Effects of some supplemented diets on the growth performance and nutrient contents of Gulsha, *Mystuscavasius* (Hamilton, 1822) in Bangladesh

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Declaration

I hereby declare that the dissertation entitled "Effects of some supplemented diets on the growth performance and nutrient contents ofGulsha,*Mystuscavasius*(Hamilton, 1822)in Bangladesh" submitted to the Department of Fisheries, University of Dhaka for the degree of Master of Science (MS) is based on self-investigation carried out under the supervisions of Dr. KanizFatema, Professor and Chairperson, Department of Fisheries, University of Dhaka, Dhaka- 1000, Bangladesh and Nahid Sultana ,Scientific Officer, Zoology Section, Biological Research Division, BCSIR Laboratories, Dhaka.

I also declare that this or any part of this work has not been submitted for any other degree anywhere. All sources of knowledge used have been duly acknowledged.

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Certificate

We certify that the research work embodied in this thesis entitled “Effects of some supplemented diets on the Growth performance and nutrient contents ofGulsha,Mystuscavasius(Hamilton, 1822) in Bangladesh” submitted by Md. Farukul Islam, roll number: 801, session: 2015-16, registration number: 2011-912-770 has been carried out under our supervision.

This is further to certify that it is an original work and suitable for the partial fulfillment of the degree of Master of Science (MS) in Fisheries from the Department of Fisheries, University of Dhaka.

We wish every success in his life.

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February,2017 TheAuthor

Abstract

Gulsha (*Mystuscavasius*) is the most common aquaculture fish species in Bangladesh. The fish is consumed by the people for their rich protein and higher nutrient content. The objective of the present study was to estimate the growth performance and nutrient content of gulsha (*Mystuscavasius*). To estimate the effects of different diets on growth performance and nutrient content of gulsha .An experiment was conducted for fourth month in concrete small pond located in BCSIR (Bangladesh Council of Scientific and Industrial Research), Dhaka. This study was conducted during the month of June, 2016 to February, 2017. The nutrient content analysiswas done in Soil and Environmental section,Biological Research Division, BCSIR, Dhaka. The growth performance of gulshawas found to be maximum with rice bran +spirulinaand lowest with commercial feed. It can be stated that the growth performance of gulshacould be improved by rice bran + spirulina. The findings of the investigation showed that Condition factor found in treatment T1 was higher than of at fourth month culture period. Specific growth rate of treatment T1 with rice bran + spirulinawas higher than treatment T2 with commercial feed at fourth month of rearing. Treatment 1 has the best proximate composition and nutrient content for human nutritional interest and thus it was found to be better option to produce healthy fish for human consumption. The findings of this research suggest that raising gulsha with rice bran and Spirulina in ponds could help in better growth performance and improve nutrient content of gulsha.

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List of abbreviations and symbols

ADG Average daily gain

BCSIR Bangladesh Council of Scientific and Industrial Research

BW Body weight

DoFDepartment of Fisheries

WHOWorld Health Organization

FCR Feed conversion ratio

FAO Food Agricultural Organization

Icddrb International Centre for Diarrhoeal Disease Research, Bangladesh

INFOODS International Network of Food Data Systems

AOAC Analysis of the Association of official Analytical Chemists

SEM Standard error of mean

H_2SO_4Sulphuric Acid

C_2H_5OH Ethanol

NaoHSodium hydroxide

K Condition factor

SGR Specific Growth rate

TL Total length

± Plus -minus

% percentage

gGram

p^HConcentration of hydrogen ion

Chapter 1

Introduction

Introduction

1.1 Background of the study

Bangladesh has the third largest aquatic fish biodiversity in Asia, after China and India, with about 800 species in fresh, brackish and marine waters (Hussain and Mazid, 2001) through which a large number of big rivers, the Meghna, the Brahmaputra, the Surma, the Karnaphuli and the Padma flow. A good number of small rivers also flow through this country. The fisheries sector includes open water bodies such as rivers, canals, lakes, etc. and closed water bodies such as ponds and flood-control polders.

Small indigenous species (SIS) of fish in our country is considered to those which grow to a maximum length of about 25 cm or 9 inch at maturity (Hossain *et al.* 1999). In the past, various SIS of fish were abundant in the rivers, *beels*, canals, streams and ponds. These are usually caught by the subsistence fisherman that provided a large portion of the animal protein intake of them. Fish also contribute about 58% to the nation's animal protein intake. The poor people of the country, those who have no income generating option in the wet season, usually depend on the fisheries and other resources.They catch fishes from nearby waters for their own consumption and sell the surplus to the market, which helps them to survive during the lean period. SIS and self-recruiting species (SRS) is the main, indeed the only source of the protein andmost of the fat soluble vitamins for the rural people who represent more than80% of the total population (Hossain *et al.*2002)

SIS and self-recruiting species (SRS) is the main, indeed the only source of the protein and most of the fat soluble vitamins for the rural people who represent more than 80% of the total population (Hossain *et al.*, 2002). Many of these valuable indigenous species have been threatened or endangered. Since 1970s the abundance of small indigenous fish species has been declining.over 150 species have been classified as SIS (Amin *et al.*2010).

The growth in biomass of fish in intensive and semi-intensive culture system depends on various factors notably feeding regimes. Fish growth at different stages is largely governed bythekindof feeding, feeding frequency, feed intakeandits ability to absorb the nutrient.One problem confronting by fish culturists is to obtain a balance between rapid fish growth and optimum use of the supplied feed. Among these, feeding frequency is an important factor for the survival and growth of fish at the early stage (Hung et al.2002).

Fish is the major protein sources in the diet of the Bangladeshi people. Fish contributes about 60% of the available protein in the diet and the rest 40% protein comes from livestock and poultry. It indicates the importance of fish in contributing to the level of nutrition of the people of Bangladesh (DOF, 1998; FAO, 1992). In spite of having large fisheries resources, Bangladesh is facing an acute malnutrition problem due to the shortage of animal protein supply in our diet. The present per capita fish consumption is only about 21 g/day (DOF, 1995) whereas 38 g/day is the required amount. This is due to rapid human population growth and decline of catch from inland open water area. There is a little prospect for additional yield from open water capture fisheries. Only the culture fisheries, seems to be dependable means of achieving increased yield. In order to meet the need for vast increase in animal protein supplies, animal breeders introduced new high-yielding varieties of live stocks, aqua culturist introducing new methods of fin fish, shell fish and crustacean culture to enable animal protein production to keep pace with the increase in population.

1.2 Nutrients in fish

Fish makes a vital contribution to the survival and health of a significant portion of the World's population. Fish is especially important in the developing world. In some of Asia's poorest countries (Bangladesh, Cambodia) people derive as much as 75% of their daily protein from fish. Often referred to as "rich food for poor people," fish provides essential nourishment, especially quality proteins and fats (macronutrients), vitamins and minerals (micronutrients). Second, for those involved in fisheries, aquaculture and fish trade, fish is a source of income which can be used to purchase other additional food items. Though this brief emphasizes the former, fish contributes to food security as an important accompaniment to rice based diets in Bangladesh.

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 Proteins

Proteins are important for growth and development of the body, maintenance and repairing of worn out tissues and for production of enzymes and hormones required for many body processes. The importance of fish in providing easily digested protein of high biological value is well documented. In the past this has served as a justification for promoting fisheries and aquaculture activities in several countries. On a fresh weight basis fish contains a good quantity of protein, about 18 - 20%, and contains all the eight essential amino acids including the sulphurcontaining lysine, methionine, and cysteine. As most maize based diets lack these compounds, ruralhouseholds in Africa dependent on maize greatly benefit by increasing their fish consumption. Fish also complements cassava based diets which are generally low in protein.

1.2.3 Fats

The fat content of fish varies depending on the species as well as the season but, in general, fish have less fat than red meats. The fat content ranges from 0.2% to 25%. However, fats from fatty fish species contain the polyunsaturated fatty acids (PUFAs) namely EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) (omega 3 fatty acids) which are essential for proper growth of children and are not associated with the occurrence of cardiovascular diseases such as coronary heart disease. In other studies, omega 3 fatty acids have also been associated with reduced risk of preterm delivery and low birth weight. The fat also contributes to energy supplies and assists in the proper absorption of fat soluble vitamins namely A, D, E, and K.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).

1.3Minerals

The minerals present in fish include iron, calcium, zinc, iodine (from marine fish), phosphorus, selenium and fluorine. Fish are the important sources of essential minerals such as zinc, copper, iron, magnesium, sodium, calcium, phosphorus, sodium and potassium (Nettleton, 1985; Banu et al. 1985; Nurullah et al. 2003). These minerals are highly 'bioavailable' meaning that they are easily absorbed by the body. Iron is important in the synthesis of hemoglobin in red blood cells which is important for transporting oxygen to all parts of the body. Iron deficiency is associated with anemia, impaired brain function and in infants is associated with poor learning ability and poor behavior. Due to its role in the immune system, its deficiency may also be associated with increased risk of infection.

