization and proximate composition among dietary treatments. All the statistical analyses were performed using SPSS program version 16.00.

RESULTS

Experimental diets were performed on the culture of *A. testudineus for* 75 days in the laboratory condition. The formulated feed was set as the control. Formulated feed was also fed fish to compare performances with those fed with supplemented diet. Detailed results of this study on the proximate composition of fish and experimental diet, survival of fish, growth performance *A. testudineus* in tanks on two experimental diets as recorded during the period of investigation were presented under the following heading.

Table

3.1 Water quality parameter of the tanks during the experiments of two types of food.

Limnological Parameters	**T_1**	**T_2(*Spirulina*)**
Temperature	22.39 ± 0.31^a	22.43 ± 0.26^a
PH	6.81 ± 0.24^a	6.79 ± 0.19^a
DO	5.26 ± 0.23^a	5.19 ± 0.29^a

Table 3.2Composition of the test (diet) feed (%) per 1 kg feed

Ingredients	**Diet-I**	**Diet- II (*Spirulina*)**
Fish meal	20	37
Soybean meal	15	25
Wheat flour	27	20
Corn meal	32	12
Vitamin	3	3
Oil	2	2
Salt	1	1

Table3.3Proximate composition (%)of ingredients used in experimental diets.

Ingredients(%)	**Fish meal**	**Soybean meal**	**Wheat flour**	**Corn meal**
Moisture	7.30	11.56	11.94	10.67
Ash	22	7.48	3.61	1.42
Crude protein	57.98	34.16	13.07	7.36
Crude lipid	11.45	33.81	25.98	5.16

Table 3.4Proximate composition of two experimental feed (dry weight basis)

Items	**Feed I (control)**	**Feed II**
Moisture (%)	13.04	12.20±.09
Protein (%)	31.12	56.23±1.15
Lipid (%)	9.97	7.53±0.088
Ash (%)	16.11	14.95±1.31

3.1 Proximate composition of fish

The proximate composition of experimental fish fed experimental diet was determined at the initial and at the end of the experiment. The initial proximate composition of fish was 79.89±0.47%, 16.17±0.28%, 2.53% and 1.41±0.23% for moisture, protein, lipid and ash respectively.

Table 3.5 Whole body proximate composition of *A. testudineus* fed with two experimental diets (% fresh weight basis, ± SEM)

Items	**Diet I**	**Diet II**
Moisture (%)	79.09±0.857[a]	74.18±0.834[b]
Protein (%)	16.28±0.098[a]	19.63±0.131[b]
Fat (%)	2.57±.017[a]	4.21±.008[b]
Ash (%)	2.06±0.027[a]	1.98±0.012[b]

3.1.1Moisture Content in Fish

Figure3.1 depicted the percentage of moisture in *A.testudineus* species fed with two kinds of diet. The higher moisture content was found for T_1(79.09±0.857%) and lower for T_2(74.18±0.834%).There is a significant difference between two treatments for moisture content in fish (P>0.05).

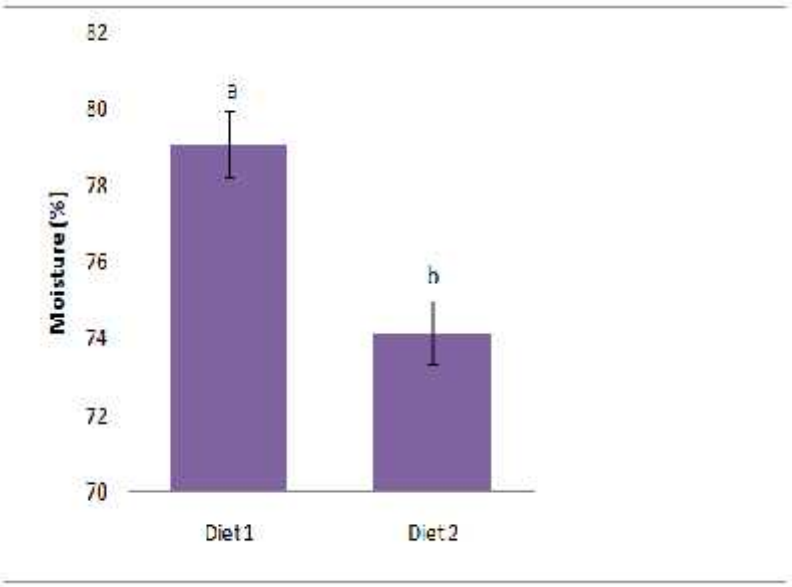

Fig. 3.1 Moisture content (%), (Mean ± SEM) in *A. testudineus* cultured for 75 days fed with two experimental diets.Bars with different letters indicate significant difference (P>0.05).

3.1.2 Protein Content in Fish

Percentage of crude protein for *A.testudineus* species under study was compiled in Figure 3.2. In present study protein content was found to be in the range of16.28 -19.63%. The higher protein content being present inT_2(19.63±0.131%), while the lower being present in T_1(16.28±0.098%).So there is a significant difference between two treatments for protein content in fish (P>0.05).

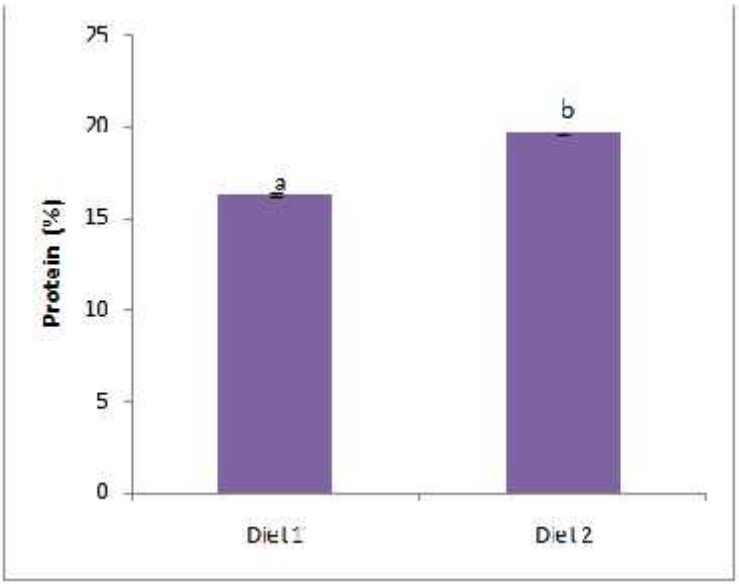

Fig. 3.2 Protein (%), (Mean ± SEM) in *A. testudineus* cultured for 75 days fed two experimental diets.Bars with differentletters indicate significant difference(P>0.05).

3.1.3 Lipid content in Fish

All experimental fish had higher lipid contents than the initial lipid composition.Table3.5 shows the the difference between two treatmeant. Lipid content was found to be in the rage of (2.57-4.21)%. There were significant differences between T_2 (4.21±.008%) andT_1 (2.57±.017%)(P>0.05).

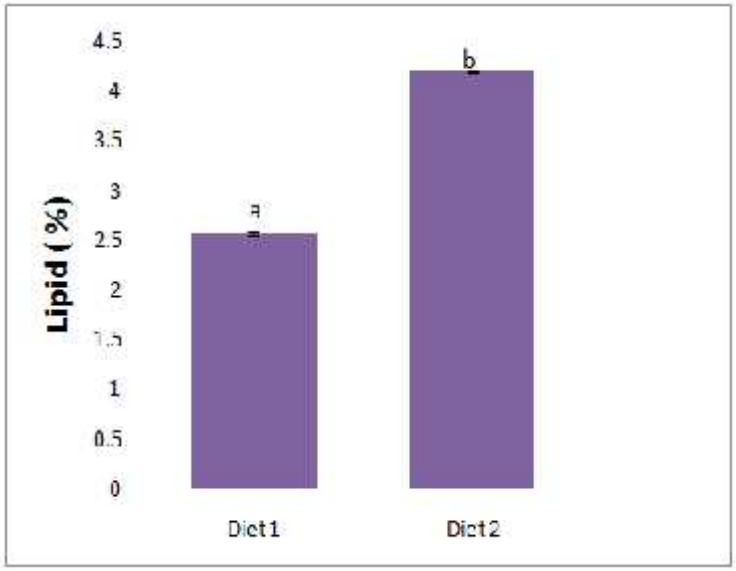

Fig. 3.3 Lipid (%),(Mean ± SEM) in *A. testudineus*cultured for 75 daysfed with two experimental diets.Bars with different letters indicate significant difference(P>0.05).

