# Evaluation of the effects of feed attractants *(Spirulina* and ekangi) on growth performance, feed utilization and body composition of fingerlings of stinging cat fish, *Heteropneustes fossilis* (Bloch, 1792)

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# CERTIFICATE

This is to certify that this thesis entitled **"Evaluation of the effects of feed attractants (*Spirulina* and ekangi) on growth performance, feed utilization and body composition of fingerlings of stinging cat fish, *Heteropneustes fossilis* (Bloch, 1792)"** submitted by Md. Shawkat Ali 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.

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April 3, 2014 Author

# ABSTRACT

Asian catfish (local name shingi or shing), *Heteropneuestes fossilis* as a fish was chosen for this study due to high survival rate, low diseases and high demand to the people as a food. An experiment was conducted for sixty days to evaluate the effects of *Spirulina* and ekangi used as feed additives on growth performance and body composition of stinging fish, *H. fossilis* at Department of Fisheries, University of Dhaka. There were nine aquariums under three treatments. Fingerlings of *H. fossilis* with initial weight 5.23±0.64g, 4.84±0.75g and 6.64±0.96g and initial length 10.23±.47cm, 10.2±0.91cm, and 11.76 ±0.27cm for three treatments i.e. $T_1$, $T_2$ and $T_3$ respectively fed on three different experimental diets. Three types of experimental diets *viz.* diet I containing control diet without addition of feed additives, diet II control diet with 1% S*pirulina,* diet III control diet with 1% ekangi. All the water quality parameters specifically- Temperature (ºC), Dissolved oxygen (DO), and pH in the aquariums were highly monitored and maintained. The change in growth and feed utilization by the shing fish for three different experimental diets has been assessed by the determination of condition factor (K), survival rate (SR %), specific growth rate (SGR %), feed conversion ratio (FCR), feed efficiency and average daily gain (ADG) and protein efficiency ratio (PER). The lowest food conversion ratio (FCR) was found for $T_2$ (diet II) while the highest was measured in $T_1$ (diet I). The FCR value of $T_3$ (diet III) was also lower than $T_1$. The values of protein efficiency ratio (PER), condition factor (K), average daily gain (ADG) and specific growth rate (SGR) were greater in $T_2$ and $T_3$ than $T_1$. Fish fed with the control diet had the lowest protein and fat content. Ash content increased significantly when fish fed supplemented diet as compared with the control treatments. The study with different feed attractants revealed that the net weight gain and feed intake were significantly higher in $T_2$ and $T_3$ than $T_1$. Result of the current study showed that supplementation of *Spirulina* and ekangi with control diet as feed attractants had significant positive effects on the FCR, SGR, and ADG and PER.

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| **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 |
| LSD | Least significance Differences |
| $T_1$ | Treatment 1 |
| $T_2$ | Treatment 2 |
| $T_3$ | Treatment 3 |

# INTRODUCTION

## 1.1 General Background

Bangladesh has the third largest aquatic fish biodiversity in Asia with about 800 species in fresh, brackish and marine water (Hossain and Mazid 2001). The country has a total 265 freshwater species and 475 marine water species and 12 exotic species (DoF 2012). Fisheries sector contributes about 3% of total export earning, 4.43% of GDP (Gross Domestic Product) and 22.23% of agricultural sector in 2010-2011 (DoF 2012). Annual total fish production in 2010-2011 in Bangladesh is about 30, 61,687 MT (DoF 2012). Fish also contributed about 60% to the nation's animal protein (DoF 2012). At present annual fish intake by an individual is 18.94 kg and the annual fish demand is 32.72 metric tons (DoF 2012). Malnutrition problem in Bangladesh can be reduced by increasing the production of fish.

Among the air-breathing catfishes, stinging catfish (*H. fossilis*) is very popular and high valued fish in Bangladesh. This fish is locally known as Shingi or Shing. It is considered to be highly nourishing, palatable and tasty and well preferred because of its less spine, less fat and high digestibility in many parts of Indian subcontinent (Khan *et al.* 2003). The species is very high content of iron (226 mg 100 $g^{-1}$) and fairly high content of calcium compared to many other freshwater fishes. Due to high nutritive value the fish is recommended in the diet of sick and convalescents. Being a lean fish it is very suitable for people for whom animal fats are undesirable (Rahman *et al.* 1982). It is a very hardy fish and can survive for quite a few hours outside the water due to presence of accessory respiratory organs. It can tolerate slightly brackish water. The fish adapts well to hypoxic water bodies and to high stocking densities (Dehadrai *et al.* 1985). This fish was abundantly available in open water system of floodplains, canals, beel and haors of Bangladesh. But due to over exploitation and ecological changes in its natural habitats; this species have become threatened. Indiscriminate destructive practices have caused havoc to aquatic bio-diversity in Bangladesh (Hussain and Mazid 2001).

Presently, *H. fossilis* is one of the threatened fish in Bangladesh (IUCN Bangladesh 2000). This fish has enormous aquaculture potential and it could be easily grown in ponds and small ditches. Culture of *H. fossilis* has yet been well flourished in

Bangladesh due to lack of appropriate culture technique. Considering its status of threatened status, high market value and high consumer demand it is essential to develop suitable culture technique. The culture technique will helpful to prevent the fish from being extinct and at the same time this delicious tasty fish will be available for the rural and urban people.

*H. fossilis* is considered suitable for culture, because of their high tolerance to adverse environmental conditions, their relatively fast growth and resistance to disease, excellent quality of its firmly textured flesh and finely appetizing fish to the consumers (Corpei 2001).

*H. fossilis* is the most economically important freshwater food fish species. It is highly valued as an excellent aquaculture species for intensive culture because of its ability to resist diseases, tolerance to crowding and wide range of environmental conditions. For the very efficient air breathing organ the survival rate of this fish is high. This also helps the fish to grow abundantly in oxygen depleted water, even in shallow mini ponds. It is easy to keep them alive for longer period in captivity and also transport them alive in semi-dry containers. Sometimes doctors suggest the patients of anemia to have large amount of *H. fossilis* in their food menu (Bhatt 1968). Induced spawning and fry production of *H. fossilis* is easy (Ramswamy and Sunderaraj 1956, Thakur *et al*. 1974). This fish contains very high protein, high iron and low fat content. Propagation and culture of this fish is very important. *Heteropneustes fossilis* has several common names like Asian stinging cat fish or Fossil cat but in our country this fish is locally called Shing fish. In many Asian countries this fish is commercially as well as aquaculturally an important species (Akand *et al.* 1989). It is an indigenous species to Indo- Pak-Bangladesh sub-continent. This cat fish used to belong to the family Heteropneustidae for many years but very recently it has been moved into the Clariidae family (Diogo *et al.* 2003). For high nutritious value, taste and flavor *H. fossilis* has a high market value and consumer preference.

## 1.2 Nutrients in Fish

Fish contributes enormously to the supply of both macro and micronutrients in our diet. It is considered to be the potential source of proteins and many other micronutrients, such as vitamins and minerals. Fish have some unusual composition features that do not

have appreciable carbohydrates. For all practical purposes, the caloric values of fishes are based only on the fat and protein 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 important ecological measure of condition that integrates both feeding condition and habitat quality. Proximate composition can also have important 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).

### 1.2.1 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 perishability of fish (Clucas and Ward 1996).

### 1.2.2 Protein

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). Fish supplies not only abundant amount of protein, but also kinds of protein most efficiently used by the body. 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).

### 1.2.3 Fat (Lipid)

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 300mg 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 omega-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).

### 1.2.4 Ash (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 necessary nutrients and in a form that can easily be consumed by the fish which will result in more efficient 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.

## 1.3 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 reported to have positive effects on growth performance, feed utilization, lipid metabolism, carcass quality, stress tolerance and disease resistance (Mustafa and Nakagawa 1995; Nakagawa and 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 to improve growth, feed utilization, lipid metabolism, body composition, stress responses, liver function and disease resistance in ayu, *Plecoglossus altivelis*.

Microalgal species are of great value because of their high bioactive materials content, including polyunsaturated fatty acids, β-carotene and 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).

## 1.4 *Spirulina* as a feed additive

*Spirulina* has been one of the most widely used micro-algal species in aqua feeds 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 like 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*.

### 1.1.1 Nutritional food value

The use of *Spirulina* as complementary feed in various sector of aquaculture resulting fast growth factors, enhancing the pigmentation and immunity systems. 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 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 |
| Moisture | 3-7 |
| Fiber | 8-10 |

### 1.4.2 Biochemical composition of *Spirulina*

**Protein & Amino acids**

*Spirulina* contains 60-70% protein along with phenolic acids, tocopherols, carotenes and linolenic acids 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:

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

- **Tryptophane:** Increases utilization of B vitamins, improves nerve health.
- **Valine** : Stimulates muscle coordination.

**Cabohydrates**

*Spirulina* contains about 15 -21 % carbohydrates in the form of Glucose, fructose, sucrose, rhamnose, mannose, 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**

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

**Essential fatty acids**

*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).

**β-carotene and vitamins**

*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.

### 1.4.4 Nutritional 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 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* 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. *Spirulina* provides phycocyanin, a source of biliverdin which is among the most potent of all intra-cellular antioxidants.

**Immunomodulatory Property**

*Spirulina* is an effective immune modulator. It 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.

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. *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.

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

*Spirulina* is rich in a brilliant 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 marine organisms), *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 appearance is the most important characteristic in case of shrimps and fishes for choice & demand in food market. *Spirulin*a diet promotes 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. Astaxanthin is predominant carotenoid associated with the red body colour of the black tiger prawn *Penaeus monodon*. *Spirulina platensis* and *S. pacifica* strain contains 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. 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.

**1.5 Ekangi as a feed attractant**

Feed attractants are substances which are added in trace amounts in the fish diets to enhance feed intake and to fulfill the nutrient requirement. Fish primarily detect feeds through sight or olfaction (Jones 1992). Dietary feeding stimulants are essential to elicit an acceptable and rapid feeding response. Two types of feeding stimulants may be considered for use within aquaculture feeds. Natural ingredient sources which exhibit attractant or feeding stimulant properties or the use of the purified or synthetic chemical derivatives which are responsible for the attractant property of natural ingredient sources. Paul *et al*. (2004) reported use of the combination of plant based attractant, in the feed of *Labeo rohita* fry at 1% level of inclusion.