1.3.1 Calcium

Fish, especially the small fish that are consumed along with bone, are excellent sources of calcium (Nettleton, 1985). Calcium is a relatively inert mineral element elements usually associated with bones and tooth formation (Whedon, l982). Calcium plays an important role in the formation and maintenance of bone and tooth structure (Anderson et al. 1982). Calcium is essential for the normal growth, bone formation, blood coagulation, milk formation, vitamin D absorption etc. The body of an adult contains about 1200g of Ca, at least 99% of this amount is present in the skeleton, where calcium salts help in a cellular matrix to provide the hard structure of the bones and teeth (Stanley Davidson et al.1975).

Calcium is required for strong bones (formation and mineralization) and for the normal functioning of muscles and the nervous system. It is also important in the blood clotting process. Vitamin D is required for its proper absorption. The intake of calcium, phosphorus and fluorine is higher when small fish are eaten with their bones rather than when the fish bones are discarded. Deficiency of calcium may be associated with rickets in young children and osteomalacia (softening of bones) in adults and older people. Fluorine is also important for strong bones and teeth.

1.3.2 Potassium

All fish-including lean, fatty, farmed, wild-caught, freshwater and saltwater types contain potassium. The amount of potassium get from fish depends entirely on its variety, but most fish are good sources of the nutrient, meaning they deliver at least 10 percent of the daily value for potassium per serving. Small indigenous species stand out as rich source of potassium. Potassium is essential for normal body function. Potassium is necessary for muscle function, the transmission of nerve impulses and for carbohydrate and protein metabolism.Dietary deficiency of potassium as it is present in a large number of foods. Deficiency can occur when there are large losses resulting from excessive urination or from prolonged vomiting and diarrhea.Potassium will also affect blood pressure under certain circumstances. If you are prone to high blood pressure it would be beneficial to decrease sodium intake and increase your dietary intake of potassium, by eating more foods that have low amounts of sodium and significant amounts of potassium, such as fruits and vegetables. The use of potassium salts as substitutes for common salt (sodium chloride) is potentially dangerous, and overuse can be fatal.

1.3.3 Phosphorus

Phosphorus is an important constituent in fish body tissue. The total amount constitutes about one per cent of the body weight. The amount of phosphorus in the body is exceeded only by calcium. In bones the proportion of calcium to phosphorus is much higher than that of calcium. Most of this phosphorus is in organic combinations.Phosphorus is one mineral that performs a number of important functions. It combines with calcium to form a relatively insoluble compound calcium phosphate, which gives strength and rigidity to bones and teeth. Phosphorus like calcium is needed not only for the growth of skeleton but also for its maintenance. The utilization of many nutrients that enter the body involves the formation and degradation of phosphorus containing compounds. The phosphorus-containing lipo-protein facilitates the transport of fats in the circulation. A series of phosphorus compound are formed in the utilization of carbohydrates in the body. It is vital to the fundamental process of metabolism in the body. Phosphorus is a constituent of the nucleoproteins the substances that control heredity.

1.3.4 Magnesium

Magnesium represents another type of essential mineral found in fish. Magnesium in the body works together with calcium to form the minerals that make up bones. In addition, magnesium aids in proper muscle functioning, maintaining the health of heart, and may prevent the development of type 2 diabetes, according to the University of Maryland Medical Center. Fish rich in magnesium include cod, salmon, halibut and mackerel, so including these foods into your diet may prove beneficial for your health

1.3.5 Iron

Iron, especialiy the dark fleshed fish are good source of iron.Iron is an important mineral that is involved in various bodily functions, including the transport of oxygen in the blood.

Iron is lost from the body through shedding intestinal cells, sweat and blood loss. About one third of the world's population is iron deficient. Menstruating women are at greater risk than men and postmenopausal women for iron deficiency. Bioavailability as an animal-source food (FAO and WHO, 2004). This may have important policy implications given the public health significance of iron deficiency in Bangladesh, with prevalence recently estimated at 10.7% in preschool aged children and 7.1% in adult women (ICDDRB , 2013), and the well documented negative effects of deficiency on physical and cognitive development, pregnancy outcomes, morbidity and mortality.

1.3.6 Manganese

Manganese is a trace mineral that is present in tiny amounts in fish body. It is found mostly in bones, the liver, kidneys, and pancreas. Manganese helps the body form connective tissue, bones, blood clotting factors, and sex hormones. It also plays a tale in fat and carbohydrate metabolism, calcium absorption, and blood sugar regulation. Manganese is also necessary for normal brain and nerve function. Manganese is a component of the antioxidant enzyme superoxide dismutase (SOD), which helps fight free radicals. Antioxidants, such as SOD can help neutralize free radicals and reduce or even help prevent some of the damage they cause. Low levels of manganese in the body can contribute to infertility, bone malformation, weakness and seizures.

1.4Biology ofGulsha (*Mystuscavasius*)

The biology of a freshwater fish, *M. cavasius* with particular reference to its length frequency, breeding and food has been described. Length frequency distribution gave an indication of 4 modes during the quarter, July–September.Both the sexes attain maturity when they are approximately 10 cm long. Females grow larger than the males and are more abundant in the population. The spawning of this fish seems to take place during August and September. Maturing ovaries of females show only one batch of eggs which is probably shed in a single spawning act. The condition factor of the fish has no correlation either with the seasonal changes in maturity or with the feeding rhythm. The fish has an omnivorous habit and consumes all types of food available in the habitat. The feeding is high during the monsoonand winter months and low during the summer months.

1.4.1 Etymology

The generic name is probably derived from the Latin mystax, meaning moustache,in reference to the long barbels. It was a very old genus that has included many catfishes from throughout the world at one time or another. The specific epithet comes from the local name of the fish (kavasi).

1.4.2Size

Maximum lenth recorded 40cm (Talwar and jhingran,1991) and recorded weight of 10 kg in india.

1.4.3 Identification

Fishes of the genus *Mystus*Scopoli are small to medium-sized bagride catfishes occurring in South Asia. Roberts, (1994) recognized *Mystus* to have an elongate cranial fontanel reaching up to the base of the occipital process, long maxillary barbel, very long adipose fin, 11–30 gill rakers on the first gill arch and 37–46 total vertebrae, about equally divided between abdominal and caudal regions. The author included only eight species under the genus. Mo, (1991) characterized the genus to have a thin needle-like first infraorbital, twisted and thickened metapterygoid loosely attached to the quadrate by means of ligament or a small extent of cartilage. Jayaram andSanyal (2003) and Ferraris, (2007) respectively listed 44 and 33 species of *Mystus* as valid.Maxillary barbels, in adults, extend posteriorly beyond the caudal fin base, but in young specimen, do not extend beyond the anal fin. Color is grayish with a more or less well-defined midlateral longitudinal stripe. A dark spot emphasized by a white or pale area along its ventral margin located just anterior to the first dorsal spine.

1.4.4 Sexual dimorphism

Bhatt,(1971)has documented that male and female of *Mystusvittatus*can be distinguished externally with the presence ofgenital papilla in males which is absent in female fishes.

The papilla becomes very prominent during the breeding season.Females are comparatively larger in size than the males.

1.4.5Distribution

Lowland rivers in most major basins of the Indian subcontinent (Pakistan, Nepal, India, Sri Lanka, Bangladesh and Myanmar), including but not limited to the Indus, Brahmaputra-Ganges, Krishna, Cauvery, Irrawaddy, Salween and Tenasserim. Reports of this species from the Chao Phraya and Mekong basins, Malaysia, and Indonesia are basedon misidentifications of *M. albolineatus* or *M. singaringan* occurs in Thailand, but only in the Salween basin.

1.4.6 Feeding

Gulsha fish adapts to a wide variety of frozen and prepared food in the aquarium.

1.5 Review of Literature

Ahmed et al.(2012) have studied on the influence of commercial feeds on growth performance of *Labeorohita*(rohu) fingerlings was investigated in 42 days culture trials. Twenty fingerlings were maintained in well aerated 95 L glass tanks in triplicate. Three different commercial feeds, rice bran as control (T 1); Miracle (T 2) and Tokyo (T 3) were purchased from the local market. Fishwere fed @ of 3 % of their wet body weight twice a day at 12 hours interval for 6 weeks. Fish growth parameters (lengthand weight) were taken weekly. The amount of feed was adjusted according to the average increase in biomass of fish in each tank of all treatments. There was a significant increase (P<0.05) in average wet body weight, FCR and gross fish production of fish fed with Tokyo (T 3) when compared with Miracle (T 2) and rice bran (T 1). These findings suggest that Tokyo appears to be sufficient for obtaining optimum growth in rohu.