3.1.4 Ash Content in Fish

All experimental fish had different ash content (%) compare to the initial ash content of fish.Figure 3.4 indicates the difference of ash between treatment and control.The values of ash content were recorded after 75 days study period. The lower ash found in the T_2 (1.98±0.012). The higher ash content for $T_1$was(2.06±0.027)(P>0.05).

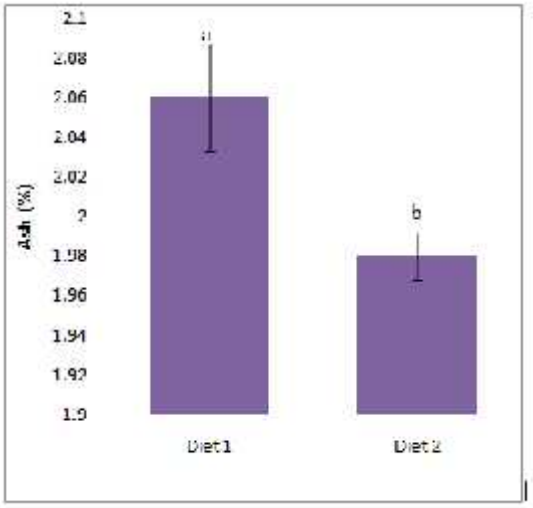

Fig. 3.4 Ash (%), (Mean ± SEM) in *A. testudineus* cultured for 75 days fed with two experimental diets.Bars with different letters indicate significant difference(P>0.05).

3.2 Growth performance

The growth performance of the experimental fish rearing 75 days fed two different experimental diet in the laboratory condition are showed in table.

Table 3.6The growth performance data (mean ± SEM) of experimental fish fed two experimental diets.

Parameters	Diet I	DietII
Initial body weight (g)	1.57±0.32[a]	1.84±0.82 [a]
Final body weight (g)	12.04±0.72[a]	17.83±0.37[b]
Initial length (cm)	3.89±0.43[a]	4.19±0.82[a]
Final length (cm)	7.9±0.15[a]	8.87±0.79[b]
Weight gain (g)	10.47±0.020[a]	15.99±0.057[b]
Percentage of Weight gain (%)	666.87±0.776[a]	869.02±0.800[b]
Average daily weight gain (ADG)	0.139±0.001[a]	0.213±0.012[b]
Specific growth rate (%)	2.71±0.170[a]	3.02±0.140[b]
Survival rate (%)	88.33±1.910[a]	93.33±0.968[b]
Condition factor (K)	2.44±0.012[a]	2.55±0.043[b]

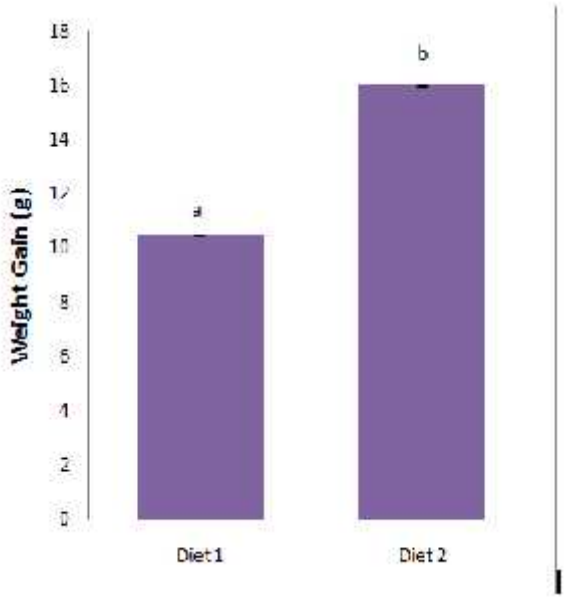

Fig. 3.5 Variation in weight gain of *A. testudineus* after 75 days fed two experimental diets.Bars (mean ±SEM) with different letters indicate significant difference(P>0.05).

3.2.1 Average daily gain (ADG, g/d)

Table and Figure 3.6 indicates the value of Average daily gain (ADG) and also shows the difference between two treatment. The values of Average daily gain (ADG) was higher in treatment 2 (0.213±0.01 g/d) and lower in control (0.139±0.001g/d).There is a significant difference between treatment and control for ADG in fish (P>0.05).

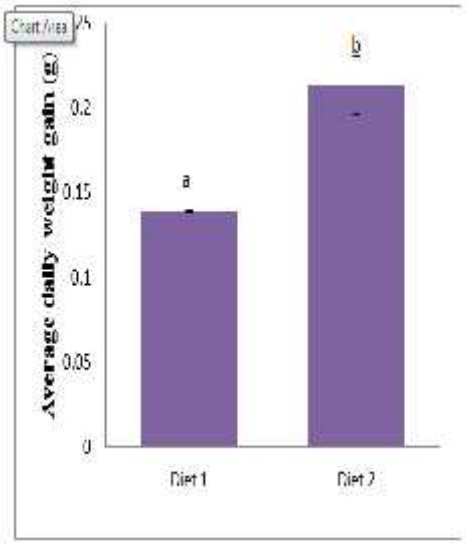

Fig. 3.6 Average dailygain of *A. testudineus* after 75 days fed with twoexperimental diets.Bars (mean ±SEM) with different letters indicate significant difference (P>0.05).

3.2.2 Specific growth rate (SGR %)

The values of Specific growth rate (SGR%) of the experimental fish*A. testudineus* rearing in six tanks fed on two different types of fish feed were estimated and the findings were different. The values of SGR% are higher for T_2 (3.02±0.140%) and for T_1 (2.71±0.170%).So there is a significant difference between treatment and control for SGR (%) in fish (P>0.05).

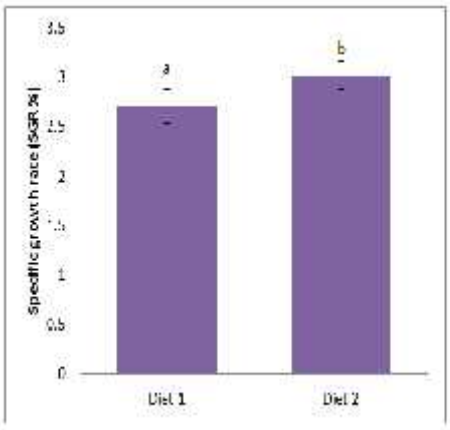

Fig. 3.7 Specific growth rate (SGR %) of the experimental fish *A. testudineus*.Bars (mean ±SEM) with different letters indicate significant difference (P>0.05).

3.2.3 Condition Factor (K)

Table 3.6 and Figure 3.8 indicates the value of condition factor and also shows the slight difference between treatment and control. Condition factor (K) of*A. testudineus* fry was (K=2.55±0.043) at$T_2$and (K =2.44±0.012) at T_1.There is a difference between two treatment (P>0.05).

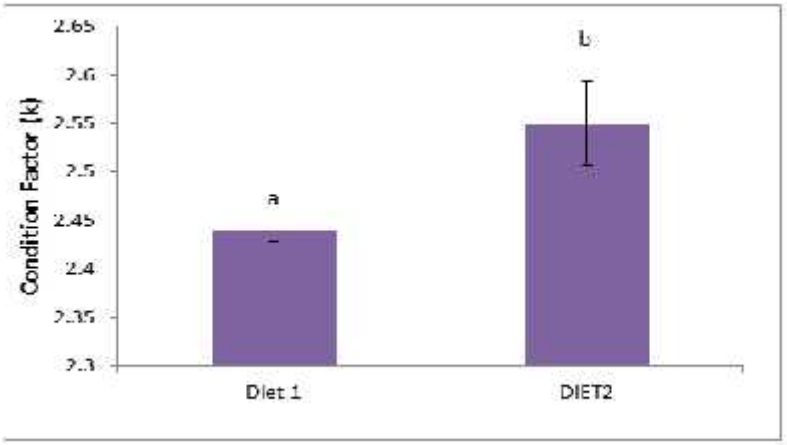

Fig. 3.8 Condition factor (K) in *A. testudineus*determined after 75 days fed withtwo experimental diets.Bars (mean ±SEM) with different letters indicate difference(P>0.05).