Mukhopadhyay and Paul (1995) reported that addition of trimethyl ammonium hydrochloride (TMAH) and addition of amino acids viz., glutamic acid, aspartic acid, serine, alanine and lysine are probable feeding stimulants in fish. Incorporation of Livol (Herbal growth promoter, Indian Herbs) in fish improves growth (Gireesha *et al.* 2002 and Maheshappa 1994). The rationale behind the use of feed additive is to improve dietary feed intake at a faster rate so as to minimize the leaching of water soluble nutrients, and wastage of feed. Several plant materials have been used for alluring fish during harvesting and angling by traditional fish farmers.

Ekangi, tambul, kharboj, chotokakla and latakasturi are the plant materials that have been used for alluring fish during harvest and angling by traditional fish farmers (Paul *et al.* 2004).

**Table 1.1** Some plants used as attractants in aquaculture

| Local name | Plant name | Parts used |
|---|---|---|
| **Ekangi** | *Kaempferia galanga* | Rhizomes |
| **Tambul** | *Zanthoxylum acanthopodium* | Seeds |
| **Kharboj** | *Cucumis melo* | Roots |
| **Chotokakla** | *Piper cubeba* | Fruits |

Fish feeding pattern, whereby preferred foods are searched for and ingested, is one of the most important behavior pattern exhibited by fish (Venkateshwarlu *et al.* 2009). Addition of attractant in fish feed have positive effect on prawn (Harpaz 1997) and rohu (Paul *et al.* 2004). Harpaz (1997) further reported that addition of an attractant to the water leads to an additional thirst for food searching activity. The increase in food searching behavior was accompanied by olfactory nerves associated with taste or feeding behavior (Nakajima *et al.* 1989). To make aquaculture profitable more number of plant based ingredients are being used for preparation of fish feed and the palatability of such feed can be increased by addition of attractants.

Ekangi contains essential oil and also known as aromatic ginger. We used ekangi with control feed at 1% to understand the chemical nature and its mode of action on the fish in laboratory condition.

**Ekangi:** *KAEMPFERIA GALANGA L.*

Family: Zingiberaceae

Bengali/Vernacular Name: Chandumula, Humula, Ekangi.

**Physiological characteristics of ekangi**

- A steam less , small rhizomatous herb
- Rhizome tuberous, aromatic.
- Root-fibers fleshy, cylindrical.
- Two leaves, spreading flat on the ground, 6.3-12.5 cm long rotund -ovate, deltoid-acuminate.
- Flowers white, 6-12 from the centre of the plan between the leaves.
- Lip broad, deeply 2 lobed, with a lilac spot at the base.

**Chemical constituents**

- Leaves and flowers contain flavonoid having antiphlogistic,vitamin P activity etc.
- Root contains Et-p-MeO-trans-cinamate as main compound.
- Rhizomes is rich in protein, amino acids,minerals,sugars, and lipid, also contains an essential oil,alkaloid,starch,gum and fatty matter(Ghani 2003). P-methoxycinamic acid and its methyl and ethyl esters have been isolated from essential oil (Rastogi and Mehrotra 1993).

## 1.6 Objectives of the Study

The overall objective of the present study was to investigate how *Spirulina* and ekangi affects the growth performance and feed utilization and biochemical composition of fingerlings of *H. fossilis* in laboratory condition.

The specific objectives were

i) Determination of the feeding response of *H. fossilis* fingerlings fed with different kinds of feed additives such as *Spirulina* and ekangi.

ii) Estimation of growth performances such as Average Daily Gain (ADG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Condition factor (K) of fingerlings of *H. fossilis* fed with different kinds of feed additives such as *Spirulina* and ekangi.

iii) Evaluation of the proximate composition (moisture, protein, lipid and ash) of *Heteropneustes fossilis* fingerlings fed with different kinds of feed additives such as *Spirulina* and ekangi.

# 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 collection of commercial feed and two different feed additives (*spirulina* and ekangi), preparation of supplemented diet, collection of fingerlings of *H. fossilis,* acclimatization of the fingerling in the laboratory condition, feeding trial of the fingerlings with the control diet without supplementing feed additives, control diet with 1% Spirulina and 1% Ekangi. Before, during and after the feeding trial the fingerling of *H. fossilis* were collected for analytical purpose as well as measuring the biological parameter required for growth performance, feed intake, feed conversion ratio (FCR), protein efficiency ratio(PER).

## 2.2 Experimental designs

A set of nine glass aquariums were used for the feeding trial of the fingerlings of *H. fossilis*. One hundred and thirty five fingerlings were stocked with density of fifteenth fish per aquariums. Nine aquariums were assigned in three treatments to one control diet without supplement and two control diets with feed additives. Fingerlings were released into the aquariums randomly.

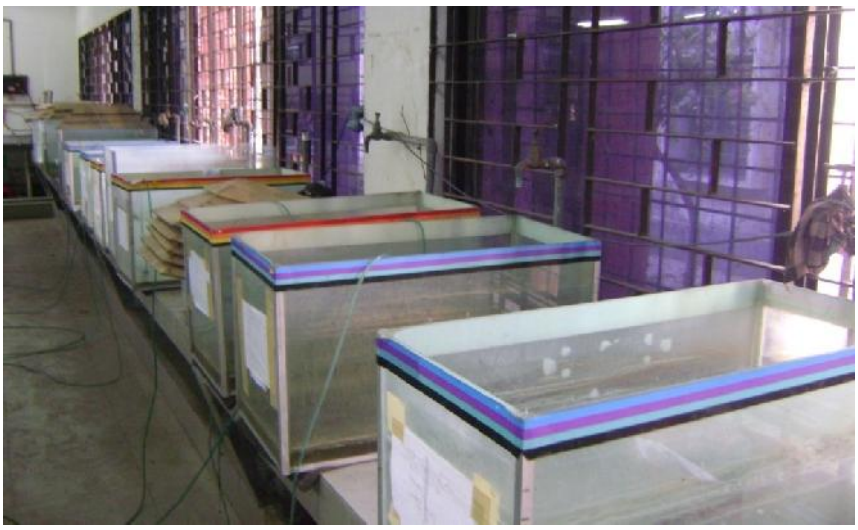


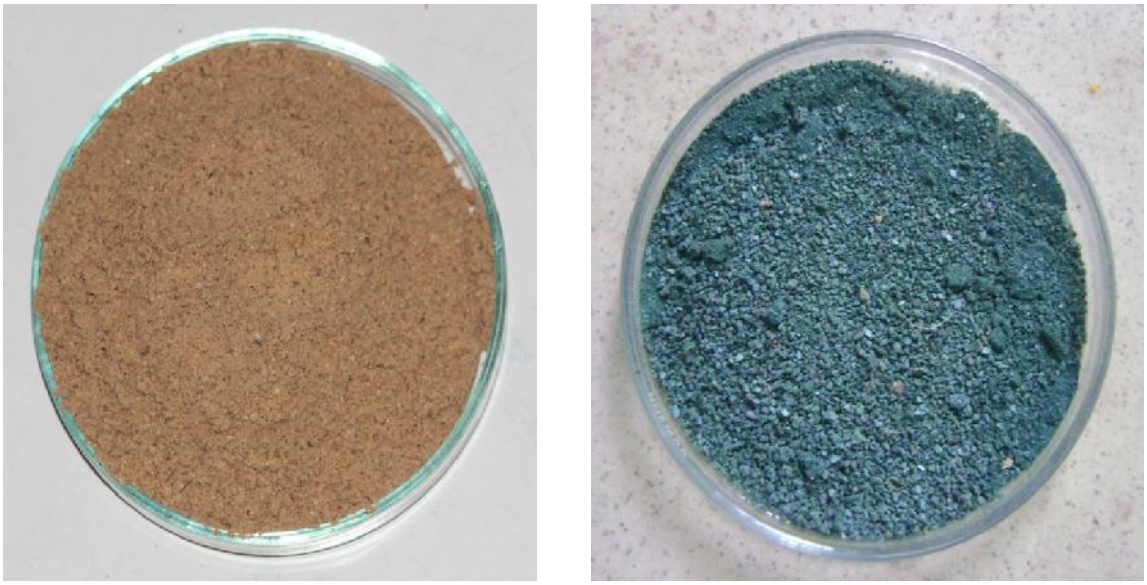
**Plate 1** Experimental system used for rearing of *H. fossilis*.

## 2.3 Collections of feed additives

Commercial feed were collected from the Fulbaria Fish and Poultry Feed Market, Dhaka.

*Spirulina* were collected from Fish Technology laboratory, BCSIR, Dhaka.

Ekangi are available in Dhaka and Kustia. Ekangi were collected from Kustia.



**2(A)** **2(B)**

**Plate 2 (A)** Ekangi powder prepared from rhizomes of the plant. **(B)** *Spirulina* powder

## 2.4 Preparation of the experimental diets

Three experimental diets were used in this experiment to evaluate the growth performance feed utilization on *H. fossilis*.

Diet –I: Commercial feed without feed additives, as control diet.

Diet-II: Control diet + 1 % *Spirulina* used as a feed additive.

Diet-II: Control diet + 1% ekangi used as a feed attractant.

Dietary ingredients of feed additives (*Spirulina* and ekangi) were ground using a laboratory grinder and then separately blended with commercial feed at fixed ratio into a homogenous doughy matter by adding water, which pelleted by pressing through a 4 mm

die in a grinding machine. The pellets were then stored in plastic containers at room temperature for further use.

## 2.5 Collection of experimental fish

Fingerlings of *H. fossilis* were collected from Water World Mathshay Hatchery, Mymensingh. Live and healthy fish were collected in December 2013 and carried in oxygenated bags with water. Fingerlings were kept in Circular tanks with adequate aeration and sufficient water. The average weight of each fingerling was 3-9 grams and average length of each species was 5-13 cm. then acclimatization for one week. During acclimatization, maintenance feed ration about 6% of body weight. After acclimatized, 135 fingerlings were kept randomly in nine glass aquariums for experiment.