Tiamiyu et al. (2013)studied the utilization of agama lizard meat meal (*Agama agama*) as dietary protein source in the diets of *Clariasgariepinus*fingerlings (MW±SE = 33.48 g±0.13). The fingerlings were fed four Isonitrogenous and Isocaloric diets containing blends of agama lizard meal in percentages of 0 (Diet I), 10 (Diet II), 20 (Diet III) and 30% (Diet IV) respectively for 8 weeks at 5% body weight. Mean weight gain (MWG) ranged from 22.85g (Diet I) to 42.80g (Diet III) and Specific Growth Rate (SGR) ranged from 0.93% per day (Diet 1) to 1.46% per day (Diet III). Feed Conversion Ratio (FCR) was between 2.61 (Diet III) and 2.96 (Diet IV). There was however no significant differences (p>0.05) in these parameters for all the test diets. Proximate composition of carcass shows significant differences ($p<0.05$) in moisture and ash contents with no significant differences observed for all the other proximate composition parameters. From the foregoing, agama lizard meal can be included at any level but it is recommended that 20% inclusion level be used.

Muralisankar et al.(2014) made observation on the suitability of three vegetable oils (sunflower oil, coconut oil and castor oil) as an alternative dietary lipid source for cod liver oil to culture Macrobrachiumrosenbergii post larvae (PLs). The experimental feeds contained 40% protein with separately incorporated three vegetable oils and cod liver oil. The feeding trial was conducted on M. rosenbergii PL for 60 days. In the final day of the experimental period, the survival rate, weight gain, length gain, specific growth rate and protein efficiency ratio of prawns showed no significance ($P >0.05$) between sunflower oil and cod liver oil incorporated feed fed groups. The coconut oil and castor oil showed lower performance when compared with cod liver oil. The present result showed biochemical accumulation of total protein, amino acids, carbohydrate and lipid in experimental groups.. The present results revealed that the sunflower oil was on par with cod liver oil. Hence, the sunflower oil can be incorporated in feed formulation for M. rosenbergii PL culture. It can be concluded that the coconut oil and castor oil are not ideal vegetable lipid source with these concentrations which produced lower performance in survival, growth and biochemical compositions of *M. rosenbergii* PL.

Ezechi et al. (2007) State that the growth response of the developmental stages of hybrid cat fish resulting from the crossing of *Heterobranchusbidorsalis* (male) and *Clariasgariepinus* (female), fed with different feed rations. Hatchlings of the hybrid catfish were cultured in plastic aquaria set up at the fish hatchery complex, NnamdiAzikiwe University, Awka. The hatchlings were fed and observed for 91 days. Different food items such as artificially compounded feed, zooplankton / live organisms *Moinasp* and *Brachionussp*, and a mixture of zooplankton (*Moinasp* and *Brachionussp*) and artificially compounded feed were administered. The mixture gave the best result with a mean weight 0.792g ± 2.73g for lowed by those fed *Moinasp* (1.93g ± 0.27g) and *Brachionussp* (1.80g ± 0.32) respectively. Hatchlings fed only artificially compounded feed exhibited the poorest growth.

Bakhtiyarl et al. (2011) have conducted a study on the the effect of different live feed and dry feed on growth and survival of *Labeorohita*at a stocking density of 20 larvae per trough having 10 L of water. The increase in weights and lengths were used as measures of growth. The duration of the experiment was 30 days. Seven dietary treatments were tested viz. Artificial diet with 45% protein (LFr1), rotifers like *Asplanchna* and *Brachionus*(LFr2), wild zooplankton (LFr3), bioenriched zooplankton (raised on Chlorella, cod liver oil and vitamin C) (LFr4), Artemia (LFr5), Chironomous (LFr6) and Oligochaetes (LFr7). Among the different treatments, larvae fed with diet LFr4 resulted in the maximum body weight gain (BWG) (13.18 ± 0.13 mg) which was significantly (P < 0.05) higher than all the treatments which followed the order ofpreference as: LFr5 (12.31 ± 0.10 mg), LFr3 (9.75 ± 0.08 mg), LFr2 (7.85 ± 0.10 mg), LFr7 (6.49 ± 0.15 mg), LFr6 (5.66 ± 0.13 mg) and LFr1 (4.99 ± 0.14 mg). The diet LFr4 recorded maximum survivability (92.0 ± 2.0%). All the values recorded for the BWG and survival were found to be significantly different (P <n 0.05) among others. Protein and lipid content of the prawn after the treatment was found to be highest.

Kalsoom et al.(2009**)** studied the Growth performance and feed conversion ratio (FCR) of wheat bran, rice broken and blood meal wereevaluated in hybrid fish *Catlacatla*x *Labeorohita*. Two replicates for each treatment (ingredient) werefollowed. The feed was supplied at the rate of 4% of wet body weight of fish fingerlings twice a day. The fishgained higher body weight (1.60 ± 0.14g) on wheat bran, followed by rice broken (1.51 ± 0.07g) and bloodmeal (1.24 ± 0.09g). The body weight of fish on wheat bran and rice broken was significantly higher (p<0.05)than those fed blood meal, while the difference between the former two groups was non significant. The totallength obtained by the fish averaged 5.06 ± 0.03, 5.05 ± 0.09 and 4.96 ± 0.32 cm on wheat bran, rice brokenand blood meal, respectively, the difference being non significant. Feed conversion ratio (FCR) was highest(poorest) on blood meal (1.68 ± 0.96), followed by that of rice broken (1.64 ± 0.64) and wheat bran (1.59 ± 0.71). The FCR value for wheat bran was significantly better than that for rice broken and blood meal. It was concluded that efficacy of wheat bran was better for the growth of hybrid fish and this supported the inclusion of this ingredient in the diet of hybrids.

Toppe et al. (2006) studied the chemical composition, content of minerals and the profiles of amino acids and fatty acids were analyzed in fish bones from eight different species of fish. Fish bones varied significantly in chemical composition. The main difference was lipid content ranging from 23 g/kg in cod (*Gadusmorhua*) to 509 g/kg in mackerel (*Scomberscombrus*). In general fatty fish species showed higher lipid levels in the bones compared to lean fish species. Similarly, lower levels of protein and ash were observed in bones from fatty fish species. Protein levels differed from 363 g/kg lipid free dry matter (dm) to 568 g/kg lipid free dm with a concomitant inverse difference in ash content. Ash to protein ratio differed from 0.78 to 1.71 with the lowest level in fish that naturally have highest swimming and physical activity. Saithe (*Pollachiusvirens*) and salmon (*Salmosalar*) were found to be significantly different in the levels of lipid, protein and ash, and ash/protein ratio in the bones. Only small differences were observed in the level of amino acids although species specific differences were observed. The levels of Ca and P in lipid free fish boneswere about the same in all species analyzed. Fatty acid profile differed in relation to total lipid levels in the fish bones, but some minor differences between fish species were observed.

Islam and Joadder, (2005) recorded the proximate composition and seasonal variation in proteins, lipid, ash and related substances of freshwater gobi. The results indicated that the proximate composition of the lish depends on season but also to a great extent in reaction to sex and reproductive cycle. The results also showed that *G. giuris* is a 'low fat-high protein'fish. The highest amount was moisture and lipid than those of the male fish (female>male) except protein and carbohydrate in male fish (male>female).

Hartman and Margraf(2008) noted that relationships between the various body proximate components and dry matter content were examined for live species of fishes, representing anadromous, marine and freshwater species: chum salmon *Oncorhynchusketa*, Chinook salmon *Oncorhyncustshawytscha*, brook trout *Salvelinusfontinalis,* bluefish *Pomatomussaltatrix* and striped bass *Moronesaxatilzls*. The dry matter content or per cent dry mass of these fishes can be used to reliably predict the per cent composition of the other components. Therefore, with validation it is possible to estimate fat, protein and ash content of fishes from per cent dry mass information, reducing the need for costly and time-consuming laboratory proximate analysis. This approach coupled with new methods of non-lethal estimation of per cent dry mass, such as bioelectrical impedance analysis, can provide non-destructive measurements of proximate composition of fishes.

Hossain et al. (1999) worked withtwenty three small indigenous fish species (SIS) in the size range of 3-18 cmwere analysed for proximate composition and minerals (Ca and P) content toe1aluate their nutritive value. The moisture content of different species rangedbetween 71.00 and 81.94%. Small sized fishes showed highermoisture content. The muscle protein content among the species varied widely(16.16-22.28%). In general, the muscle protein content of fishes showed highervalue than the whole carcass protein content. The carcass lipid content variedbetween 1.87 and 9.55% and showed an inverse relationship with the moisturecontent. The gross energy content ranged from 19.51-27.30 Kg/g on dry matterbasis. In the present study, the calcium and phosphorus contents rangedbetween 0.85-3.20% and 1.01-3.29% respectively. The calcium and phosphorusratio (Ca/P) varied between 0.44 and 2.00. From the nutritional point of view, itshowed that the SIS are good source of protein and minerals especially calciumand phosphorus.