3.3 Feed utilization

Feed utilization of *Anabas testudineus* fed two experimental diets was performed as feed efficiency, food conversion ratio (FCR) and protein efficiency ratio respectively. There was a significant effect of two experimental diets on those performances of feed utilization (P<0.05).

Table 3.7Feed utilization data (mean± SEM) of *A. testudineus* fed two experimental diets.

Items	Diet I	Diet II
Feed conversion ratio (FCR)	1.26±0.212[a]	1.10±0.105[b]
Feed efficiency	79.35±0.759[a]	90.548±0.624[b]
Protein efficiency ratio (PER)	0.735±0.029[a]	0.905±0.008[b]

3.3.1 Feed conversion ratio (FCR)

The Feed conversion ratio of *A. testudineus* kept in different tanks and fed on two different experimental diet have been calculated after 75 days study period. The higher FCR (1.26±0.212) was found in the control (Diet I) while the lower (FCR1.10±0.105) was measured in T_2 (Diet II).So there is a significant difference between treatment and control for FCR (%) in fish (P>0.05).

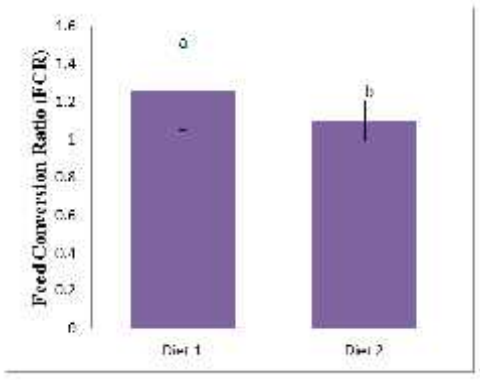

Fig. 3.9 Feed Conversion Ratio (FCR), (Mean ± SEM) of *A. testudineus* fry cultured for 75 days fed two experimental diets.Bars with different letters indicate significant difference (P>0.05).

3.3.2 Protein efficiency Ratio (PER %)

The values of protein efficiency ratio the experimental fish rearing in six tanks for 75 days fed on two different experimental diets have been estimated after the study period. The values of PER for T_2 was (0.905±0.008) which was significantly higher than T_1 (0.735±0.029).There is a significant difference between treatment and control for PER (%) in fish (P>0.05).

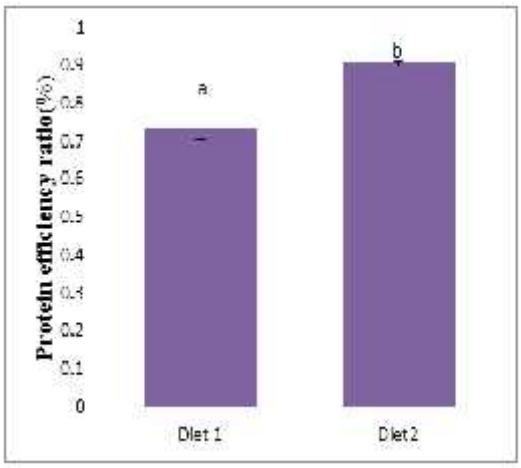

Fig. 3.10 Protein efficiency ratio(%) oftwo experimental diets fed by *A. testudineus* observed in a laboratory condition.Bars (mean ±SEM) with two letters indicate significant difference (P>0.05).

4.3.3 Feed efficiency (%)

Table 3.7 and Figure 3.11 indicates the value of feed efficiency and also shows the difference between treatment and control. The values of feed efficiency ranges had been calculated after 75 days study period. The higher value was observed for T_2(90.548±0.624%) and lower feed efficiency T_1 (79.35±0.759). So there is a significant difference between two treatment for feed effeciency (%) in fish (P>0.05).

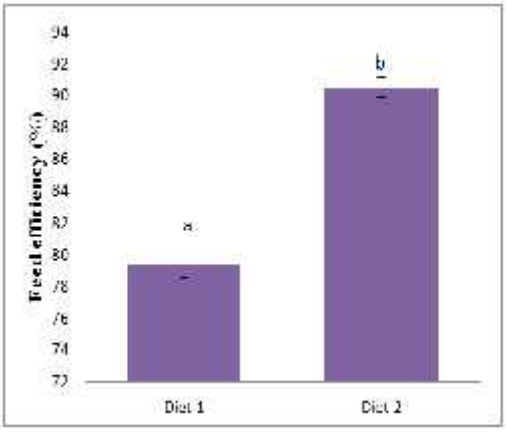

Fig. 3.11 Feed efficiency (%),(Mean ± SEM) of diet supplemented with and without feed additives fed by *A. testudineus* observed in a laboratory test.Bars with two letters indicate significant difference (P>0.05).

3.3.4 Survival rate (%)

Survival rate of fish were significantly different between experimental diets. The feed intake in control diet were significantly lower than the other treatment diets ($P<0.05$). The average survival rate of fish wasfor (88.33±1.910%)in fish fed with control feed and (93.33±0.05%)in fish fed with *Spirulina*.

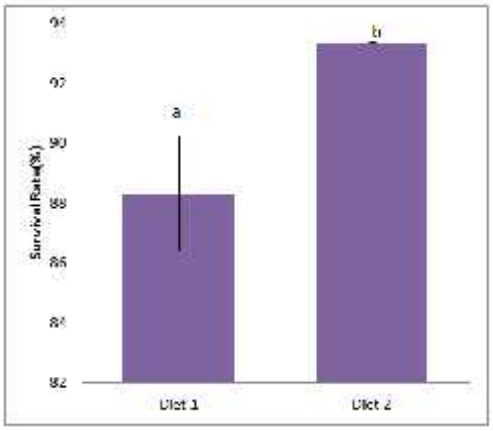

Fig. 3.12 Survival rates(%), (mean ±SEM) of *A. testudineus* fed supplemented without and with feed additives observed in a laboratory condition.Bars with two letters indicate significant difference ($P>0.05$).

DISCUSSIONS

For successful culture of any fish suitable fish feed is one of the most essential prerequisites.Availability of ingredients of feed or quality feed are the vital factors that affect commercial fish culture.In this study locally available fish ingredients are collected and prepared feed as Diet I and Diet II.Between two diet, DietII contains higher protein percentage (on dry matter basis).The Study had two aspects:growth performancesand body composition, of climbing perch (*A.testudineus*). High growth performances in terms of total length (TL), body weight (BW), specific growth rate (SGR), feed conversion ratio (FCR), average daily gain (ADG) and condition factor (K) were recorded between two types of treatment.

The results obtained from this experiment showed that the growth responses of fish fed with DietIon term of weight gain (WG), daily weight gain (DWG), percentage of weight gain (PWG), specific growth rate (SGR %), survival rate (SR), and also the feed efficiency such as feed intake (FI), protein efficiency ratio (PER) were decreased compare to Diet II. In this experiment, fish fed diets containing *Spirulina* showed the best growth rate, feed efficiency,feed conversion ratio (FCR), and significantly higher percentage of weight gain thanDietI(control diet)

Growth performance

The growth rate of the *A. testudineus* fry were shown in table 3.3. Total biomass of fry was increases in T_2. Average weight gain in $T_2$was 15.99±0.57g where average weight gain (10.47±0.23g) was found in T_1.Statistical data showed that there were significant differences among two treatments.Weight gain in control diet was significantly lower than diet II ($P<0.05$). Statistical analysis showed that lower bound and upper bound at 95% confidence for two treatments was 15.747 and 16.245. F values between groups were 9.672. Statistically there was no significant among treatments fed with two experimental diets.

Survival rate (%)

The survival rate of this experimental fish was high in comparison with other fishes as the fish has accessory respiratory organ. At the time of experiment (rearing fish in the tanks which having tap water) the survival rate is comparatively lower than the natural water body as the tap water contained slightly higher iron (Fe) amount than need. Survival rate of fish were significantly different between experimental diets. These controls had problem with feed intake, may be related with commercial feed contained in the diet that caused low palatability. The feed intake in control diet were significantly lower than diet II($P<0.05$). The average survival rate of fish was (88.33± 1.910%)in fish fed with control diet and (93.33± 0.968%) in fish fed diet with*Spirulina*.