**Classification**

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Siluriformes
Family: Heteropneustidae
Genus: *Heteropneustes*
Species: *H. fossilis*

**Plate no. 3** Sample of experimental fish (*H. fossilis)* during study period.

## 2.6 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 16.00- 17.00 pm. The feeding trial continued for a period of 60 days. Fish were randomly sampled 5% 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 fingerlings were also determined and dead fish were removed from aquariums.

## 2.7 Water quality measurement

Water quality such as pH, dissolved oxygen was monitored in the tanks regularly following different procedures. 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 aquariums every week during experiment by pH meter.

## 2.8 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) and condition factor (K) of fish.


**Plate no. 4 A**. Measurement of experimental fish, **B.** Weighing of experimental fish.

### 2.8.1 Average daily gain (ADG)

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

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

### 2.8.3 Specific growth rate (SGR %)

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

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

### 2.8.4 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)

## 2.9 Feed utilization

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

### 2.9.1 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 *H. fossilis* fed control diet, diet II and diet III were determined at the end of the experiment as follow:

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

### 2.9.2 Protein efficiency ratio (PER)

Protein efficiency ratio was determined by the following formula:

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

### 2.9.3 Survival rate (%)

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

$$\text{Survival rate (\%)} = \frac{\text{Final no. of fish survived}}{\text{No. of actual fish stocked}} \times 100$$

### 2.9.4 Feed efficiency (%)

Feed efficiency was determined by the following formula:

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

## 2.10 Biochemical Analyses of Fish

**Sample preparation**

After 60days 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.

### 2.10.1 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:

$$\text{Moisture (\%)} = \frac{\text{Initial weight (g)} - \text{final weight (g)}}{\text{Weight of the sample}} \times 100$$

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

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

### 2.10.2 Estimation of protein content

#### a) Principle

Protein content can be measured by estimating the nitrogen content of the material and 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 15$^{th}$ edition 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

**2.10.3 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**

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

**2.10.4 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**

- Porcelain crucibles.
- Crucible furnace
- Drier
- Gas burner
- 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:**

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

## 2.11 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 *H. fossilis for* 60 days in the laboratory condition. The commercial feed was set as the control. Commercial 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 *H. fossilis* in aquariums on three experimental diets as recorded during the period of investigation were presented under the following heading.

**Table 3.1** Proximate composition of three experimental diet (dry weight basis, ±SEM)

| **Items** | **Diet I (control)** | **Diet II** | **Diet III** |
|---|---|---|---|
| Moisture (%) | 32 | 35.4±0.11 | 32.3±0.09 |
| Protein (%) | 12 | 10.93±1.2 | 11.84±1.02 |
| Lipid (%) | 5 | 4.57±1.52 | 4.98±0.096 |
| Ash (%) | 18 | 18.44±0.088 | 18.62±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 carcass composition of fish was 79.03±0.44%, 16.4±0.38%, 2.47% and 1.31±% for moisture, protein, lipid and ash respectively.

**Table 3.2** Whole body proximate composition of *H. fossilis* fed with three experimental diets (% fresh weight basis, ± SEM)**.**

| **Items** | **Diet I** | **Diet II** | **Diet III** |
|---|---|---|---|
| Moisture (%) | 79.13±0.046 | 73.66±0.053 | 78.63±0.20 |
| Protein (%) | 16.42±0.215 | 17.84±0.385 | 16.77±0.159 |
| Fat (%) | 2.43±.045 | 3.03±.126 | 3.013±0.059 |
| Ash (%) | 2.25±0.142 | 2.87±0.184 | 2.35±0.07 |

### 3.1.1 Moisture Content in Fish

Fig.3.1 depicted the percentage of moisture in *H.fossilis* species fed with different kinds of diet. Moisture content was found to be in the range of 73.61-79.13g %. The highest moisture content was found for $T_1$ (79.13±0.046 %) and lowest for $T_2$ (73.66±0.0536%). Moisture content for $T_3$ was 78.63±0.202% which is higher than $T_2$ but lower than $T_1$.There is no significant difference among these treatments for moisture content in fish.

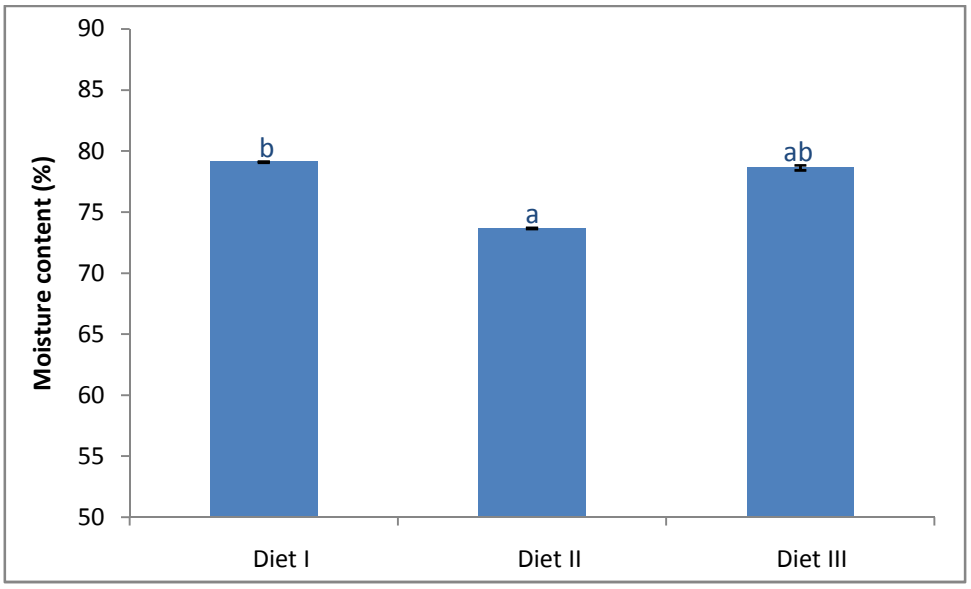



**Fig. 3.1** Moisture content (%), (Mean ± SEM) in *H. fossilis* cultured for 60 days fed with three experimental diets. Bars (mean ±SEM) different letters indicate significant difference.

### 3.1.2 Protein Content in Fish

Percentage of crude protein for *H.fossilis* species under study was compiled in Figure: In present study protein content was found to be in the range of 16.42-17.84%g. The highest protein content being present in $T_2$ (17.84±0.385%), while the lowest being present in $T_1$ (16.42±0.215%). In $T_3$, the crude protein content was found 16.73±0.159%, which was significantly higher than $T_1$ but lower than $T_2$.

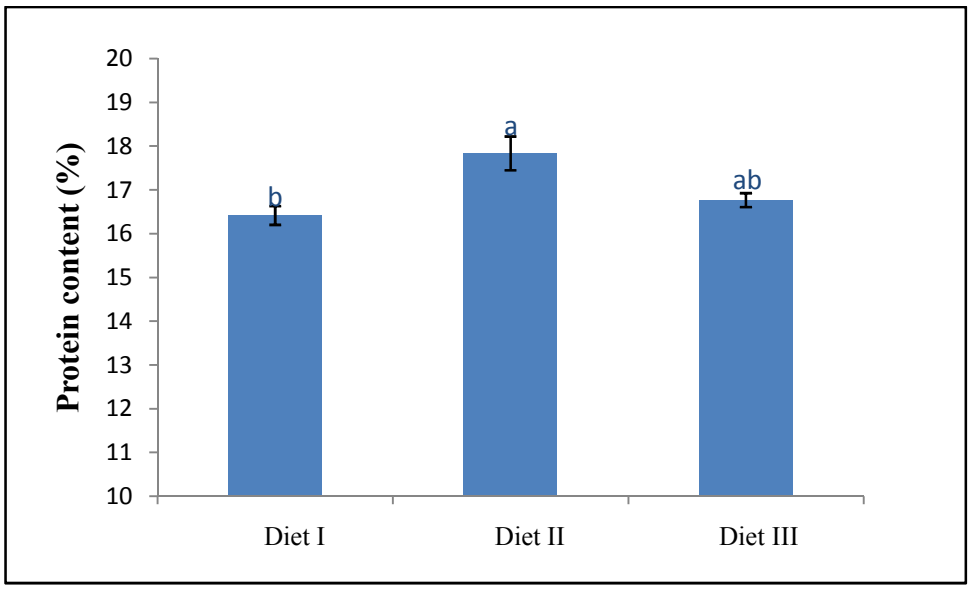



**Fig. 3.2** Protein content (%), (Mean ± SEM) in *H. fossilis* cultured for 60 days fed three experimental diets. Bars (mean ±SEM) different letters indicate significant difference.

### 3.1.3 Lipid content in Fish

All experimental fish had higher lipid contents than the initial lipid composition. Lipid content was found to be in the rage of 3.35-1.57 %. There were no significant differences between $T_2$ (3.03±0.126%) and $T_3$ (3.013±0.059%). But there were significant differences between control (2.43±.045%) and treatments.

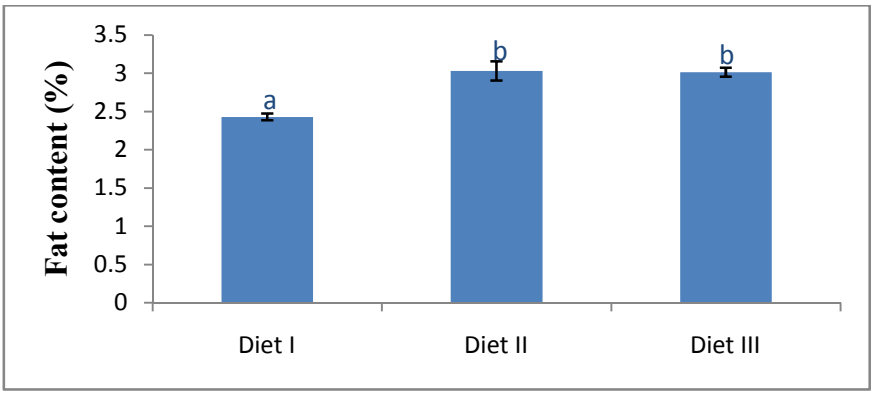



**Fig. 3.3** Lipid Content (%), (Mean ± SEM) in *H. fossilis* cultured for 60 days fed with three experimental diets. Bars (mean ±SEM) different letters indicate significant difference.