1.6 Justification of the study

In Bangladesh, fish is an irreplaceable animal-source food in the diet of millions, both in terms of quantity - accounting for approximately 60% of animal protein intake at 18.1 kg consumed per person per year - and frequency of consumption, far exceeding that of any other animal-source food (Belton et al. 2014). The country possesses diverse and abundant aquatic resources with 267 freshwater fish species (Thilsted, 2010), and an annual production of 3.1 million tonnes (Belton and Thilsted, 2014). While remaining largely successful in increasing supply to meet the demand of a growing population, the extent to which the growth of aquaculture has been able to mitigate reduction in dietary diversity and micronutrient intake from the diverse but waning capture fisheries sector, focusing on only a few select large species, is questionable (Belton et al. 2014).

Despite improvement in some food and nutrition security indicators (Jpgsph and HKI, 2012), malnutrition, largely caused by inadequate micronutrient intake, remains widespread with 41% of children under five years suffering from stunted growth (Niport et al. 2013). The fisheries and aquaculture sector has been recognized as a key resource in tackling food and nutrition security issues and features prominently in the national development agenda (Government of the PeoplesRepublic of Bangladesh, 2005a,b, 2006, 2011). Knowledge of the nutrient composition of important foods is an invaluable tool in understanding the links between foodproduction, access and nutrient intakes, and in devising policies and programmessuch as development of improved production technologies, to ensure that food supply optimally fulfils population nutrient requirements. However, despite the clear importance of fish in the Bangladeshi diet, existing composition data is not enough. The primary objective of this study was to document comprehensive nutrient composition profiles of Gulsha (Mystuscavasius).The Gulsha, *(Mystuscavasius)* naturally occurs in Bangladesh, India, Pakistan, Ceylon, Myanmar, Srilanka, Thailand, Cochin-China, Tongking, Souththern China, Philippines, Polynesia and Malaysia (Talwar and Jhingram, 1991).

The reasons behind the greater expansion of Gulshaculture in Bangladesh are: a) The fish can tolerate our environment easily b) It is cultivable under high stocking density c) It is cultivable in deep or shallow water; d) They can survive in low oxygen level; e) It can be marketed at live condition.

Considering all those prospects of demand, Gulsha was selected to conduct this research study. Many researchers have worked on the growth performance and nutrient contents of Gulsha (*Mystuscavasius)*collected from various sources all over the world and determined the growth performance and nutrient profile of Gulsha. But there is not any research regarding the growth performance and nutrient contents of Gulsha (*Mystuscavasius)* by culture with different supplemented diets.

1.7 Objectives of the study

The objectives of the study are:

- To estimate the nutrient and proximate composition of *Mystuscavasius.*
- To find out the growth performances by using different types of feed.
- To compare the growth and nutrient composition of *Mystuscavasius* reared in different tank with different kinds of feed.

Chapter 2

Materials and Methods

Chapter 2

Materials and Methods

2.1 Experimental fish

The selected specimen fish is *mystuscavasius*(Hamilton, 1822) locally knows as Gulsha.

Scientific classification

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Siluriformes

Family: Bagridae

Plate 1: *MystusCavasius*

Genus:*Mystus*

Speces: *M. cavasius*

2.2 Collection of sample

Fry of gulsa (*Mystuscavasius*) were collected from Adorsho Hatchery, Treshal,Mymenshings. Live fish were collected in June 2016 and carried in oxygenated bags with sample water.

2.3 Study place:

The experiment was carried out in the wet laboratory at Zoology Section, Biological Research Division, Bangladesh council of Scientific and Industrial Research (BCSIR).

2.4 Experimental Design

Ponds are situated near to the office building where four small concrete ponds are used for the experiment. Each of the small ponds is ten feet in length and six feet in width and depth is about two and one-fourth feet. All the small ponds are filled up with tap water and labeled according to experimental design. Each of the ponds is filled up with tap water in the quantity of about 650 liters. In total 1060 fingerlings of gulshaare stocked in all of the small ponds and each of the small ponds containing about 265 fingerlings. After bringing the fry first acclimatized for sometimes in clean water. Then fish was released slowly into the pond. No feed give on first day. From the second day of stocking, feed given at a rate of 10% of body weight of the fish. Feed are supplied regularly on the basis of body weight of the fish. Feed quantity increment in every subsequent five month is done by 8%, 7% and 6% respectively. Treatment-1 was feeding with the rice bran and spirulina, Treatment-2 was feeding with the commercial feed.

Plate 2: An experimental set up of aquaculture small concrete ponds for Gulsa culture in BCSIR, Dhaka.

2.5Materials and Instruments

a) P^H meter

b) Beaker

c) DO meter

d)Thermometer

e) Digital electric balance

f) Conductivity meter

g) Bowls

h) Measuring steel scale

i) Petridish

J) Filter paper

k) Small ponds

l) Live spirulina

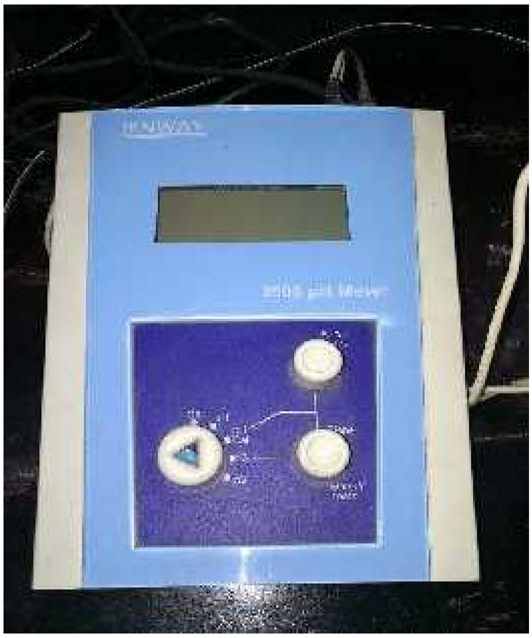
m) Conical Flask

n) Gulshafry

o) Oven

q) Rice bran

q) Commercial feed (Saudi bangla)



A: Electric balance

B: P^H meter

C: Saudi bangla feed

D: Rice bran

Plate 3: Showing the Electric balance (A) digital p^H meter (C), Saudi bangla feed (C) and Rice bran (D).

2.6 Measurement of physio-chemical parameters

Physio - chemical parameters were recorded at every 7 days interval.

Water temperature

Water temperature (°C) from each tank was recorded by using ordinary thermometer.

Dissolve oxygen (mg/l)

Dissolved oxygen of the tank water was recorded in mg/**l** with the help of a dissolved oxygen meter (Model Oxi 3150i, Germany) from the laboratory at Zoology Section, Biological Research Division, Bangladesh council of Scientific and Industrial Research (BCSIR)

P^H

$P^H$was measured by digital pH meter (Jenway, Model 3020,United Kingdom).

2.7 Measurement of growth performance of fish

Fish sample preparation

Growth of the sample was measured after 30days interval. Lengths of the fingerlings were measured with measuring scale and weight with weighting balance. 20 samples were measured randomly at a time and measured. After recording length and weight the fingerlings were then slowly released into the labeled tank water. Feed were supplied as per experimental design. The tank was also cleaned per month and clean water was supplied in each of the tanks. Growth of the fingerlings was measured consequently five times at the interval of 30 days. Experiment was continuedupto 5 month.

2.7.1 Condition Factor (K %)

Condition factor is the volume of fishrelative to its length and taken to mean the well being of an individual fish in respect to its habitat where its lives so as to understand how well a given habitat supports life of an individual in terms of nutritional requirements and other environmental conditions (Weatherly, 1987). The condition factor is used in studies of fisheries biology to indicate the well-being degree of fish in the environment in which they live and to verify if they make good use of the foods available (Weatherly, 1987). Sexual differences, age, changes in seasons gonad maturity levels, nutritional levels and maturity of fishes can influence the condition factor (K) value (Lagler, 1952.Kotos, 1990).