These findings have more or less similarities with the findingsofRinna*et al* (2013) . Their study had maximum survivality (94%) observed in *T.trichopterus* fed with 30gk-1 *Spirulina* diet.

Akand and Haque (1989) in a study had 82 to 93% survival rate of the shingi fish during the feeding trial.

Niamat and Jafri (1984) they have also got 100% survival rate of shingi fish, *H. fossilis*in a study with formulated pelleted feed.

Mustafa *et.al*(1995) in a study with red sea bream with dietary algae observed survival rate ranged from 77.8-87.8% these findings are within our observed value of survival rate of *H. fossilis.*

Lower and Upper value at 95 % confidence level was in 77.26 and 97.09 for T_1 and T_2 respectively. From ANOVA table, F value between groups was 10.439.statistically there was no significant difference among treatments ($P<0.05$).

Averge daily gain (ADG)

The average daily gain in T_2(0.213±0.01) was higher than the control (0.139±0.001g/d).ADG value depends on several climatic factors including temperature, salinity, DO, light intensity, water current and other various factors such as availability of feed, stocking density and what not.

Increased ADG of the fish suggested that the fish were able to regulate osmotic pressure of the body fluid; this was in agreement with suggestions of Nikolsky (1963), the more the osmo-regulatory adaptation, lesser the difference between the compositions and pressures of the internal fluid of the organism and its external environment.

In 1989,Sangrattanakhul (1989) found the value of ADGin *Anabas testudineus* is ranging from 0.10- 0.12g this findings are more or similarities with us.

Specific growth rate (%)

It has been observed that SGR was decreased at the end of the experiment in case of treatment and control. This finding resembles the Medawar's (1945) fifth law "the specific growth rate declines more and more slowly as the organism increases in age"and "Organism age fastest when they are young" is expressed by Medawar's (1945) fifth law.

Minot (1908) was the first person to recognize that for most animals the specific growth rate is highest early in life and that it typically decreases with increasing age, becoming zero in some animals.

The values of Specific growth rate (SGR %) of the experimental fish *A. testudineus* were estimated and the findings were different. The maximum SGR (%) for T_2 (3.01±0.140%) fed with control diet containing *Spirulina* where minimum for T_1.From ANOVA data F value between treatment was 2.139.There was statistically significance between treatments (P>0.05).

Condition factor (K)

The condition factor was high in bothT_1 andT_2. However the condition factor was(2.44±0.012%) and (2.55±0.043%) in T_1 and T_2 respectively.The value of K is influenced by age of fish, sex, season,stage of maturation, fullness of gut, type of food consumed,amount of fat reserve and degree of muscular development.

Rahman *et al.* (1997) in a study on the survival and growth of cat fish after giving selected supplemental feeds got the values of condition factor between 0.81-0.87 which are not similar with our finding values.

Feed conversion ratio

The highest FCR was found in the control (diet I) (1.26±0.212) while the lower was (1.10±0.105) in T_2 (diet II containing *Spirulina* as a feed additive). The lower the FCR the better the feedconversion to fish flesh. From this point of view the *Spirulina* containing diet (diet II) gives the better result than control diet.FCR values slightly increased in commercial diets because the utilization of commercial diet was lower than other diets.As the good quality of protein in the diet increases, the FCR gets smaller. This means that it takes less feed to produce a kilogram of fish.

Potongkam (1972) reported that FCR of climbing perch fed on trash fish and pellet were 2.07 and 1.89, respectively.

Doolgindachabaporn (1994) found that the FCR value of *Crypinus carpio* ranges from 1.8 to 3.0 and Akand *et.al* 1989 found FCR value 2.0 to 2.7 in case of *H.fossilis.*
Watanabe*et al.*1990 mentioned that feed supplemented with *Spirulina* powder improved the feed conversion ratio and growth rates for striped jack, *Pseudocaranx dentex*. These results may possibly due to the improved feed intake and nutrient digestibility.
According to Catacutan and Coloso (1997) found that FCR ranged from 1.21 to 1.65 in sea bass fed with diets varying carbohydrate and lipid levels which are more or less similarities with our finding values.

Protein efficiency ratio (PER)

The values of PER for T_2 was 0.905±0.008 which was higher than control diet.The fish groups fed with experimental diets (diet II) showed a higher feed intake rate than control diet during the experimental periods. This might be due to the attractive color, racy flavor and good nutrient composition of the experimental diets.
From this point of view, addition of *Spirulina* in the formulated feed shows better PER.

Corpei(2001)found that food conversion ratio and PER were better when the fish were maintained on artificial diets with 10% and 20% dried algae.

A 3% supplementation of *Spirulina* meal in moist pellets reconfirmed the efficacy of *Spirulina* in improving the growth performances and feed utilization of feed inred sea bream fish (Mustafa *et al.*1994).

Feed efficiency (FE)

The value of FE is higher inT_2(90.548±0.624) than control diet but more effective.

Takeuchi *et al.*2002found that juvenile tilapia fed solely on the alga show a lower feedefficiency and protein efficiency ratio than commercial-diet-fed tilapia.The feed efficiency is higher (37.04±8.11%) in T_2.The protein utilization of control diet was lower as PER also lower in fish fed with control diet.

Mustafa *et al. (*1995) found 51.5 to 62.3% feed efficiency while working with red sea bream fed on feed having protein 38.5-39.3%.

Aksnes *et al.*(1997) found 58 to 66% feed efficiency in his growth performance study of gilthead sea bream *Sparus aurata*[Linnaeus, 1758] with high quality fish meal.

Proximate composition

According to the size and age of the fish, proximate composition showed variations for twoexperimental diets. The final body moisture was statistically significant ($P<0.05$) between $T_1$and T_2. The percentage of moisture in experimental fish carcass 79.09±0.857% in fish fed with diet I and 74.18±0.834% in fish fed with diet II. Fish fed diet containing control diet was slightly higher moisture content than fish fed diet containing *Spirulina*.

Catacutan and Coloso (1995) reported that high protein content on a dry matter basis as well as the high moisture content was affected by feed quality.

In a study (Stansby 1954), moisture content for fresh water fish was reported to be in the range of 72.1-83.6 %, with a mean of 77.64%.

In another study, Rubbi *et al*. (1987) investigated the moisture content for twenty-seven species of fresh water fish, where moisture content was found to be in the range of 72.18-83.65%.

The final body protein content was statistically significant ($P<0.05$). In present study carcass protein (19.63±0.098) was higher in fish fed diet containing *Spirulina*.Statistical data showed that F value F value between treatments was 7.46

Rahman *et al.* (2007) found 37% crude protein when they carried out their study, effects of dietary vitamin C on the feed utilization and growth performance in climbing perch.Nandeesha *et al. (*1998)recorded no difference in themoisture and protein content in carcasses of common carp fed on diets incorporated with up to 55%*Spirulina* powder. Govindan (1985) also demonstrated a range of 9-25% protein for freshwater and marine fish.

Fish fed higher dietary protein showed a higher carcass lipid. A similar trend for crude lipid has been reported by Murari *et al. (*1985) in *Cyprinus carpio*.The fat content was higher in fish fed diet containing *Spirulina*.Total lipid concentration differs due to size of fish, portion of fish, time of fish catch, water temperature as well as season (Quazi 1989).

The effects of *Spirulina* on whole-body protein and lipidcontents are correlated with their synthesis and accumulationrate in muscle, as well as the growth rate of the organisms (Smith 1981; Fauconneau 1984; Soivio *et al.*1989 and Abdel- Tawwab *et al*. 2006). Nandeesha *et al.* (2001) stated that the effect of dietary *Spirulina*on whole-body lipid content is dependent on species ofthe *Spirulina* used.

Ash contains kinds of minerals which play important role in body structure for each organism including calcium, magnesium, phosphorus, iron, zinc and so on.It was observed from the present study, for ash content in twotreatment was 2.06±0.0272%and 1.98±0.012% for T_1 and T_2 respectively.