### 3.1.4 Ash Content in Fish

The values of ash content were recorded after 60 days study period. The highest ash found in the $T_2$ (2.87±0.184). The ash content for $T_1$ and $T_3$ were 2.25±0.142 and 2.35±0.07 respectively.

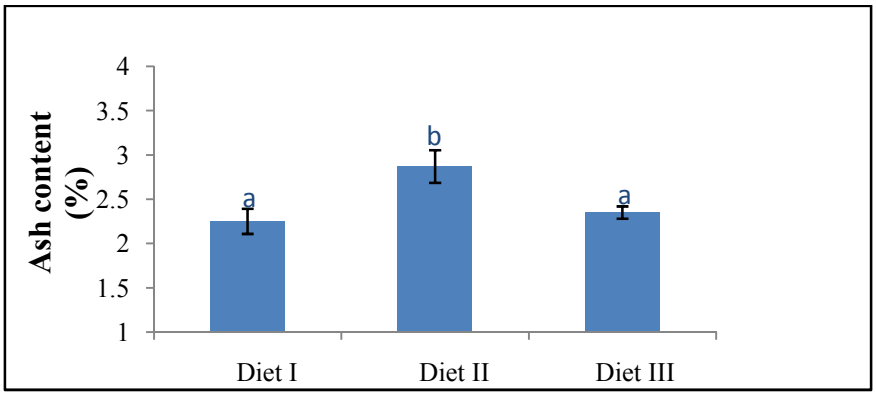



**Fig. 3.4** Ash Content (%), (Mean ± SEM) in *H. fossilis* cultured for 60 days fed with three experimental diets. Bars (mean ±SEM) different letters indicate significant difference.

## 3.2 Growth performance

The growth performance of the experimental fish rearing 60 days fed three different experimental diet in the laboratory condition are showed in table.

**Table 3.3** The growth performance data (mean ± SEM) of experimental fish fed three experimental diets.

| Parameters | Diet I | Diet II | Diet III |
|---|---|---|---|
| Initial body weight (g) | 5.23±0.64 | 4.84±0.75 | 6.64±0.96 |
| Final body weight (g) | 10.54±.93 | 11.96±.13 | 13.11±0.94 |
| Initial length (cm) | 10.23±.47 | 10.2±0.91 | 11.76±0.27 |
| Final length (cm) | 13.02±.21 | 14.96±.86 | 15±0.36 |
| Weight gain (g) | 5.31±.31 | 7.12±.41 | 6.47±0.02 |
| Percentage of Weight gain (%) | 103.11±7.57 | 150.17±19.037 | 101.33±13.67 |
| Average daily weight gain (ADG) | 0.088±0.001 | 0.12±0.01 | 0.11±.00 |
| Specific growth rate (%) | 1.75±0.07 | 1.54±0.12 | 1.58±0.12 |
| Survival rate (%) | 86.67±0.00 | 97.77±2.23 | 95.54±2.22 |
| Condition factor (K) | 0.46±0.015 | 0.36±0.032 | 0.38±0.017 |

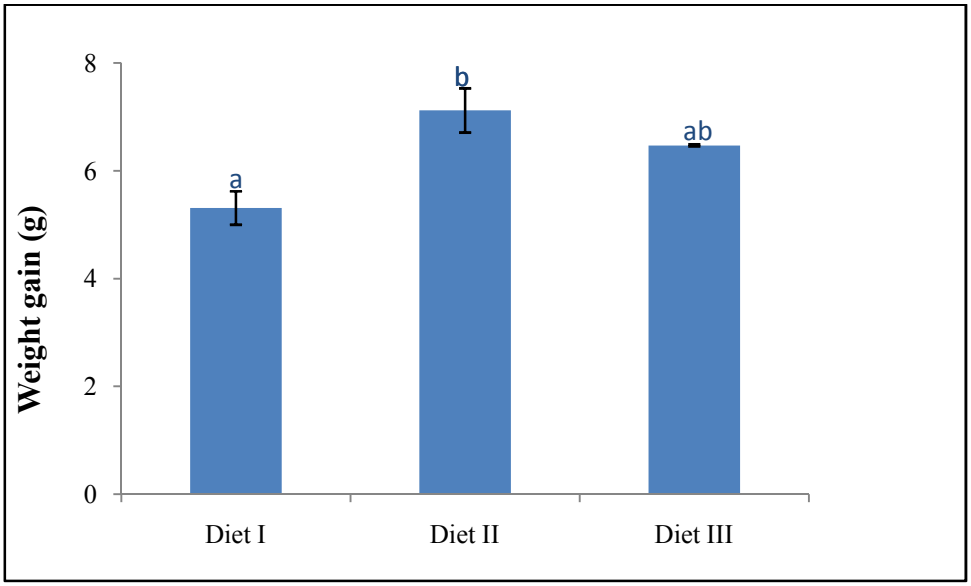



**Fig. 3.5** Variation in weight gain of *H. fossilis* after 60 days fed three experimental diets. Bars (mean ±SEM) different letters indicate significant difference.

### 3.2.1 Average daily gain (ADG, g/d)

The values of Average daily gain (ADG) was highest in treatment 2 (0.12±0.009g/d) and lowest in control (0.088±0.003 g/d). The values of average daily gain (ADG) of the experimental fish *H. fossilis* for $T_3$ (0.11±0.00 g/d) is higher than the control.

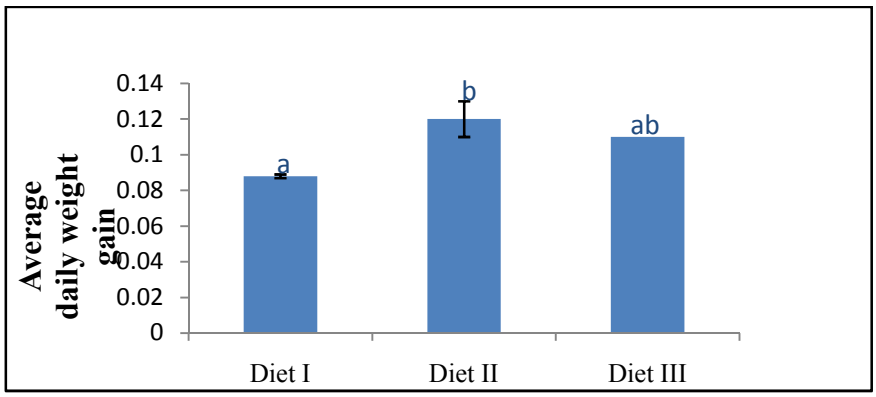



**Fig. 3.6** Average dailygain of *H. fossilis* after 60 days fed with three experimental diets. Bars (mean ±SEM) different letters indicate significant difference.

### 3.2.2 Specific growth rate (SGR %)

The values of Specific growth rate (SGR%) of the experimental fish H. fossilis rearing in nine tanks fed on three different types of fish feed were estimated and the findings were different. The values of SGR% are highest for $T_2$ (1.54±0.119 %). The values of specific growth rate for $T_3$ (diet III) (1.16±0.116%) were more or less similar with $T_1$ (1.16±0.066%).

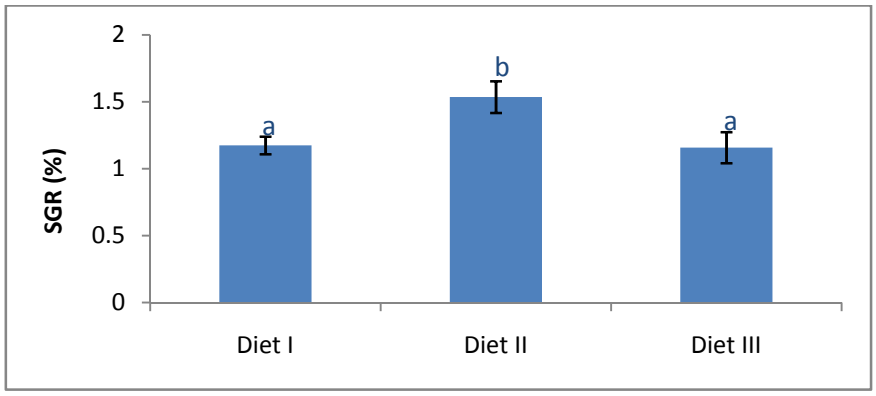



**Fig. 3.7** Specific growth rate (SGR %) of the experimental fish *H. fossilis*. Bars (mean ±SEM) different letters indicate significant difference.

### 3.2.3 Condition Factor (K)

Condition factor (K) of *H. fossilis* fingerlings was the highest (K=0.46±0.015) at $T_1$ and the lowest (K =0.38±0.017) at $T_3$. And then the K value (K =0.36±0.032) of $T_2$ was higher than $T_3$ and lower than $T_1$ (Fig 3.8).