This is the factor through which condition of the fish is expressed in numerical terms i.e. degree of plumpness or flatness is usually estimated as the condition factor. It was calculated by the following formula as suggested by Hile (1936).

$$K = (W/ L^3) \times 100$$

Where,

K= Condition factor

W= Body weight in grams

L= Body length in centimeter

2.7.2 Average Daily Gain (ADG, g/day)

Average daily gain means the increase of body weight per day. It was calculated by the following formula as suggested by Jones (1967).

$$ADG = \frac{(\text{Mean final fish weight} - \text{Mean initial fish weight})}{\text{Time } (T2-T1)}$$

Where,

T2= Final time

T1= Initial time

2.7.3 Specific Growth Rate (SGR %)

SGR mean the percentage of body increase per day. Specific growth rate was calculated by the following formula as suggested by Hopkins (1992).

$$SGR\ (\%) = \frac{(\ln WT - \ln WI)}{T - t} \times 100$$

Where,

In Wt-= Natural log of weight at time T

In W1=Natural log of initial weight

T= Final time

t = Initial time.

2.7.4 Feed Conversion Ratio (FCR)

The FCR is simply the amount of feed it takes to grow a kilogram of fish. Feed conversion ratio (FCR) was determined by the following formula as suggested by payne (1987).

$$FCR = \frac{\text{Feed (g) consumed by the fish}}{\text{Weight (g) gain of the fish } (W2 - W1)}$$

Where,

W2= Final weight

W1=Initial weight

2.7.5 Estimation of survival rate

Survivality of*Mystuscavasius*fish for each treatment was estimated on the basis of number of fish gathered at the end ofthe 1st month, 2nd month, 3rd month and 4thmonths of rearing of the fishes in the experimental ponds. .Survivalityof *Mystuscavasius*was calculated by counting the actual number of fish survived, divided by the initial number Stocked and multiplying by 100, and thus

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

2.8 Biochemical Analysis of Fish:

Sample Preparation:

After five month of rearing the sample were collected, measured and weighted. Then the samples were taken for laboratory analysis to estimate the whole body percentages of water, protein, fat ash and minerals. 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 wascollected as completely as possible. Wet weight was recorded and dried in an oven at 100°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.

2.8.1 Estimation of Moisture

Principle:

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

Apparatus:

1. Oven (100-105°C)

2. Dishes, porcelain crucible, glass Petri dish or watch glass.

3. Dessicators

4. Electric balance

Procedure:

Moisture was determined according to the AOAC (1984) method as describe by Gopalan(1976). At first, weight of the crucible was made constant and 0.8-1.0g fresh sample was taken in crucible. The crucible was then placed in an oven at loo-105°C for 5-8 hours. Then the crucible-containing sample was weighed in an electric balance (Mettler H31 AR Gallenkap, Switzerland) and heated in an oven until constant weight was found each time. The crucible was cooled in a dessicator before weighing. Then it was determined from the following formula:

Calculation:

Moisture (%) = $\frac{Initial\ weight(g) - Final\ weight(g)}{Weight\ of\ the\ sample}$ x 100

Where,

Initial weight= sample weight + crucible weight (before heating)

Final weight= sample weight + crucible weight (after heating

Estimation of Moisture Factor:

Moisture Factor = $\frac{(100 - Moisture\ content)}{100}$

2.8.2 Estimation of Ash

Principle:

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

Materials and equipment:

i) Porcelain crucible

ii) Crucible furanace

iii) Dryer

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 6OO-700°C till the residue become white. The crucibles were cooled in a dessicator and weighed.

Calculation:

% of Ash = $\frac{Final\ weight(g) - crucible\ weight(g)}{weight\ of\ sample}$ X 100 X moisture factor

2.8.3 Estimation of Fat:

Principle:

Fat was determined according to the modified method described by Folcheta1. (1957). Anhydrous chloroform methanol mixture was used to extract the fat from the dry sample.

Reagents:

Chloroform-methanol mixture (2:1): Chloroform was mixed with methanol in the ratio of 2: l.

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 40 ml of chloroform: methanol (2:1) solution was added. The sample was allowed to stand for overnight and was filtered.

Filter 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 (20 ml) was added. The separating funnel was vigorously shaken for proper mixing and allowed to stand for 4-6 hours. The lower phase was 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 a 25 ml beaker and allowed to air dry and then dried in an oven at 105°C for the determination of total lipid was determined gravimetrically. Fat content was calculated by followed formula.

Calculation:

(%) of fat = $\frac{weight\ of\ extract(g)}{sample\ weight(g)}$ x 100 x moisture factor

2.8.4 Estimation of Protein:

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 taken into consideration in the present investigation. The estimation of nitrogen was made by modified Kjeldahl method (1990.988.05 in ofiicial method of Analysis of the Association of ofiicial Analytical Chemists. Fifteenth edition.pg.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 [$(NH4)_2SO_4$]. 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%.

2. Concentrated Sulphuric Acid (H_2SO_4): Concentrated 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 upto 100ml.

4. Receiver Solution:

Ten gmof boric acid was added in 500ml de-ionized water in a one liter volumetric flask, heated it on a medium setting until the boric acid was dissolved. An amount of 002g bromocresol green was dissolved with 4ml ethanol (C_2H_5OH) in another beaker. An amount of 0.014g Methyl red was dissolved with 4ml ethanol (C_2H_5OH) in another beaker. Bromocresolgreen and Methyl red solution was then transferred into that volumetric flask. 0.5ml 1N NaOH was also added and the total volume was made 1000ml with de- ionized

5. N/70 H_2SO_4

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 in weighing paper and measured accurately. This sample was poured into a 500ml clean and dry Kjeldahl flask, to which 10g of digestion mixture and 12-15 ml of concentrated H_2SO_4 added. To avoid frothing and bumping, 2-5 glass beeds were placed inside the flask. A blank was carried with all reagents except sample material for the comparison. The flasks were then heated in a Fume hood Digestion Chamber at 400°C until the Solution became colorless.

2.Distillation

The distilling set of Kjeldahl apparatus was thoroughly washed with distilled water before starting the distillation. In a measuring cylinder 60ml of 40% NaOH was taken and it was carefully poured down the side of the Kjeldahlflask.The mouth of the flask was closed with stopper containing connective tube, which was ultimately connected to the ammonia-receiving flask containing 25ml 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:

$$\% \text{ of Nitrogen} = \frac{(S-B)\times A\times C\times Factor}{Weight\ of\ sample} X\ 100$$

Where,

S=Titration reading for sample

B= Titration reading for blank

A= Strength of N/70 H280

C= Digest taken for distillation

Factor= 0.0002 (1ml of N/70 H_2SO_4)= 0.0002 g N)

The total N content was then converted into crude by multiplying with the factor 6.25

%of Crude Protein = NX 6.25

A: Digestion chamber

B: Distillation chamber

Plate 4: Digestion chamber(A) and Distillation chamber (B) for protein estimation.

2.9Fish sample preparation for minerals analysis

Four different fish samples were collected directly from the experimental pond were kept sealed in resealable polypropylene bags. The samples were kept at room temperature .The fish was then meshed and 5g of meshed wet sample was taken into an oven and dried at 60°Cfor 48 hours. After the sample was completely dried then it was grinded. 1g of dry sample was taken into a beaker and 10 ml of nitric acid was mixed with the sample. The mixer was then put into Fume Chamber and digested at 80ºC until the solution become clear and added 5ml perchloric acid. Then the solution was diluted to 50 ml with deionized water and was filtered and collected into clean and sterilized plastic bottles for further analysis of minerals in the sample (Huq and Alam, 2005). The process of fish sample preparation is shown in the following steps:

3-5 gram of fish sample was kept in an oven at 60°C   for 48 hour

⇩

Oven dried samples were homogenized using blender

⇩

1g of the dried samples were taken and 10ml of nitric acid was mixed with the samples

⇩

The mixer was digested at 80℃ in Fume Chamber

⇩

Again added 5ml of percholric acid and the sample was digested at 80°Cin Fume Chamber

⇩

Samples were diluted to 50ml deionized water

⇩

The solution was filtered and collected into clean and sterized bottles

2.9.1 Laboratory analysis

The minerals Ca, Mg, Fe, Mn and cu were analyzed by using Atomic Absorption Spectrometer (Model No.: AA-7000, Shimadzu). The instrument was calibrated with chemical standard solutions prepared from commercially available chemicals and all reagents used for the preparation of samples were of analytical grade and deionized water was used throughout the study. The Phosphorus in the sample were measured with UV/VIS Spectrophotometry. The potassium in the sample were measured withFlame photometer.

Plate 5: Atomic Absorption Spectrometer for minerals analysis

A: UV/VIS Spectrophotometry for phosphorus analysis

B: Flame photometer for potassium analysis

Plate 6: UV/VIS Spectrophotometry for phosphorus analysis(A) and Flame photometer for potassium analysis(B).

Chapter 3

Chapter 3
Results

3.1 Effects of some supplemented diets on the growth performance of *Mystuscavasius*(Hamilton, 1822)

Physio-chemical parameters are important factor to enhance the growth of fish in aquaculture. In this investigation the average temperature of water is (28.75±.104)°C, the average of dissolved oxygen is (8.1562±.147) mg/l, the range of p^H is(8.2313±.0574).