CONCLUSION

Koi fish (*A. testudineus)* is a popular type of fish among village people due to its high nourishing quality.It is found in most of the fresh water bodies. The current study showed the dietary effect of feed additives growth performance and body composition and of *A. testudineus*during rearing and feeding trail in the laboratory condition. The growth performance and body composition from these study were significantly lower in formulated feed(control diet) ($P<0.05$) than feed with *Spirulina*. Formulated diet showed significantly adverse effect ($P<0.05$) on growth performances in term of weight gain (WG), percentage of weight gain (PWG), average daily gain (ADG), specific growth rate (SGR) and also showed adverse affect on feed efficiency when focus on feed intake (FI), feed efficiency (FE), food conversion ratio (FCR) compare to fish feed with *Spirulina.*

The protein quality of*A. testudineus*diets contained only formulated feed was decreased ($P<0.05$) when emphasized on protein efficiency ratio (PER). Percentage of protein and lipid were better in body of fish after fed with diet containing *Spirulina*than control diet.

During the study period FCR, PER and feed efficiency of the rearing *A. testudineus*showed results in favor of the use of prepared fish diet with *Spirulina*.

From this study it can be concluded that addition of *Spirulina* at 3% in the control diet have been positive effects on the growth performance and body composition.

.

To make aquaculture profitable and cost effective additional number of plant based ingredients are being used for preparation of fish feed. However, more work is necessary to understand the chemical nature of *Spirulina* and its mode of action and it has already opened a new area of research.

RECOMMENDATIONS

1. More research needed to evaluate the involvement of feed additive to physiological function of fish.

2. Further studies should be conducted on level of additive in fish.

3.As it has acted as astrong feeding effectors for *A.testudineus* at a very low inclusion level, this study would serve as afoundation for further and refined studies with *Spirulina* .

4.Current study is totally laboratory based, so further fields studies are needed to clarify the actual effect of feed additive on the fish.

REFERENCES

ABDEL-TAWWB, M., Y. A. E. KHATTAB, M. H. AHMAD and A. M. E. SHALABY. 2006. Compensatory growth, feed utilization, whole body composition and hematological changes in starved juvenile Nile tilapia, *Oreochromis niloticus* (L.). Journal of Applied Aquaculture **18**(3), 17-36.

ABDUL-TAWWAB, M., AHMAD, M. H., ABDEL-HADI, Y. M., and SEDEN, M.E.A. 2008. Use of*Spirulina* (*Arthrospira platensis*) as a growth and immunity promoter for Nile tilapia, *Oreochromis niloticus (L.)* fry challenged with pathogenic *Aeromonas hydrophila*.

AKAND, A. M., HASAN, M. R and HABIB, M. A. B. 1991. Utilization of carbohydrateand lipid as dietary energy sources by stinging catfish, (*Heteropneustes fossilis*).In: S. S.

AKAND, A.M., MIA, M.L and HAQUE, M.M. 1989. Effect of dietary protein level on growth, food conversion and body composition of Shingi *H. fossilis* (Bloch,1992). Aquaculture, **77**:175-180.

AKSNES, A., IZQUIERDO, M. S., ROBAINA, L., VERGARA, J. M. and MONTERO, D. 1997. Influence of fish meal quality and feed pellet on growth,feed efficiency and muscle composition in gilthead seabream (*Sparus aurata*). Aquaculture **153** :251-261.

ALAM, J.M., MUSTAFA, G.M. and ISLAM, M.M. 2010. Effects of some artificial diets on the growth performance, survival rate and biomass of the fry of climbing perch, *Anabas testudineus* (Bloch, 1972). Nature and Science,**8**(2): 36-41.

ALAM, J.M., MUSTAFA, G.M. and KHALEQUE, A.M. 2009. Evaluation of the effects of different dietary vitamin C levels on the body composition, growth performance and feed utilization efficiencies in stinging catfish, *Heteropeneustes fossilis* (Bloch, 1792). Journal of American Science, **5**(3): 31-40.

AOAC. 1984. Association of Official Analytical Chemist, 14th edition, Eds. Willium's.

AOAC. 2000. Association of Official Analytical Chemists. 17th Edn., AOAC,

BADAWY, T. M., IBRAHIM, E. M. and ZEINHOM, N.M. 2008.Partial replacement of fishmeal with dried microalgae *Chlorella spp.* and *Scenedesmus spp.*) In Nile Tilapia (*Oreochromis niloticus*) diets. 8th International Symposium on Tilapia in Aquaculture.

BANARJEE, M. and DEB, M. 1996. Potential of fly ash and *Spirulina* combination as a slow release fertilizer for rice field. Cientifica Jaboticabal, **24**: 55–62.

BANG, H. O., DYERBERG, J., SINCLAIR, H.M. 1980. The composition of the Eskimo food in north western Greenland. Am. J. Ciln. Nutr. **33**: 2657-2666.

BANU, C.P., NAHAR, B., BEGUM, M. and AHMED, K. 1985. Studies on the protein, riboflavin and iron content of some freshwater fish and prawn of Bangladesh.

BELAY, A., KATO, T. and OTA,Y. 1996,*Spirulina* (*Arthrospira*); Potential application as an animal feed supplement. J. Appl. Phycol. **8**: 303-311.

BHATT, V. S. 1968. Studies on the biology of some fresh water fishes. Part –Vll. *H. fossilis* (Bloch), Indian J. Fish. **15**(1&2): 99-115.

BHATT, V.B. and MADYASTHA, KM.2000. C-phycocyanin: a potent peroxyl radical scavenger in vivo and in vitro. Biochem Biophys Res Commun. **275:** 20-25.

BOONYARATPALIN, M.P., SURANEIRANAT and TUNPIBAL, T. 1998. Replacement of fish meal with various types of soybean products in diets for the asian seabass, *Lates calcarifer.* Aquaculture **161**: 67-78.

BOROWITZKA, M.A. 1997. Microalgae for aquaculture; Opportunities and constraints. J. Appl. Phycol. **9**: 393-401

CATACUTAN, M.R. and COLOSO, R.M. 1995. Effect of dietary protein to energy ratio on growth, survival, and body composition of juvenile asian sea bass, *Lates calcarifer*. Aquaculture **131:** 125-133.

CATACUTAN, M.R. and COLOSO, R.M. 1997. Growth of juvenile asian sea bass, *Lates calcarifer* fed varying carbohydrate and lipid levels. Aquaculture **149**: 137-144.

CLUCAS, I.J., WARD, A.R. 1996. Post-Harvest Fisheries Development. A Guide to Handling Preservation, Processing and Quality. Natural Resources Institute. United Kingdom.

COHEN, Z. and VONSHAK, A. 1991. Fatty acid composition of *Spirulina* and *Spirulina*-like cyanobacteria in relation to their chemotaxonomy. Phytochemistry **30**: 205-206.

CORPEI, 2001. alternative dietary protein for farmed tilapia *Orechromis spp.*Aquaculture, **179**: 149-168.

DE SILVA (ed.). Fish nutrition research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop. Asian Fisheries Society, Manila, Philippines, 93-100pp.

DEHADRAI, P.V., YUSUF, K.M. and DAS, R.K. 1985. Package and practices for increasing production of air breathing fishes. In: Aquaculture Extension Manual, New Series,Information and Extension Division of CIFRI. Indian Council of Agriculture Research,1-14pp.

DELONG, D. C., HALVER, J. E and MERTZ, E. T. 1958. Nutrition of salmonid fishes. VI. Protein requirements of Chinook salmon at twowater temperatures. J. Nutr. **65**: 589

DESILVA, S.S. and PERERA, M.K. 1985. Effects of dietary protein levels on growth, food conversion and protein use in young*Tilapia nilotica* at four salinities. Transaction of the American FisheriesSociety, **114**: 584-589.

DIOGO, R., CHARDON, M. and VANDEWALLE, P. 2003. Osteology and myology of the cephalic region and pectoral girdle of *Heteropneustes fossilis* (Teleostei: Siluriformes), with comments on thephylogenetic relationships between *Heteropneustes* and the *clariid* catfishes. Animal Biology **53**: 379-396.

DoF. Annual Report 2014. Department of Fisheries, Bangladesh, Ministry ofFisheries and Livestock, 111.

DoF. Fisheries Statistical Yearbook of Bangladesh 2014. Department ofFisheries, Bangladesh, Ministry of Fisheries and Livestock, 2-34.

DOOLGINDACHABAPORN., 1994. Physiological responses during stress and subsequent recovery at different salinities in adult pejerrey Odontesthes bonariensis. Aquaculture, **200**: 349–362.