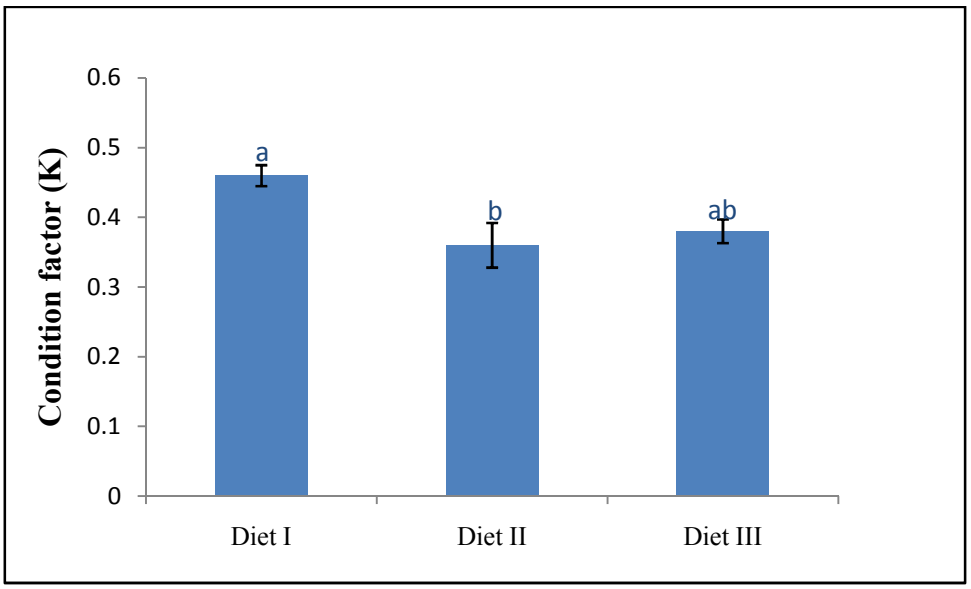



**Fig. 3.8** Condition factor (K) in *H. fossilis* determined after 60 days fed with three experimental diets. Bars (mean ±SEM) different letters indicate significant difference.

## 3.3 Feed utilization

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

**Table 3.4:** Feed utilization data (mean± SEM) of *H. fossilis* fed three experimental diets**.**

| Items | Diet I | Diet II | Diet II |
|---|---|---|---|
| Feed conversion ratio (FCR) | 1.78±0.067 | 1.43±0.102 | 1.65±0.046 |
| Feed efficiency | 28.68±2.09 | 37.04±8.11 | 28.2±3.76 |
| Protein efficiency ratio (PER) | 0.89±0.064 | 1.043±0.230 | 0.86±0.118 |

### 3.3.1 Feed conversion ratio (FCR)

The Feed conversion ratio of *H. fossilis* kept in different aquariums and fed on three different experimental diet have been calculated after 60 days study period. The highest FCR (1.78±0.066 %) was found in the control (Diet I) while the lowest (FCR 1.44±0.102 %) was measured in $T_2$ (Diet II). In $T_3$ (Diet III) the value of FCR was 1.65±0.046 % which is significantly higher than $T_2$ but lower than control.

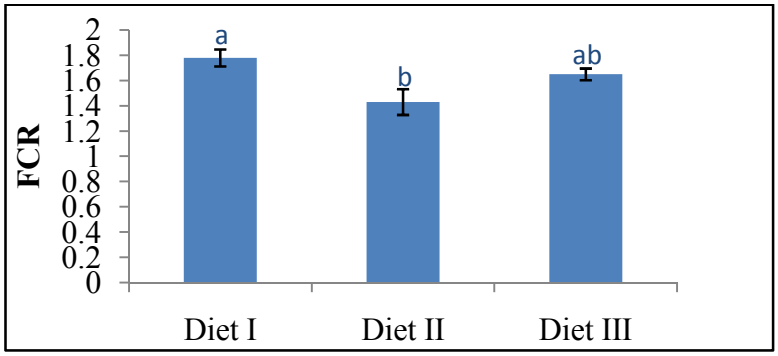



**Fig. 3.9** Feed Conversion Ratio (FCR), (Mean ± SEM) of *H. fossilis* fingerlings cultured for 60 days fed three experimental diets. Bars (mean ±SEM) different letters indicate significant difference.

### 3.3.2 Protein efficiency Ratio (PER %)

The values of protein efficiency ratio the experimental fish rearing in nine aquariums for 60 days fed on three different experimental diets have been estimated after the study period. The values of PER for $T_2$ was (1.04±0.23%) which was significantly higher than $T_1$ (control diet) (0.89±0.064) and $T_3$ (0.86±0.118)

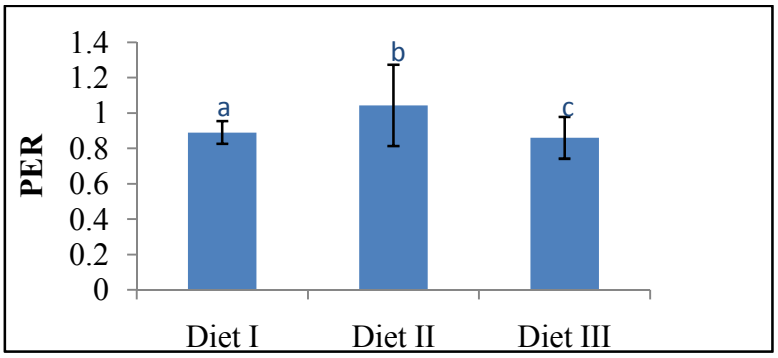



**Fig. 3.10** Protein efficiency ratio of three experimental diets fed by *H. fossilis* observed in a laboratory condition. Bars (mean ±SEM) different letters indicate significant difference.

### 4.3.3 Feed efficiency (%)

The values of feed efficiency ranges had been calculated after 60 days study period. The highest value was observed for $T_2$ (37.04±8.11%). There was no significant difference of feed efficiency between $T_1$ (28.68±2.09) and $T_3$ (28.2±3.76).

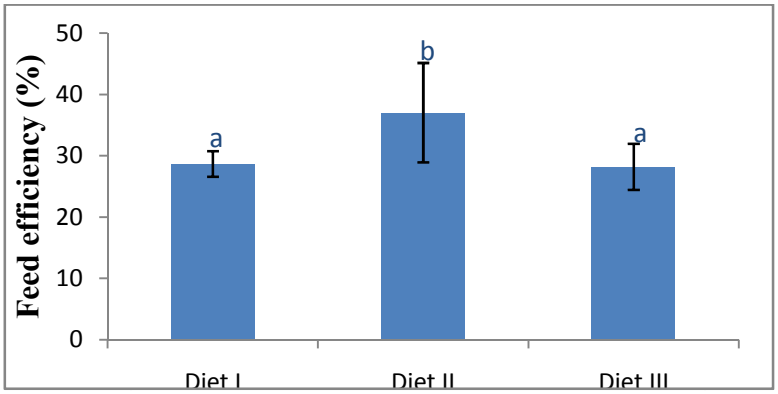



**Fig. 3.11** Feed efficiency (FE) of diet supplemented with and without feed additives fed by *H. fossilis* observed in a laboratory test.

### 3.3.4 Survival rate (%)

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 the other treatment diets ($P<0.05$). The average survival rate of fish was 86.67±0.00% in fish fed with control feed and 97.77±2.23% and 93.32±3.84% in fish fed with *Spirulina* and ekangi.

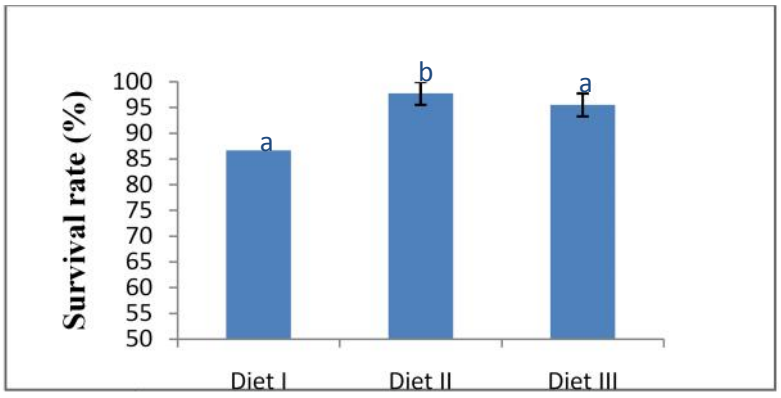



**Fig. 3.12** Survival rates of *H. fossilis* fed supplemented with and without feed additives observed in a laboratory condition.

# DISCUSSIONS

The Study had three aspects: body composition, feed utilization efficiencies and growth performances of stinging cat fish (*H. fossilis*). As *H. fossilis* is omnivorous fish, different kinds of diet are supplied to determine the effect of feeding regime on the growth performance of fish and also the proximate composition of fish. 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 among three types of treatment.

The results obtained from this experiment showed that the growth responses of fish fed with control diet on 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), food conversion ratio (FCR), protein efficiency ratio (PER) were decreased. In this experiment, fish fed diets containing *Spirulina* and ekangi showed the best growth rate, feed efficiency and significantly higher percentage of weight gain than control diet.

**Growth performance**

The growth rate of the *H. fossilis* fingerlings were shown in table 3.3. Total biomass of fingerlings was increases in $T_2$ and $T_3$ than $T_1$. Average weight gain in $T_2$ and $T_3$ were 7.12±0.41g and 6.47±0.02g respectively where lowest average weight gain (5.31±0.31g) was found in $T_1$. Statistical data showed that there were significant differences among three treatments. Weight gain in control diet were significantly lower than the other treatment diets ($P<0.05$). Statistical analysis showed that lower bound and upper bound at 95% confidence for three treatments was 5.5952 and 7.0026. F values between groups were 9.359. Statistically there was no significant among treatments fed with three 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 aquariums which having tap water) the survival rate is comparatively lower than the natural water body as the tap water contained a little bit 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 the other treatment diets ($P<0.05$). The average survival rate of fish was 86.67±2.23% in fish fed with control diet and 97.77±2.23% and 93.32±3.84% in fish fed diet with *Spirulina* and ekangi.

These findings have more or less similarities with the findings of Akand and Haque (1989). Their study had 82 to 93% survival rate of the 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.* Minimum and maximum value at 95 % confidence level was 86.67, 93.3-100,100 for T1, $T_2$ and $T_3$ respectively. From ANOVA table, F value between groups was 10.419.statistically there was no significant difference among treatments ($P<0.05$).

**Averge daily gain (ADG)**

The average daily gain in $T_2$ and $T_3$ were 0.12±0.009 (g/d) and 0.113±0.00 (g/d) after 60 days study period. The average daily gain of both treatments was higher than the control (0.088±0.005g/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 so on. 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 Nilkolsky (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 ADG in *Anabas testudineus* is ranging from 0.10- 0.12g which has similarities with our findings. Statistical data showed that F value between treatments was 0.624. Minimum and maximum value at 95 % confidence level was 0.08 and 0.09 for $T_1$ which was lower than the $T_2$ and $T_3$. Statistically there was significantly difference among three treatments ($P>0.05$).