3.1.1 Condition Factor (K, %)

Condition factor (K) of Gulsha fingerlings was the highest (K= 0.93810) in treatment T1 at fourth month and the lowest (K = 0.6063) value was found in treatment T2 at the Zero Month (Fig.1).

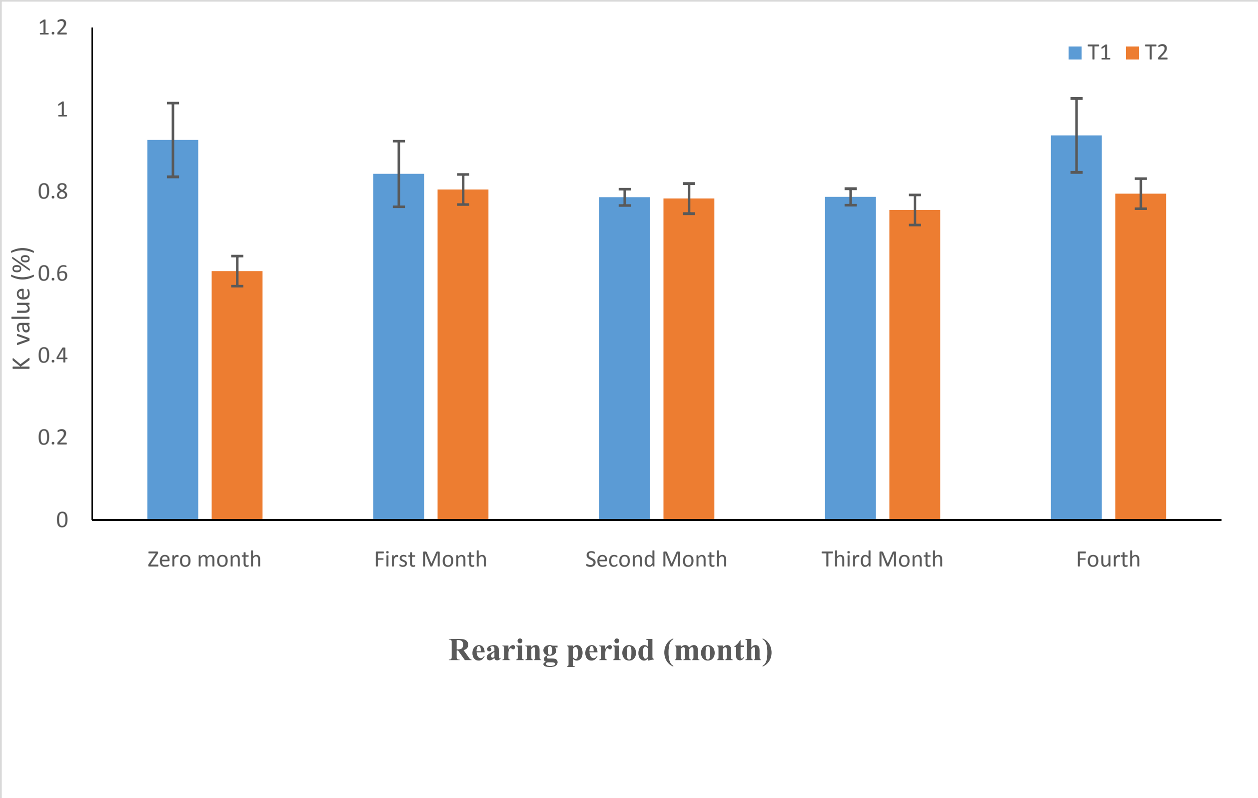


Figure1: Condition Factor, K(%),(Mean ± SEM) of Gulsha fish culture for fourth month with two treatments.

3.1.2 Average Daily Gain (ADG)

Average daily gain (ADG) 0f Gulsha fingerling was observed the highest value (ADG= 0.5272) in treatment T1 at Fourth month. During the rearing period the average daily gain was observed lowest value (ADG= 0.001) in Treatment T2 at Third month (Fig.2).

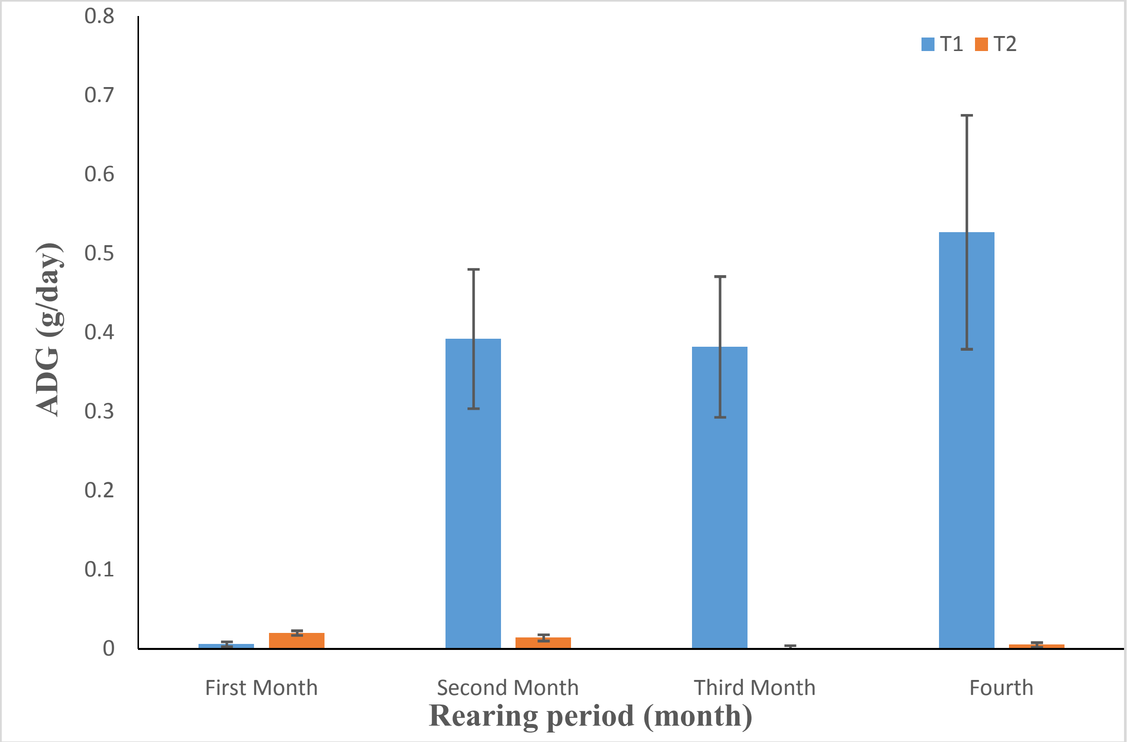


Figure 2: Average Daily Gain, ADG (Mean ± SEM) at fourth month rearing period with two different treatments.

3.1.3 Specific Growth Rate (SGR)

Specific growth rate (SGR) of Gulsha fingerling was observed the highest value (SGR= 3.396) in treatment T2 at First month. During the rearing period the average daily gain was observed lowest value (SGR=0.160) in Treatment T2 at Third month (Fig.3).

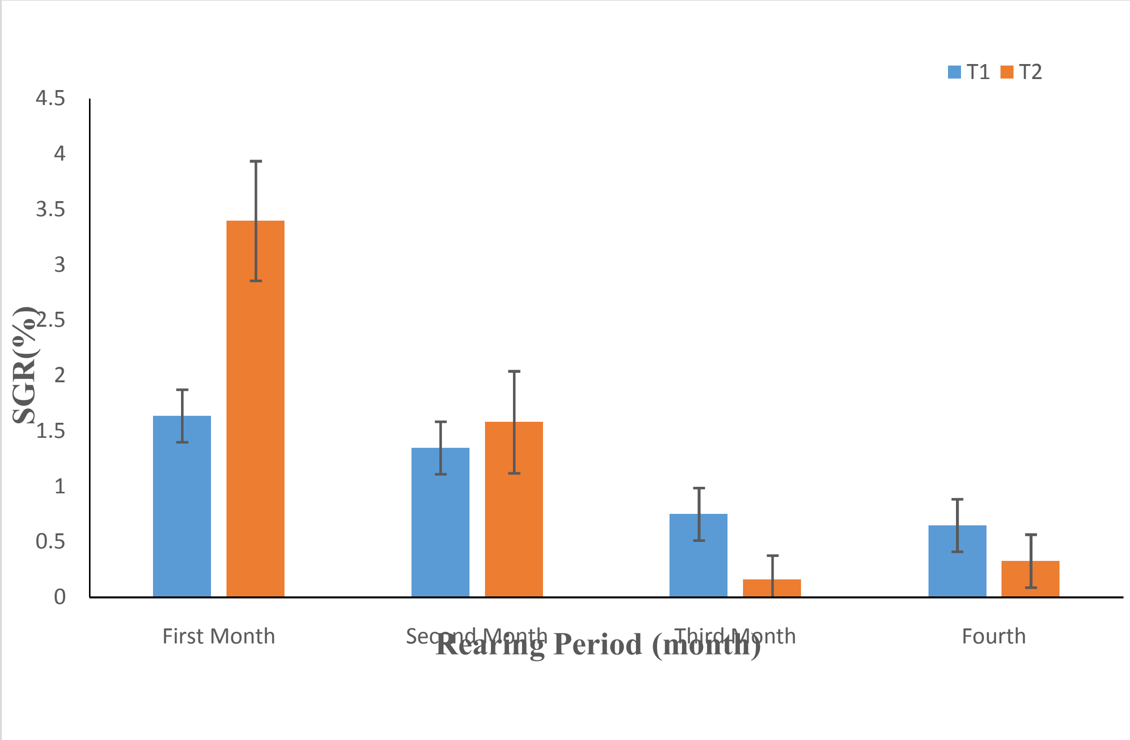


Figure 3: Specific Growth Rate, SGR(%),(Mean ± SEM) at fourth month rearing period with two different treatments.