El-HINDAWY, M. M., ABD-RAZIC,M. A., GABER, H. A. and ZENHOM, M. M. 2006. Effect of various level of dietary algae *Scenedesmus spp* on physiological performance and digestibility of Nile tilapia fingerlings. 1st Scientific Conference of the Egyptian Aquaculture Society. Sharm El-Sheikh – Sinai, Egypt, 137-149.

EMST 1985. Nutritional properties of fish oils. World Rev. Nutr. diet. 11-46.

FAUCONNEAU, B. 1984. The measurements of whole body protein synthesis in larval and juvenile carp (*Cyprinus carpio L.).* Comparative Biochemistry and Physiology **78**: 845-850.

FOLCH, M.J., LESS and SLOANE STANELY., G. H., 1957. A simple method for isolation and purification of total lipids from animal tissue. *Journal of Biological* Chemistru. **26**: 497.

GAUTHAM,K. and R ASHA RAJANI,Feeding of *Spirilina*- A Boost to the Poultry Production, Poultry magazine, Oct-12, 31-33.

GHANI, A. 2003. Medicinal plants of Bangladesh with Chemical Constituents and Uses. Asiatic Society ofBangladesh, Dhaka, Bangladesh.

GIREESHA,O., RAMESHA, T.J., VARGHESE,T.J. and MANJAPPA, K. 2002. Impact of dietary livol on the growth,biochemical composition and gut digestive enzyme activity of Catla(*Catla catla*) (Hamilton). J. of Aquacult. in the Tropics.**16**:35-42.

GIRI, S.S., SAHOO, S.K., MOHANTY, S.N. 2010. Replacements of by-catch fish meal with dried chicken viscera meal in extruded feeds: effect on growth, nutrient utilizationand carcass composition of catfish *Clarias batrachus* (Linn.) fingerlings. Aquacult. Int.**18**: 539-544.

GOODNIGHT, S.H.JR., HARRIS, W. S. 1982. Polyunsaturated fatty acids hyperlipidemia, thrombosis. Arterioclerosis **2**: 87.

GOVIDAN, T.K., 1985. "Fish processing technology". Oxford and IBH publishing Co. New Delhi, Bombay and Calcutta. **48**: 180-198

GRUNDY, S.M., BILHEIMER, D. and BLACKBURN, H. 1982. Rationale of the diet/heart statement of the American Heart Association, Circulation **65**:839A-854A.

GUROY, D., GUROY, B., MERRIFIELD, D.L., ERGUN, S., TEKINAY, A.A. and YIGIT, M. 2011. Effect of dietary *Ulva* and *Spirulina* on weight loss and body composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum), during a starvation period. J Anim Pysiol Anim Nutr (Berl). **95**: 320-327.

HARPAZ, S. 1997. Enhancement of growth in juvenile freshwater prawn, *Macrobrachium rosenbergii*, through the use of chemoattract.Aquacult.**156**: 221-227.

HOLMAN 1982. Fatty acids composition of fish oils. Academic Press N.Y.

HOSSAIN, MA. SULTANA, Z. KIBRIA ASM and KM AZIMUDDIN.2012.Optimum dietary protein requirement of a Thai strain of Climbing perch, *Anabas testudineus*(Bloch,1792) fry. Turk.J.Fish

HUSS, H.H., 1988. Fresh fish-quality changes. Food and Agriculture Organization of the United Nations Danish International Development Agency, Rome.

IUCN, Bangladesh. 2000. Red book of threatened fish of Bangladesh. The world Conservation Union. Appendix 1, 61.

JAQUOT, R., 1961. Organic constituent's fish and other aquatic animals. In: Fish as Food Ed. Borgstrom. Academic Press, New York and London. 145-209.

JONES, K.A. 1992.Fish chemoreception. In: Food Search behavior in Fish and the use of chemical Lures in commercial and sports fishing (Ed.T.J.Hara), Chapman and Hall, London, 288.

KHAN, M.N., SIAFUL ISLAM, A.K.M. and HUSSAIN, M.G. 2003. Marginal analysis of culture of stinging catfish (*Heteropneustes fossilis*): Effect of different stocking density in earthen ponds. Pakistan J. Biol. Sci. **6** (7): 666-670.

KHATOON, N., SENGUPTA, S. P., HOMECHAUDHURI and PAL, R. 2010. Evaluation of Algae Based Feed in Goldfish (*Carassius auratus*) Nutrition. Proc. Zool. Soc. **63** (2): 109–114.

LANNO, R.P., HICKLE, B.E. and DIXON, D.G. 1989. Feeding and nutritional considerations in aquatic toxicology. Hydrobiology **188-189**, 525-531.

LOVELL, R. T. 1972. Protein requirement of cagecultured channel catfish. Proceedings, Southeast Asian Association of game and fisheries commission.**26**:357-361.

LOVELL, R. T. 1989. Nutrition and Feeding of Fish. An AVI Book, Van Nostrand Reinhold, New York, 260.

MAEDA, S. and SAKAGUCHI, T. 1990. Accumulation and detoxification of toxic metal elements by algae. Introduction to Appl. Phycol. 109–136.

MAHAJAN, G. and KAMAT M. 1995. γ-Linolenic acid production from *Spirulina platensis*. Appl Microbiol Biotechnol. **43:** 466-469.

MAHESHAPPA, K. 1994. Effect of different dosages of livol on growth and body composition of rohu,*Labeo rohita* (Ham.). M.F.Sc. Thesis, University of Agricultural science, Bangalore 130 pp.

MATTY, A. and SMITH, P. 1978. Evaluation of yeast, a bacterium and an alga as a protein source for rainbow trout. I. Effect of protein level on growth, gross conversion efficiency and protein conversion efficiency. Aquaculture, **14**:235-246.

MEDWARS, P. B. 1945. Size, shape and age in"Essays on Growth and Form Presented toD' Arcy Wentworth Thompson" Oxford University Press.

MINOT, C. S. 1908. "The problem of Age, Growth and Death" Murvey.London.

MU, Y.Y., LAM, T.J. and SHIM, K.L., 2000. Protein digestibility and amino acid availability of several protein sources for juvenile Chinese hairy crab, Eriocheir sinensis H. Milne-Edwards (Decapoda Grapsidae Aquaculture Research.**31** (10): 757-765.

MUKHOPADHYAY, P.K., and PAUL, B.N. 1995. Values addition components in Aquaculture feeds. Fishing Chimes.**6**:15-16.

MURARI, T., AKIYAMA, T., WATANABE, T. and NOSE, T. 1985. Effects of dietary protein levels on performance and caracass composition of fingerling carp. Bull. Jap. Soc. Sci. Fish., **54**: 605-608.

MURRY, J., and BURT, J.R. 1992. The composition of fish. Ministry of Agriculture, Fisheries and Food, Torry Reseach Station.

MUSTAFA, M.G.and AHSAN,D.A. 2004.Growth And feed utilization of Climbing perch, *Anabas testudineus.* (Bloch) fed on formulated diet using local ingredients.Bangladesh J. 2001.**32**(2):159-164, 2004

MUSTAFA, M.G. and NAKAGAWA, H. 1995. A review: dietary benefits of algae as an additive in fish feed. Isr J Aquac-Bamidgeh **47**: 155-162.

MUSTAFA, M.G., UMINO, T. and NAKAGAWA, H. 1994. The effect of *Spirulina* feeding on muscle protein deposition in red sea bream, *Pagrus major*. J Appl Ichthyol 10, 141-145.

MUSTAFA, M.G., UMINO, T. and NAKAGAWA, H. 1997. Limited synergistic effect of dietary *Spirulina* on vitamin C nutrition of red sea bream *Pagrus major*. J Mar Biotechnol. **5**: 129-132.

MUSTAFA, M. G., WAKAMATSU, S., AKI, T., TAKEDA, T., UMINO and NAKAGAWA, H. 1995. Effects of algae meal as feed additive ongrowth, feed efficiency and body composition in Red Sea Bream. Fisheries Science **61**(1): 25-28.

NAKAGAWA, H. and MONTGOMERY, WL. 2007. Algae. In: Dietary Supplements for the Health and Quality of Cultured Fish. Nakagawa H, Sato M and Gatlin DM 3rd, eds. CABI International, Cambridge, MA, US, 133-168.