**Specific growth rate (%)**

SGR was decreased at the end of the experiment in case of both 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".

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 *H. fossilis* were estimated and the findings were different. The maximum SGR (%) for $T_2$ (1.53±0.119%) fed with control diet containing *Spirulina* where minimum for $T_1$ and $T_3$. From ANOVA data F value between treatment was 3.135.There was statistically significance among treatments (P>0.05).

**Condition factor (K)**

The condition factor was highest in $T_1$ (0.462± 0.015%). However the condition factor in the $T_2$ (0.36±0.032%) was more or less similar with $T_3$ (0.38±0.017%). The values of condition factor of the fish ranged from 0.36-0.46. 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.

**Feed conversion ratio**

The highest FCR was found in the control (diet I) (1.783±0.066) while the lowest was measured in $T_2$ (diet II containing *Spirulina* as a feed additive). In $T_3$ (Diet III containing ekangi as a feed attractant) the value of FCR was significantly higher than treatment 2 but lower than control. The lower the FCR the better the feed conversion to fish flesh. From this point of view the *Spirulina* containing diet (diet II) gives the better result than control diet. Diet containing ekangi also showed lower FCR value than control diet indicate that the diet II gives the better result than the 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.

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. 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.

**Protein efficiency ratio (PER)**

The values of PER for $T_2$ was 1.043±0.23 % which was higher than control diet. The values of PER for $T_3$ was also higher than control. The fish groups fed with two experimental diets (diet I and diet III) 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* and ekangi in the commercial feed shows better PER.

Dawah, *et al.* 2002 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 in fish (Mustafa *et al.*1994).

Takeuchi *et al.* 2002 found that juvenile tilapia fed solely on the alga show a lower feed efficiency 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 so PER also lower in fish fed with control diet. The range of feed efficiency was 21.1-52.09% in this experiment.

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 three experimental diets. The final body moisture was statistically significant ($P<0.05$) between T2 and T3. The percentage of moisture in experimental fish carcass 79.13±0.046% in fish fed with diet I and 73.66±0.053% 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 (17.84±0.385) was highest in fish fed diet containing *Spirulina*. The final body protein content was also increase in $T_3$ (16.73±0.159) than control diet. Statistical data showed that F value F value between treatments was 0.868.

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 the moisture 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 Murali *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 lipid contents are correlated with their synthesis and accumulation rate 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 of the *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 different treatment fingerlings was 2.43±0.045%, 3.03±0.126% and 3.01±0.059% for $T_1$, $T_2$ and $T_3$ respectively.

In the previous study (Valverde *et al.* 2000) but lower than the values determined by Banu *et al*. (1981), where the content has been shown to be 67 mg% for Shol and 13.74 mg% for Aeir.

# SUMMARY

An experiment was conducted for sixty days to evaluate the effects of *Spirulina* and ekangi used as feed additives on growth performance and body composition of stinging fish, *H. fossilis* at Department of Fisheries, University of Dhaka. Three types of experimental diets *viz.* diet I containing control diet without addition of feed additives, diet II control diet with 1% S*pirulina,* diet III control diet with 1% ekangi. The change in growth and feed utilization by the shing fish for three different experimental diets has been assessed by the determination of condition factor (K), survival rate (SR %), specific growth rate (SGR %), feed conversion ratio (FCR), feed efficiency and average daily gain (ADG) and protein efficiency ratio (PER). Fingerlings of *H. fossilis* with initial weight 5.23±0.64g, 4.84±0.75g and 6.64±0.96g and initial length 10.23±.47cm, 10.2±0.91cm, and 11.76 ±0.27cm for three treatments i.e. $T_1$, $T_2$ and $T_3$ respectively fed on three different experimental diets. The experiments consisted of collection of commercial feed and two different feed additives (*Spirulina* and ekangi), preparation of supplemented diet, collection of fingerlings of *H. fossilis,* acclimatization of the fingerling in the laboratory condition, feeding trial of the fingerlings with the control diet without supplementing feed additives, control diet with 1% Spirulina and 1% Ekangi. Before, during and after the feeding trial the fingerling of *H. fossilis* were collected for analytical purpose as well as measuring the biological parameter required for growth performance, feed intake, feed conversion ratio (FCR), protein efficiency ratio(PER).The initial proximate composition of the sample species determined before rearing and feeding trial.At the initial stage proximate composition of the sample species kept at different aquariums had been found variations due to difference in size(Length- weight difference). After 60 days study period proximate composition was determined to evaluate the overall progress. Results revealed that fish fed diet I and diet II influence the final carcass composition of the fishes.

Highest survival rate (95.54±2.22%) was obtained for $T_2$. Statistical analysis data indicated that Survival rate was greater when fish fed ekangi supplemented diet.lowest survival was found for $T_1$ (86.67%).

The highest FCR was found in the control (diet I) (1.783±0.066) while the lowest was measured in $T_2$ (diet II containing *Spirulina* as a feed additive). In $T_3$ (Diet III containing

ekangi as a feed attractant) the value of FCR was significantly higher than treatment 2 but lower than control. 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* and ekangi in the commercial feed shows better PER

# CONCLUSION

The current study showed the dietary effect of feed additives on the feed utilization efficiency, body composition and growth performance of *H. fossilis* during rearing and feeding trail in the laboratory condition. The growth performance and feed utilization from these study were significantly lower in commercial feed ($P<0.05$) than feed with *Spirulina* and ekangi. Commercial 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).

The protein quality of *H.fossilis* diets contained Commercial 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* and ekangi than control diet.

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

From this study it can be concluded that addition of *Spirulina* at 1% in the control diet have been positive effects on the growth performance and feed utilization.
In case of addition of ekangi in the diet it also can be concluded that incorporation of 1% ekangi in the control diet can be improved growth of fish due to attractiveness to the feed and increases feed efficiency.

To make aquaculture profitable more number of plant based ingredients are being used for preparation of fish feed and the palatability of such feed can be increased by addition of attractants. However, more work is necessary to understand the chemical nature and its mode of action and it opens new area of research.

# RECOMMENDATIONS

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

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

3. Although, at present there is no economical advantage exists in replacing commercial feed attractants by *Spirulina* and ekangi, it has acted as a strong feeding effectors for *H.fossilis* at a very low inclusion level, this study would serve as a foundation for further and refined studies with *Spirulina* and ekangi.

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

# REFERENCES

Abdel-Tawwab, 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 carbohydrate and 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 two water 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 Fisheries Society, **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 the phylogenetic relationships between *Heteropneustes* and the *clariid* catfishes. Animal Biology **53**: 379-396.

DoF 2012. Annual Report 2011-2012. Department of Fisheries, Bangladesh, Ministry of Fisheries and Livestock, 111.

DoF 2012. Fisheries Statistical Yearbook of Bangladesh 2011-2012. Department of Fisheries, 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 of Bangladesh, 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 utilization and 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.

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

HUSSAIN, M. G. and MAZID, M. A. 2001. Genetic improvement and conservation of carp species in Bangladesh. Bangladesh Fisheries Research Institute and International Center for Living Aquatic Resources Management.

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 to D' 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 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, MD. G., WAKAMATSU, S., AKI, T., TAKEDA, T., UMINO and NAKAGAWA. 1995. Effects of algae meal as feed additive on growth, 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.

NETTLETON, A., 1985. Nutrients and Substances in Fresh Sea Food. In: Seafood nutrition facts. Issues and 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. and THILSTED, 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 AOAC Int. **84**: 1708-1714.

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 dietary lipid levels on growth and carass composition of *ompok pabda* (Siluridae) fry. Indian J. of Animal Nutr. **28**:198-202 .

PAUL, B.N., RANGACHARYULU, P.V., SARKAR, S.N. and MUKHOPADHYAY, P.K.1996. Effect of feed color on the growth performance and activity of digestive enzymes in 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 feeding climbing perch, *Anabas testudenius* (Bloch) with ground trash fish and pellets. Department of Fisheries Annual Report, Bangkok, Thailand.

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.

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 degree Thesis. Kasetsart University. Bangkok, Thailand.74.

SHABIR, A.,M., ASHRAF K., NAJAR A.M. and MANSOOR 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. 2009 Plants 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. and SAKAI, 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

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Diet I | 3 | 79.1300 | .08000 | .04619 | 78.9313 | 79.3287 | 79.05 | 79.21 |
| Diet II | 3 | 73.6667 | .08963 | .05175 | 73.4440 | 73.8893 | 73.61 | 73.77 |
| Diet III | 3 | 52.9500 | 44.48044 | 25.68080 | -57.5455 | 163.4455 | 1.59 | 78.98 |
| Total | 9 | 68.5822 | 25.25240 | 8.41747 | 49.1715 | 87.9929 | 1.59 | 79.21 |

**Table 9.2** ANOVA of final body moisture

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 1144.421 | 2 | 572.210 | .868 | .467 |
| Within Groups | 3957.048 | 6 | 659.508 | | |
| Total | 5101.469 | 8 | | | |

**Table 9.3** Multiple Comparisons of final body moisture

| | (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| LSD | Diet I | Diet II | 5.46333 | 20.96836 | .803 | -45.8444 | 56.7711 |
| | | Diet III | 26.18000 | 20.96836 | .258 | -25.1277 | 77.4877 |
| | Diet II | Diet I | -5.46333 | 20.96836 | .803 | -56.7711 | 45.8444 |
| | | Diet III | 20.71667 | 20.96836 | .361 | -30.5911 | 72.0244 |
| | Diet III | Diet I | -26.18000 | 20.96836 | .258 | -77.4877 | 25.1277 |
| | | Diet II | -20.71667 | 20.96836 | .361 | -72.0244 | 30.5911 |