3.1.4 Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) of Gulsha fingerling was observed the highest value (FCR= .964±0.217) in treatment T2 at third month. During the rearing period the average daily gain was observed lowest value (FCR=0.75±0.539)) in Treatment T2 at First month (Fig.4).

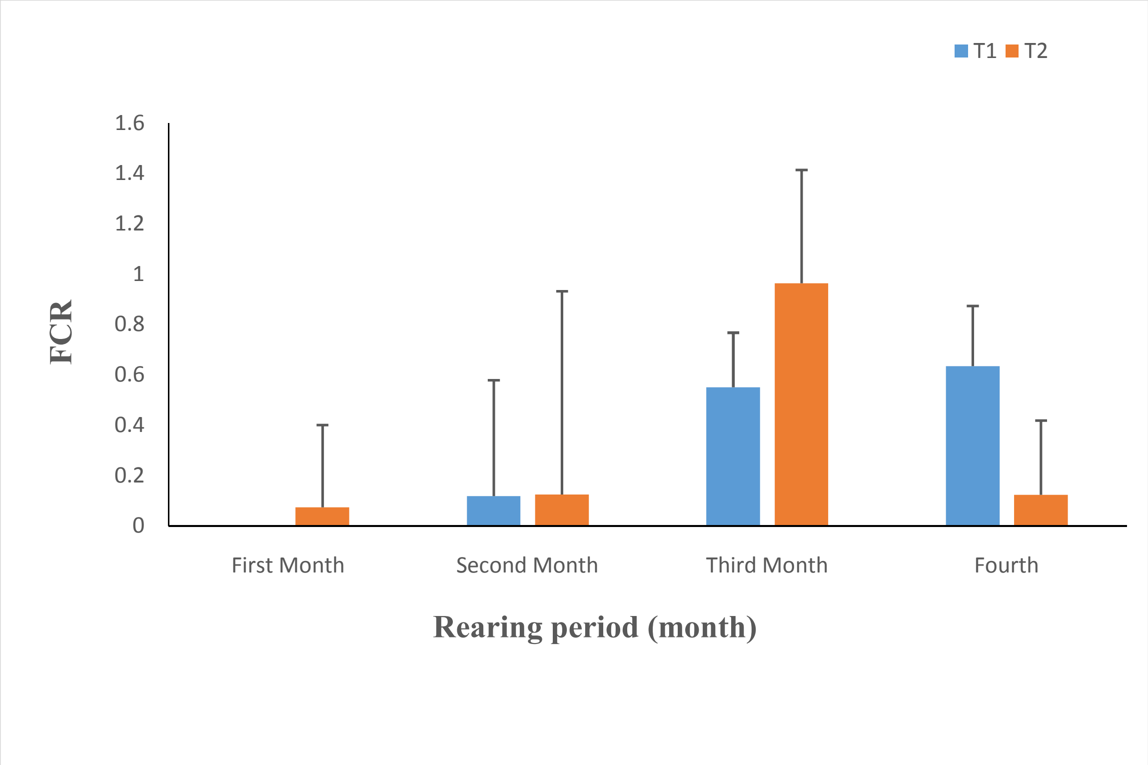


Figure 4:Feed Conversion Ratio, FCR (Mean ± SEM) at fourth month rearing period with two different treatments.

3.1.5 Survival Rate (%)

The values of survival rate of the experimental fish Gulsha(*Mystuscavasius*) rearing in four small ponds fed on two different types of feed have been represented in appendice table 5 at the end of 1st, 2nd, 3rd , and 4th month culture period. The values of survival rate of fish are expressed in percentage showing in Fig.5. The values of survival rate highest in treatment T2 during culture period.

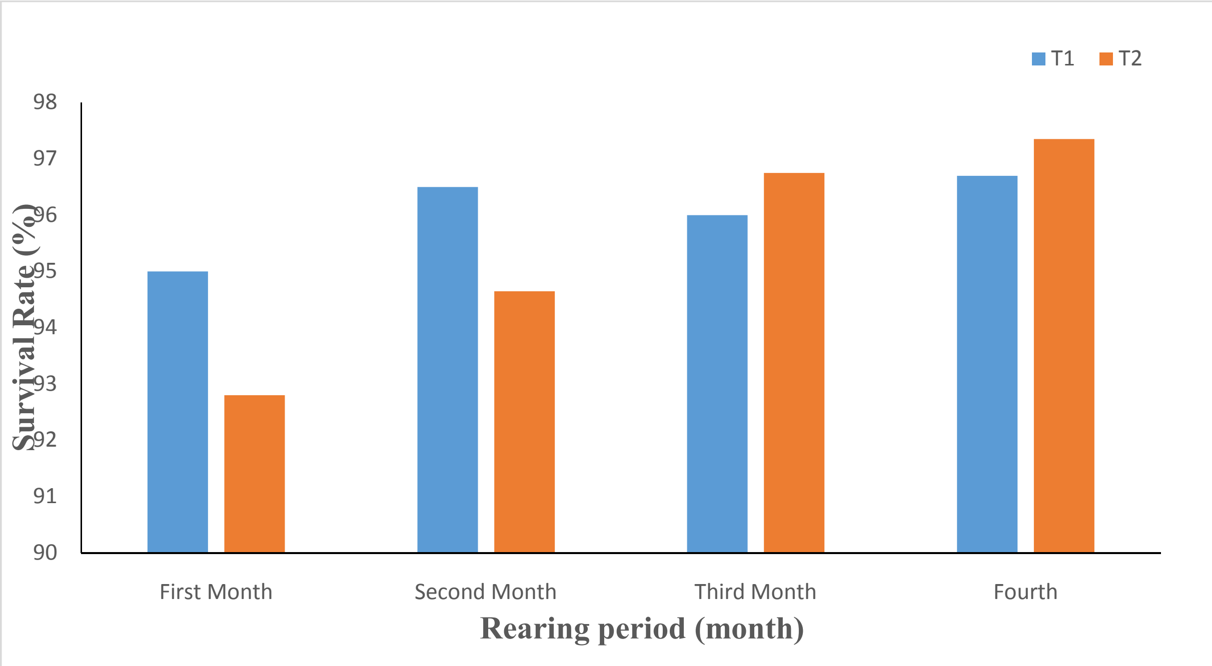


Figure 5: Survival rate of gulsha fish cultured for fourth month with two different treatments.

3.2 Proximate Analysis of fish sample

3.2.1 Moisture content in fish

Percentage of moisture in gulsha fish fed with different kinds of feeds depicted by Fig.6. Moisture content was found to be in the range of (76.30 – 78.80)% .From this it was observed that moisture content in tilapia fingerlings fed with commercial feed was highest 78.69% at the treatment T2.