NAKAJIMA, K., UCHIDA, A. and ISHIDA, Y.1989. A New Feeding Attractant,Dimethyl- propiothein,for freshwater fish Nippon Suisan Gakkaishi. **55**:689-695.

NANDEESHA, MC, GANGADHARA, B., MANISSERY, JK. and VENKATARAMAN, LV. 2001. Growth performance of two Indian major carps, catla (*Catla catla*) and rohu (*Labeo rohita*) fed diets containing different levels of *Spirulina platensis*. Bioresour Technol**. 80**: 117-120.

NANDEESHA, M.C., GANGADHARA, B., VARGHESE, T.J. and KESHAVANATH, P. 1998. Effect of feeding *Spirulina platensis* on the growth, proximate composition and organoleptic quality of common carp, *Cyprinus carpio L*. Aquac Res. **29:** 305-312.

NARGIS, A. and HOSSAIN M.A.1987.Food and feeding habit of koi fish (*Anabaas testudineus* Bloch.Anabantidae: Perciformes) Bangladesh J.Agri.**12**(2):121-127

NETTLETON, A.1985. Nutrientsand Substances in Fresh Sea Food. In: Seafood facts.Issuesand marketing of nutrition of fish and shellfish. Van Nostrand Reinold, New York. 25-64.

NIAMAT, R. and JAFRI, A. K. 1984. Growth response of the siloroid, *H. fossilis* Bloch, fed pelleted feed. Aquaculture. **37**: 223-229.

NIKOLSKY, G.V. 1963. The Ecology of Fishes. Academic Press, London, 352. ISBN: 597.0500000000000.

NILSON, 1946. The value of fish shellfish. Food Research.**30**:177.

NRC (National Research Council) 1993. Nutrient requirements of fish. Committee on Animal Nutrition. Board on Agriculture. National Research Council. National Academy Press. Washington DC, USA.

NURULLAH, M., KAMAL, M., WAHAB, M.A., ISLAM, M.N., AHASAN, C.T. andTHILSTED, S.H., 2003. Nutritional quality of some small indigenous fish species of Bangladesh.pp. 151-158.

OKAMURA, H. & AOYAMA, I. 1994. Interactive toxic effect and distribution of heavy metals in phytoplankton. Toxicol. & Water Quality.**9**: 7–15.

OLIW, E., GRASTROM, E. and ANGGARD, E. 1983. The prostaglandins and essential fatty acids. In: Pace-Asciak, C. and E, Granstrom, Eds. Prostaglandins and essential fatty acids. In: Pace-Asciak, C. and E, Granstrom, Eds. Prostaglandins and related substances Elsevier, Amsterdam.

ÖTLES, S. and PIRE R. 2001. Fatty acid composition of *Chlorella* and *Spirulina* microalgae species. J AOACInt. **84**: 1708-171

PANDEY,A.1987.Some aspects of ecophysiology of*Anabastestudineuts*(Bloch).Ph.D.Thesis,Bhagalpur, University of Bhagalpur,India.

PAOLETTI, C., PUSHPARAJ, B. and TOMASELLI, L.F. 1975. Ricerche sulla nutrizione minerale di *Spirulina platensis*. Atti XVII Congr. Naz Microbiol.**2**: 833–839.

PAUL, B.N., DATTA,A.K., DAS, D.N., CHATTOPADHAY, D.N.,GIRI,S.S. and MOHANTY, S.N. 2011. Effect of dietarylipid levels on growth and carass compositionof *ompok pabda* (Siluridae) fry. Indian J. ofAnimal Nutr. **28**:198-202 .

PAUL, B.N., RANGACHARYULU,P.V.,SARKAR, S.N. and MUKHOPADHYAY, P.K.1996. Effect of feed color on thegrowth performance and activity of digestive enzymesin Rohu (*Labeo rohita)* fry. J. Aqua. **4**:15-18

PAUL, B.N., SARKAR, S.N., MOHANTY, S.N. and MUKHOPADHYAY, P. K.2004. Effect of dietary attractant on feed utilisation and growth of Rohu *Labeo rohita* (Ham.) fry. Animal Nutr. and Feed Technol.**4**: 145-152.

POTONGKAM, K. 1972. Experiment on feedingclimbing perch, *Anabas testudenius* (Bloch)with ground trash fish and pellets. Department ofFisheries Annual Report, Bangkok, Thailand.23-27

PROMYA, J. and CHITMANAT C. 2011. The effects of *Spirulina platensis* and *Cladophora* algae on the growth performance, meat quality and immunity stimulating capacity of the African Sharptooth Catfish (*Clarias gariepinus*). Int J Agric Biol. **13**:77-82.

QUAZI., S., NAHAR., B.HOSSAIN., A. AND MOHIDUZZAMAN., M., 1989. Total lipid and cholesterolin Fish. Bangladesh. J. Nutrition. **2**(1): 39-40.

QURESHI M.A. and ALI, R.A. 1996. *Spirulina platensis* exposure enhances macrophage phagocytic function in cats. Immunopharmacol Immunotoxicol. **18**:457-463.

RAHMAN, M.A., GHEYASUDDIN, H., RASID, M.H. and CHOUDHURY, M.F.Z. 1982. Limnological studies for four ponds. Bangladesh J. Fish.**2-5**(1- 2):25-35.

RAHMAN, M.A., GHEYASUDDIN, H., RASID, M.H. and CHOUDHURY, M.F.Z. 1982. Proximate composition and native quality of freshwater zeol fishes of Bangladesh. Bangladesh J. Fish. **2-5**: 34-43.

RAHMAN., M.A., BHADRA, A., BEGUM, N. AND HUSSAIN, M. G. 1997. Eflects of some selective supplemental feeds on the survival and growth of catfish (*Clarias batrachus Lin.*) fry. Bangladesh J. Fish. Res.,**l**(2):55-58.

RAMSWAMY, L. S. and SUNDERARAJ, B. I. 1956. Induced spawning in the Indian catfish, *H. fossilis* with pituitary injection. Science,**123**:1080.

RASMUSSEN, R.S., 2001. Quality of farmed salmonids with emphasis on proximate composition, yield and sensory characteristics. Aquaculture Research.**32**: 767-786.

RASTOGI, R. and MEHROTRA, B. N. 1993. Compendium of Indian Medicinal plants. Vol. **3**. Central Drug Research Institute, Lucknow and National Institute of Science Communication, New Delhi, India.

REDDY, C.M., BHAT V.B., KIRANMAI G., REDDY, M.N., REDDANNA, P. and MADYASTHA, K.M. 2000. Selective inhibition of cyclooxygenase-2 by C-phycocyanin, a biliprotein from Spirulina platensis. Biochem Biophys Res Commun.**277**: 599-603.

RINNA HAMLIN.S, JANSI.M and VASUDHEVAN.I. 2013.Effect of dietary *Spirulina platensis* on feeding parameters of blue gourami, *Trichogaster trichopterus International Journal of Research in Fisheries and Aquaculture 2013; 3(3): 103-106.*

RUAN, J.S., LONG, C.S. and Guo, B.J. 1988. *Spirulina* prevented damage induced by radiation. J. Genetics. **10**: 27–30. (In Chinese).

RUBBI, S.F., RAHMA, M.M. and JAHAN, S.S. 1987. Studies on the proximate composition and quality of some commercial species of fresh water fish.

SANGRATTANAKHUL, C. 1989. Effect of Pelletized diets containing various levels of protein on growth and survival of Climbing Perch,*Anabas testudineus* (Bloch). Master degreeThesis. Kasetsart University.Bangkok,Thailand.74.

SHABIR, A.,M., ASHRAF K., NAJAR A.M. andMANSOOR A. *Spirulina*-Nutritional research,Aqua International ,Feb-10,page 22-27.

SMITH, M. A. K. 1981. Estimation of growth potential by measurement of tissue protein synthetic rates in feeding and fasting rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Biology.**19:** 213-220.

SOIVIO, A., NIEMISTO, M. and BACKSTROM, M. 1989. Fatty acid composition of Coregonus muksun Pallas: Changes during incubation, hatching, feeding and starvation. Aquaculture.**79**: 163-168.

SRIVASTAVA, C.V.L. 1985. A textbook of Fishery and Indian fisheries, Kitab Mohal, Allahbad, India, pp. 47-86.

STANSBY, M.E. 1954. Composition of certain species of freshwater fish. Food Res. **16**: 231-234.