**Table 9.4** Homogenous test of final body moisture

| | Treatment | N | Subset for alpha = 0.05 |
|---|---|---|---|
| | | | 1 |
| Tukey B[a] | Diet III | 3 | 52.9500 |
| | Diet II | 3 | 73.6667 |
| | Diet I | 3 | 79.1300 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

**Table 9.5** Descriptive statistics of final body protein

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Diet I | 3 | 16.4267 | .37287 | .21528 | 15.5004 | 17.3529 | 16.03 | 16.77 |
| Diet II | 3 | 17.8500 | .65368 | .37740 | 16.2262 | 19.4738 | 17.24 | 18.54 |
| Diet III | 3 | 16.7367 | .27592 | .15930 | 16.0512 | 17.4221 | 16.53 | 17.05 |
| Total | 9 | 17.0044 | .76210 | .25403 | 16.4186 | 17.5902 | 16.03 | 18.54 |

**Table 9.6** ANOVA of final body protein

| | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 3.361 | 2 | 1.681 | 7.848 | .021 |
| Within Groups | 1.285 | 6 | .214 | | |
| Total | 4.646 | 8 | | | |

**Table 9.7** Multiple Comparisons of final body protein

| | (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| Tukey HSD | D-I | D-II | -1.42333* | .37785 | .022 | -2.5827 | -.2640 |
| | | D-III | -.31000 | .37785 | .705 | -1.4693 | .8493 |
| | D-II | D-I | 1.42333* | .37785 | .022 | .2640 | 2.5827 |
| | | D-III | 1.11333 | .37785 | .058 | -.0460 | 2.2727 |
| | D-III | D-I | .31000 | .37785 | .705 | -.8493 | 1.4693 |
| | | D-II | -1.11333 | .37785 | .058 | -2.2727 | .0460 |
| LSD | D-I | D-II | -1.42333* | .37785 | .009 | -2.3479 | -.4988 |
| | | D-III | -.31000 | .37785 | .443 | -1.2346 | .6146 |
| | D-II | D-I | 1.42333* | .37785 | .009 | .4988 | 2.3479 |
| | | D-III | 1.11333* | .37785 | .026 | .1888 | 2.0379 |
| | D-III | D-I | .31000 | .37785 | .443 | -.6146 | 1.2346 |
| | | D-II | -1.11333* | .37785 | .026 | -2.0379 | -.1888 |

*. The mean difference is significant at the 0.05 level.

**Table 9.8** Homogenous of final body protein

| | Treatment | N | Subset for alpha = 0.05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey HSD[a] | D-I | 3 | 16.4267 | |
| | D-III | 3 | 16.7367 | 16.7367 |
| | D-II | 3 | | 17.8500 |
| | Sig. | | .705 | .058 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

**Table 9.9** Descriptive statistics of final body lipid

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Diet I | 3 | 2.4333 | .07767 | .04485 | 2.2404 | 2.6263 | 2.37 | 2.52 |
| Diet II | 3 | 3.0333 | .21779 | .12574 | 2.4923 | 3.5744 | 2.79 | 3.21 |
| Diet III | 3 | 3.0133 | .10263 | .05925 | 2.7584 | 3.2683 | 2.90 | 3.10 |
| Total | 9 | 2.8267 | .32109 | .10703 | 2.5799 | 3.0735 | 2.37 | 3.21 |

**Table 9.10** ANOVA of final body lipid

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .697 | 2 | .348 | 16.331 | .004 |
| Within Groups | .128 | 6 | .021 | | |
| Total | .825 | 8 | | | |

**Table 9.11** Multiple Comparisons of lipid

| | (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| LSD | Diet I | Diet II | -.60000* | .11926 | .002 | -.8918 | -.3082 |
| | | Diet III | -.58000* | .11926 | .003 | -.8718 | -.2882 |
| | Diet II | Diet I | .60000* | .11926 | .002 | .3082 | .8918 |
| | | Diet III | .02000 | .11926 | .872 | -.2718 | .3118 |
| | Diet III | Diet I | .58000* | .11926 | .003 | .2882 | .8718 |
| | | Diet II | -.02000 | .11926 | .872 | -.3118 | .2718 |

*. The mean difference is significant at the 0.05 level.

**Table 9.12** Homogenous of final body lipid

| | Treatment | N | Subset for alpha = 0.05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey B[a] | Diet I | 3 | 2.4333 | |
| | Diet III | 3 | | 3.0133 |
| | Diet II | 3 | | 3.0333 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

**Table 9.13** Descriptive statistics of final body ash

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Diet I | 3 | 2.2500 | .2451 | .14154 | 1.6410 | 2.8590 | 2.01 | 2.50 |
| Diet II | 3 | 2.8933 | .1814 | .10477 | 2.4425 | 3.3441 | 2.76 | 3.10 |
| Diet III | 3 | 2.3500 | .1212 | .07000 | 2.0488 | 2.6512 | 2.22 | 2.46 |
| Total | 9 | 2.4978 | .3417 | .11393 | 2.2351 | 2.7605 | 2.01 | 3.10 |

**Table 9.14** ANOVA of final body ash

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .719 | 2 | .360 | 10.012 | .012 |
| Within Groups | .215 | 6 | .036 | | |
| Total | .935 | 8 | | | |

**Table 9.15** Multiple Comparisons of final body ash

| | (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| LSD | Diet I | Diet II | -.64333* | .15473 | .006 | -1.0219 | -.2647 |
| | | Diet III | -.10000 | .15473 | .542 | -.4786 | .2786 |
| | Diet II | Diet I | .64333* | .15473 | .006 | .2647 | 1.0219 |
| | | Diet III | .54333* | .15473 | .013 | .1647 | .9219 |
| | Diet III | Diet I | .10000 | .15473 | .542 | -.2786 | .4786 |
| | | Diet II | -.54333* | .15473 | .013 | -.9219 | -.1647 |

*. The mean difference is significant at the 0.05 level.

**Table 9.16** Homogenous of final body ash

| | Treatment | N | Subset for alpha = 0.05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey B[a] | Diet I | 3 | 2.2500 | |
| | Diet III | 3 | 2.3500 | |
| | Diet II | 3 | | 2.8933 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

## Appendix B

## Statistical data of fish growth performance

**Table 9.17** Descriptive statistics of weight gain

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Diet I | 3 | 5.306 | .5351 | .30899 | 3.9772 | 6.6362 | 4.69 | 5.65 |
| Diet II | 3 | 7.123 | .7252 | .41874 | 5.3216 | 8.9250 | 6.32 | 7.73 |
| Diet III | 3 | 6.466 | .0351 | .02028 | 6.3794 | 6.5539 | 6.43 | 6.50 |
| Total | 9 | 6.298 | .9154 | .30515 | 5.5952 | 7.0026 | 4.69 | 7.73 |

**Table 9.18** ANOVA of weight gain

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 5.077 | 2 | 2.539 | 9.359 | .014 |
| Within Groups | 1.627 | 6 | .271 | | |
| Total | 6.704 | 8 | | | |

**Table9.19** Multiple Comparisons of weight gain

| | (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| LSD | Diet I | Diet II | -1.81667* | .42523 | .005 | -2.8572 | -.7762 |
| | | Diet III | -1.16000* | .42523 | .034 | -2.2005 | -.1195 |
| | Diet II | Diet I | 1.81667* | .42523 | .005 | .7762 | 2.8572 |
| | | Diet III | .65667 | .42523 | .173 | -.3838 | 1.6972 |
| | Diet III | Diet I | 1.16000* | .42523 | .034 | .1195 | 2.2005 |
| | | Diet II | -.65667 | .42523 | .173 | -1.6972 | .3838 |

*. The mean difference is significant at the 0.05 level.

**Table 9.20** Homogenous of weight gain

| | Treatment | N | Subset for alpha = 0.05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey B[a] | Diet I | 3 | 5.3067 | |
| | Diet III | 3 | 6.4667 | 6.4667 |
| | Diet II | 3 | | 7.1233 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

**Table 9.21** Descriptive statistics of average daily gain (ADG)

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| D-I | 3 | .0870 | .00794 | .00458 | .0673 | .1067 | .08 | .09 |
| D-II | 3 | .4167 | .50521 | .29168 | -.8383 | 1.6717 | .12 | 1.00 |
| D-III | 3 | .6033 | .85448 | .49333 | -1.5193 | 2.7260 | .11 | 1.59 |
| Total | 9 | .3690 | .54555 | .18185 | -.0503 | .7883 | .08 | 1.59 |

**Table 9.22** ANOVA of average daily gain (ADG)

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .410 | 2 | .205 | .624 | .567 |
| Within Groups | 1.971 | 6 | .328 | | |
| Total | 2.381 | 8 | | | |

**Table 9.23** Multiple Comparisons of average daily gain (ADG)

| | (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| LSD | D-I | D-II | -.32967 | .46796 | .508 | -1.4747 | .8154 |
| | | D-III | -.51633 | .46796 | .312 | -1.6614 | .6287 |
| | D-II | D-I | .32967 | .46796 | .508 | -.8154 | 1.4747 |
| | | D-III | -.18667 | .46796 | .704 | -1.3317 | .9584 |
| | D-III | D-I | .51633 | .46796 | .312 | -.6287 | 1.6614 |
| | | D-II | .18667 | .46796 | .704 | -.9584 | 1.3317 |

**Table 9.24** Homogenous of average daily gain (ADG)

| | Treatment | N | Subset for alpha = 0.05 |
|---|---|---|---|
| | | | 1 |
| Tukey B[a] | D-I | 3 | .0870 |
| | D-II | 3 | .4167 |
| | D-III | 3 | .6033 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

**Table 9.25** Descriptive statistics of SGR

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Diet I | 3 | 1.1753 | .11494 | .06636 | .8898 | 1.4609 | 1.06 | 1.28 |
| Diet II | 3 | 1.5363 | .20682 | .11941 | 1.0226 | 2.0501 | 1.35 | 1.76 |
| Diet III | 3 | 1.3760 | .19480 | .11247 | .8921 | 1.8599 | 1.21 | 1.59 |
| Total | 9 | 1.3626 | .21914 | .07305 | 1.1941 | 1.5310 | 1.06 | 1.76 |

**Table 9.26** ANOVA of SGR

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .196 | 2 | .098 | 3.135 | .117 |
| Within Groups | .188 | 6 | .031 | | |
| Total | .384 | 8 | | | |

**Table 9.27** Multiple Comparisons of SGR

| | (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| LSD | Diet I | Diet II | -.36100* | .14448 | .047 | -.7145 | -.0075 |
| | | Diet III | -.20067 | .14448 | .214 | -.5542 | .1529 |
| | Diet II | Diet I | .36100* | .14448 | .047 | .0075 | .7145 |
| | | Diet III | .16033 | .14448 | .310 | -.1932 | .5139 |
| | Diet III | Diet I | .20067 | .14448 | .214 | -.1529 | .5542 |
| | | Diet II | -.16033 | .14448 | .310 | -.5139 | .1932 |

*. The mean difference is significant at the 0.05 level.