STOTT, A.E., TAKEUCHI, T. and KOIKE, Y. 2004. Performance of a new artificial abalone hatchery culture system in terms of settlement of larvae and growth and survival of post-larvae *Haliotis discuss* (Reeve). *Fish. Sci*.**70**: 1070–1081.

TAKEUCHI, T., LU, J., YOSHIZAKI, G. and SATOH, S. 2002. Effect on the growth and body composition of juvenile tilapia *Oreochromis niloticus* fed raw *Spirulina*. Fish Sci. **68**: 34-40.

THAKUR, N. K., PAL, R. N. and KHAN, H. A. 1974. Embryonic and larval development of *H. fossilis* (Bloch), J. Inl. Fish. Soc . India. **6**:33-44.

TITUS, B.G., KULMACZ, R.J. and LANDS, W.E.M. 1982. Selective destruction and removal of heme from Prostaglandin H. Syntase. Arch. Biochem. Biophys. Demic. **214**:824.

TONGSIRI, K., MANG-AMPHAN and YUWADEE, P. 2010 .Effect of Replacing Fishmeal with *Spirulina* on Growth, Carcass Composition and pigment of the Mekong Giant Catfish. Asian Journal of Agricultural Sciences.**2**(3): 106-110.

VALVERDAE, I.M., PERIAGO, M.J., SANTAELLA, M. and ROS, G. 2000. The content and nutritional significance of minerals on fish flesh in the presence and absence of bone.

VENKATARAMAN, N., and CHARI, A.P., 1951. The Elementary Chemical Composition of Marine Organisms (Efton and Settlov, traslators) Yale Univ. Press. New Haven, 463-566.

VENKATESHWARLU, G.,A.P. MURALIDHAR, R. RAMESH and A.K. PAL. 2009Plants traditionally used in fish harvests & angling potential feed attractants in aquaculture. Indian. J. of Traditional Knowledge. **8**:539-542.

Washington, DC. 21-447.

WATANUKI, H., K. OTA,K., MALIN, A. C., TASSAKKA, A. R., KATO, T. andSAKAI, M. 2006. Immunostimulant effects of dietary *Spirulina platensis* on carp, *Cyprinus carpio*. Aquaculture.**258**: 157–163.

Appendix A

Statistical data of proximate composition

Table 9.1 Descriptive statistics of final body moisture

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
diet1	3	77.9967	1.48561	.85772
diet2	3	76.9433	1.44500	.83427

T-Test

	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
diet1	90.935	2	.000	77.99667	74.3062	81.6871
diet2	92.228	2	.000	76.94333	73.3537	80.5329

Table 9.2 ANOVA of final body moisture

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.414	1	2.207	.793.	.473.
Within Groups	.732	4	.		
Total	5.146	5			

Descriptive Statistics

	N	Range	Minimum	Maximum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
protein diet_1	3	.32	16.60	16.92	16.7933	.09821	.17010	.029
protein_2	3	.43	19.48	19.91	19.7367	.13094	.22679	.051
Valid N (listwise)	3							

Table 9.3 Descriptive statistics of final body protein

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
protein diet_1	3	16.7933	.17010	.09821
protein_2	3	19.7367	.22679	.13094

T-Test

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
protein diet_1	171.001	2	.000	16.79333	16.3708	17.2159
protein_2	150.734	2	.000	19.73667	19.1733	20.3000

Table 9.4ANOVA of final body protein

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.573	1	2.207	7.46.	.022.
Within Groups	.561	4	.181.		
Total	4.134	5			

Table 9.5 Descriptive statistics of final body lipid

One-Sample Statistics				
	N	Mean	Std. Deviation	Std. Error Mean
Ldiet1	3	2.5367	.03055	.01764
Ldiet2	3	4.1967	.01528	.00882

T-Test

One-Sample Test						
	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Ldiet1	143.815	2	.000	2.53667	2.4608	2.6126
Ldiet2	475.857	2	.000	4.19667	4.1587	4.2346

Table 9.6ANOVA of final body lipid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.572	1	.357	12.336.	.007
Within Groups	.126	4	.027		
Total	.698	5			

Table 9.7 Descriptive statistics of final body ash

One-Sample Statistics				
	N	Mean	Std. Deviation	Std. Error Mean
ashdiet1	3	2.1067	.04726	.02728
ashdiet2	3	1.9667	.02082	.01202

T-Test

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
ashdiet1	77.211	2	.000	2.10667	1.9893	2.2241
ashdiet2	163.637	2	.000	1.96667	1.9150	2.0184

Table 9.8ANOVA of final body ash

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.756	1	.370	11.06.	.013
Within Groups	.203	4	.039		
Total	.959	5			

Descriptive Statistics

	N	Range	Minimum	Maximum	Mean		Std. Deviation	
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	variance
wgdiet1	3	.07	10.43	10.50	10.4667	.02028	.03512	.036
wgdiet2	3	.20	15.90	16.10	15.9967	.05783	.10017	.048
Valid N (listwise)	3							

Appendix B

Statistical data of fish growth performance

Table 9.9 Descriptive statistics of weight gain

One-Sample Statistics				
	N	Mean	Std. Deviation	Std. Error Mean
wgdiet1	3	10.4667	.03512	.02028
wgdiet2	3	15.9967	.10017	.05783

T-Test

One-Sample Test						
	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
wgdiet1	516.213	2	.000	10.46667	10.3794	10.5539
wgdiet2	276.610	2	.000	15.99667	15.7478	16.2455

Table 9.10ANOVA of weight gain

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.756	1	2.76.	9.672.	.018
Within Groups	1.235	4	.372		
Total	6.991	5			

T-Test

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
sgrdiet1	3	2.713	.279.	.170
sgrdiet2	3	2.973	.302	.140

Table 9.11 Descriptive statistics of SGR

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
sgrdiet1	4.067	2	.000	.653	2.66	2.79
sgrdiet2	5.739	2	.000	.712	3.06	2.85

Table 9.12ANOVA of SGR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.189	1	.971	2.179	.135
Within Groups	.177	4	.358		
Total		5			

Table 9.13 Descriptive statistics of survival rate

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
srvdiet1	3	85.4867	3.30849	1.91016
srvdiet2	3	92.9233	1.67739	.96844

T-Test

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
srvdiet1	44.754	2	.000	85.48667	77.2679	93.7054
srvdiet2	95.951	2	.000	92.92333	88.7565	97.0902

Table 9.14 ANOVA of survival rate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	209.361	1	77.211	10.439	.101
Within Groups	61.820	4	9.752		
Total	271.181	5			

Appendix C

Statistical data of feed utilization

Descriptive Statistics

	N	Range	Minimum	Maximum	Mean		Variance
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
fcrdiet1	3	.66	1.21	1.87	1.4467	.21216	.135
fcrdiet2	3	.35	1.10	1.45	1.3067	.10588	.034
Valid N (listwise)	3						

Table 9.15 Descriptive statistics of FCR

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
fcrdiet1	3	1.4467	.36747	.21216
fcrdiet2	3	1.3067	.18339	.10588

T-Test

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
fcrdiet1	6.819	2	.021	1.44667	.5338	2.3595
fcrdiet2	12.341	2	.007	1.30667	.8511	1.7622

Table 9.16ANOVA of FCR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.153	1	.093	4.742	.078
Within Groups	.107	4	.017		
Total	.260	5			

Table 9.17 Descriptive statistics of PER

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
perdiet1	3	.7527	.05173	.02987
perdiet2	3	.8867	.01528	.00882

T-Test

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
perdiet1	25.200	2	.002	.75267	.6242	.8812
perdiet2	100.539	2	.000	.88667	.8487	.9246

Table 9.18ANOVA of PER

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.086	1	.039	.499	.719
Within Groups	.521	4	.063		
Total	.607	5			

Table 9.19 Descriptive statistics of feed efficiency

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
feffdiet1	3	78.1667	1.31561	.75957
feffdiet2	3	89.5633	1.08214	.62478

T-Test

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
feffdiet1	102.909	2	.000	78.16667	74.8985	81.4348
feffdiet2	143.353	2	.000	89.56333	86.8751	92.2515

Table 9.20ANOVA of feed efficiency

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	139.506	1	67.190	.838	.463
Within Groups	528.819	4	93.071		
Total	668.325	5			