**Table 9.28** Homogenous of SGR

| | Treatment | N | Subset for alpha = 0.05 |
|---|---|---|---|
| | | | 1 |
| Tukey B[a] | Diet I | 3 | 1.1753 |
| | Diet III | 3 | 1.3760 |
| | Diet II | 3 | 1.5363 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size =3.00

**Table 9.29** Descriptive statistics of condition factor (K)

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Diet I | 3 | .460 | .02646 | .0152 | .3943 | .5257 | .43 | .48 |
| Diet II | 3 | .356 | .05508 | .0318 | .2199 | .4935 | .30 | .41 |
| Diet III | 3 | .383 | .02887 | .0166 | .3116 | .4550 | .35 | .40 |
| Total | 9 | .400 | .05745 | .0191 | .3558 | .4442 | .30 | .48 |

**Table 9.30** ANOVA of condition factor (K)

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .017 | 2 | .009 | 5.672 | .041 |
| Within Groups | .009 | 6 | .002 | | |
| Total | .026 | 8 | | | |

**Table 9.31** Multiple Comparisons of condition factor

| | (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| LSD | Diet I | Diet II | .10333* | .03186 | .018 | .0254 | .1813 |
| | | Diet III | .07667 | .03186 | .053 | -.0013 | .1546 |
| | Diet II | Diet I | -.10333* | .03186 | .018 | -.1813 | -.0254 |
| | | Diet III | -.02667 | .03186 | .435 | -.1046 | .0513 |
| | Diet III | Diet I | -.07667 | .03186 | .053 | -.1546 | .0013 |
| | | Diet II | .02667 | .03186 | .435 | -.0513 | .1046 |

*. The mean difference is significant at the 0.05 level.

**Table 9.32** Homogenous of condition factor (K)

| | Treatment | N | Subset for alpha = 0.05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey B[a] | Diet II | 3 | .3567 | |
| | Diet III | 3 | .3833 | .3833 |
| | Diet I | 3 | | .4600 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

**Table 9.33** Descriptive statistics of survival rate

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Diet I | 3 | 86.67 | 0.00000 | 0.000 | 86.6700 | 86.6700 | 86.67 | 86.67 |
| Diet II | 3 | 97.77 | 3.86825 | 2.233 | 88.1574 | 107.3759 | 93.30 | 100.0 |
| Diet III | 3 | 95.53 | 3.85093 | 2.223 | 85.9871 | 105.1196 | 93.33 | 100.0 |
| Total | 9 | 93.33 | 5.77207 | 1.924 | 88.8932 | 97.7668 | 86.67 | 100.0 |

**Table 9.34** ANOVA of survival rate

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 206.948 | 2 | 103.474 | 10.419 | .011 |
| Within Groups | 59.586 | 6 | 9.931 | | |
| Total | 266.534 | 8 | | | |

**Table 9.35** Multiple Comparisons of survival rate

| | (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| LSD | Diet I | Diet II | -11.09667* | 2.57306 | .005 | -17.3927 | -4.8006 |
| | | Diet III | -8.88333* | 2.57306 | .014 | -15.1794 | -2.5873 |
| | Diet II | Diet I | 11.09667* | 2.57306 | .005 | 4.8006 | 17.3927 |
| | | Diet III | 2.21333 | 2.57306 | .423 | -4.0827 | 8.5094 |
| | Diet III | Diet I | 8.88333* | 2.57306 | .014 | 2.5873 | 15.1794 |
| | | Diet II | -2.21333 | 2.57306 | .423 | -8.5094 | 4.0827 |

*. The mean difference is significant at the 0.05 level.

**Table 9.36** Homogenous of survival rate

| | Treatment | N | Subset for alpha = 0.05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey B[a] | Diet I | 3 | 86.6700 | |
| | Diet III | 3 | | 95.5533 |
| | Diet II | 3 | | 97.7667 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

# Appendix C

## Statistical data of feed utilization

**Table 9.37** Descriptive statistics of FCR

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Diet I | 3 | 1.783 | .11504 | .0664 | 1.4976 | 2.0691 | 1.67 | 1.90 |
| Diet II | 3 | 1.436 | .17616 | .1017 | .9991 | 1.8743 | 1.25 | 1.60 |
| Diet III | 3 | 1.650 | .07937 | .0458 | 1.4528 | 1.8472 | 1.59 | 1.74 |
| Total | 9 | 1.623 | .18861 | .0628 | 1.4784 | 1.7683 | 1.25 | 1.90 |

**Table 9.38** ANOVA of FCR

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .183 | 2 | .092 | 5.442 | .045 |
| Within Groups | .101 | 6 | .017 | | |
| Total | .285 | 8 | | | |

**Table 9.39** Multiple Comparisons of FCR

| | (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| LSD | Diet I | Diet II | .34667* | .10600 | .017 | .0873 | .6061 |
| | | Diet III | .13333 | .10600 | .255 | -.1261 | .3927 |
| | Diet II | Diet I | -.34667* | .10600 | .017 | -.6061 | -.0873 |
| | | Diet III | -.21333 | .10600 | .091 | -.4727 | .0461 |
| | Diet III | Diet I | -.13333 | .10600 | .255 | -.3927 | .1261 |
| | | Diet II | .21333 | .10600 | .091 | -.0461 | .4727 |

*. The mean difference is significant at the 0.05 level.

**Table 9.40** Homogenous of FCR

| | Treatment | N | Subset for alpha = 0.05 | |
|---|---|---|---|---|
| | | | 1 | 2 |
| Tukey B[a] | Diet II | 3 | 1.4367 | |
| | Diet III | 3 | 1.6500 | 1.6500 |
| | Diet I | 3 | | 1.7833 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

**Table 9.41** Descriptive statistics of PER

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Diet I | 3 | .8933 | .11015 | .06360 | .6197 | 1.1670 | .78 | 1.00 |
| Diet II | 3 | 1.043 | .39879 | .23024 | .0527 | 2.0340 | .68 | 1.47 |
| Diet III | 3 | .8633 | .20404 | .11780 | .3565 | 1.3702 | .64 | 1.04 |
| Total | 9 | .9333 | .24531 | .08177 | .7448 | 1.1219 | .64 | 1.47 |

**Table 9.42** ANOVA of PER

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .056 | 2 | .028 | .393 | .691 |
| Within Groups | .426 | 6 | .071 | | |
| Total | .481 | 8 | | | |

**Table 9.43** Multiple Comparisons of PER

| | (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| LSD | Diet I | Diet II | -.15000 | .21746 | .516 | -.6821 | .3821 |
| | | Diet III | .03000 | .21746 | .895 | -.5021 | .5621 |
| | Diet II | Diet I | .15000 | .21746 | .516 | -.3821 | .6821 |
| | | Diet III | .18000 | .21746 | .439 | -.3521 | .7121 |
| | Diet III | Diet I | -.03000 | .21746 | .895 | -.5621 | .5021 |
| | | Diet II | -.18000 | .21746 | .439 | -.7121 | .3521 |

**Table 9.44** Homogenous of PER

| | Treatment | N | Subset for alpha = 0.05 |
|---|---|---|---|
| | | | 1 |
| Tukey B[a] | Diet III | 3 | .8633 |
| | Diet I | 3 | .8933 |
| | Diet II | 3 | 1.0433 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

**Table 9.45** Descriptive statistics of feed efficiency

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Diet I | 3 | 28.68 | 3.62641 | 2.093 | 19.6748 | 37.6918 | 25.00 | 32.25 |
| Diet II | 3 | 36.81 | 14.1134 | 8.148 | 1.7536 | 71.8731 | 24.26 | 52.09 |
| Diet III | 3 | 28.20 | 6.51383 | 3.760 | 12.0187 | 44.3813 | 21.10 | 33.90 |
| Total | 9 | 31.23 | 9.01430 | 3.004 | 24.3032 | 38.1612 | 21.10 | 52.09 |

**Table 9.46** ANOVA of feed efficiency

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 140.520 | 2 | 70.260 | .827 | .482 |
| Within Groups | 509.541 | 6 | 84.923 | | |
| Total | 650.061 | 8 | | | |

**Table 9.47** Multiple Comparisons of feed efficiency

| | (I) Treatment | (J) Treatment | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| LSD | Diet I | Diet II | -8.13000 | 7.52434 | .321 | -26.5414 | 10.2814 |
| | | Diet III | .48333 | 7.52434 | .951 | -17.9281 | 18.8947 |
| | Diet II | Diet I | 8.13000 | 7.52434 | .321 | -10.2814 | 26.5414 |
| | | Diet III | 8.61333 | 7.52434 | .296 | -9.7981 | 27.0247 |
| | Diet III | Diet I | -.48333 | 7.52434 | .951 | -18.8947 | 17.9281 |
| | | Diet II | -8.61333 | 7.52434 | .296 | -27.0247 | 9.7981 |

**Table 9.48** Homogenous of feed efficiency

| | Treatment | N | Subset for alpha = 0.05 |
|---|---|---|---|
| | | | 1 |
| Tukey B[a] | Diet III | 3 | 28.2000 |
| | Diet I | 3 | 28.6833 |
| | Diet II | 3 | 36.8133 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.