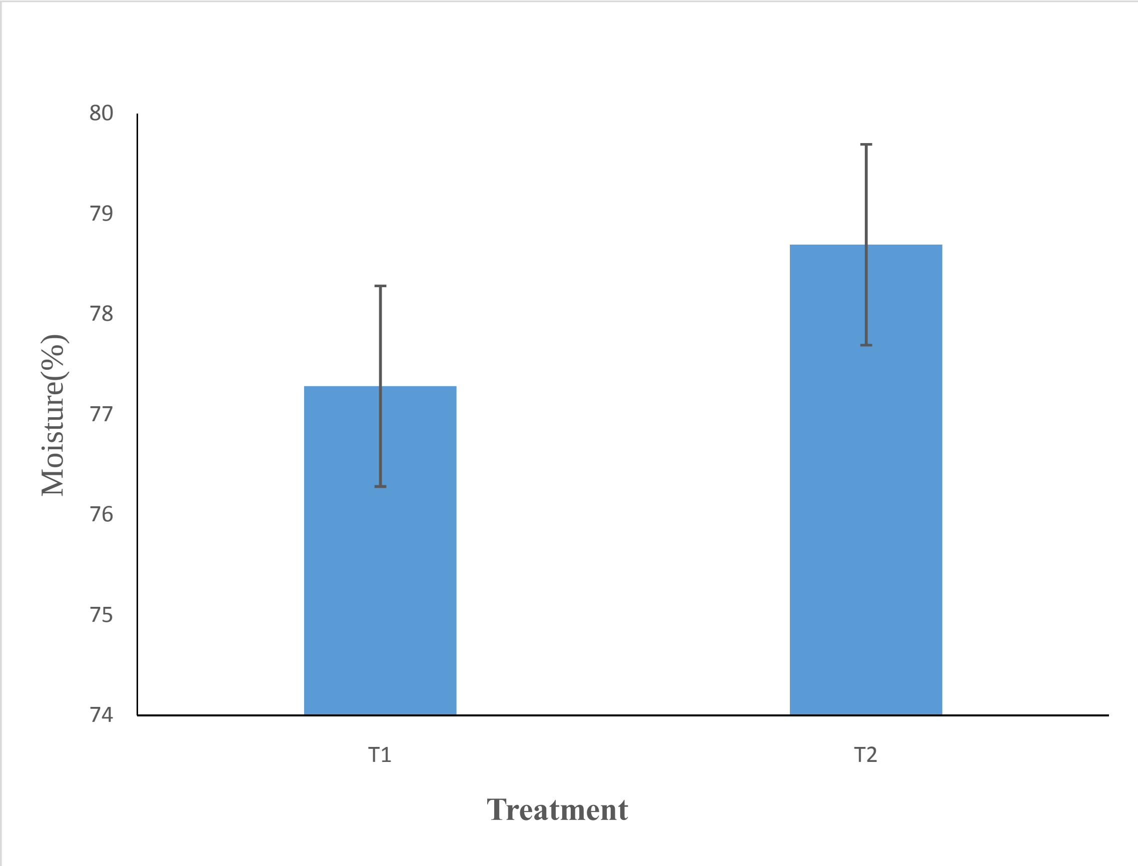


Figure 6: Moisture Content (%),(Mean± SEM) in gulsha fish cultured for fourth month with two different treatments.

3.2.2 Ash Content in Fish

Ash content was present in the Fig.7. Ash content in different fish fingerlings range from 2.92 to 3.82%. The highest ash content was found in fish fed with the rice bran andspirulina. While the lowest ash content present in fish fed commercial feed. Ash content was found the highest point at T1 and the lowest one in T2.

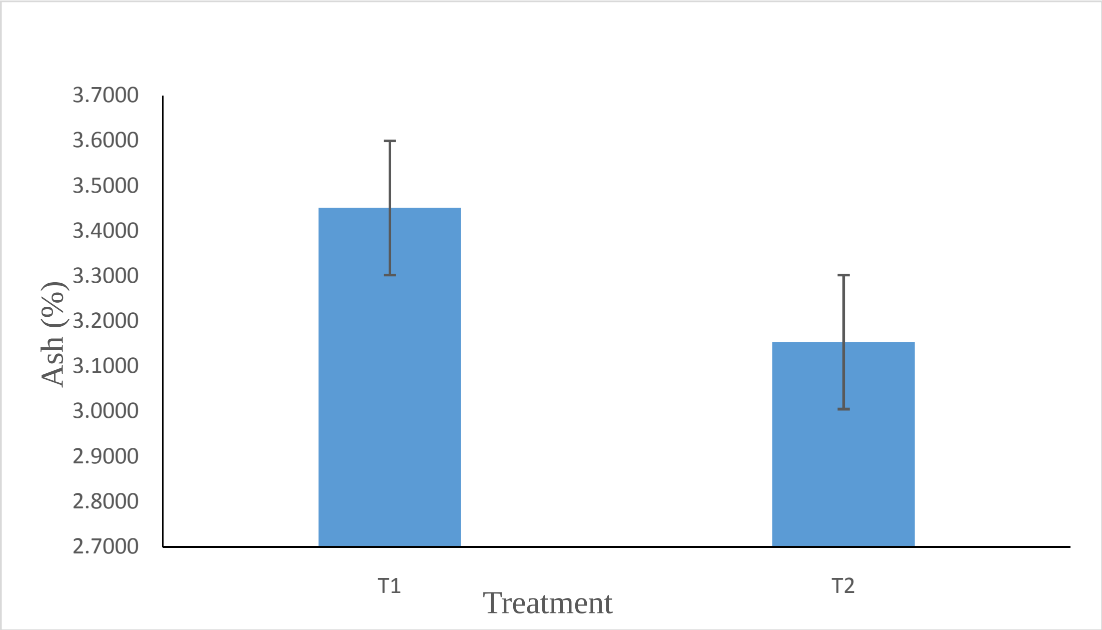


Figure 7: Ash content,(Mean± SEM) in gulsha fish cultured for fourth month with two different treatments.

3.2.3 Lipid Content in Fish

The Percentage of fat in gulsha fingerlings fed with different feedshowen by Figure 8. Lipid content was found to be in the range of (4.616-2.09) %. The highest percentage was found in fish fed with rice bran and spirulina (4.306%) while the lowest percentage was present in fish fed with commercial feed (2.172%) of total fat respectively.

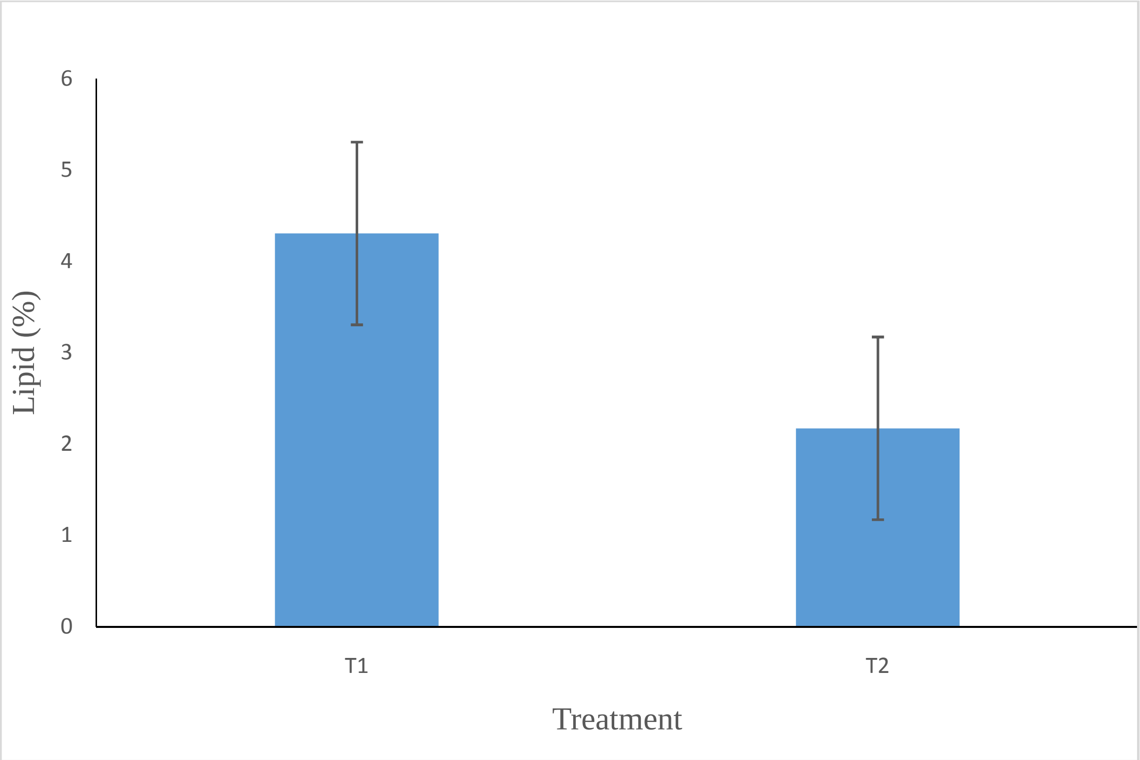


Figure 8: Lipid content,(Mean± SEM) in gulsha fish cultured for fourth month with two different treatments.

3.2.4Protein Content in Fish

Percentage of crude protein for gulshaspecies under study was compiled in Figure 9. In present study protein content was found to be in the range of (14.90 -15.05)%, the highest content being present T1 (15.05%), while the lowest being present in T2 (14.85%).

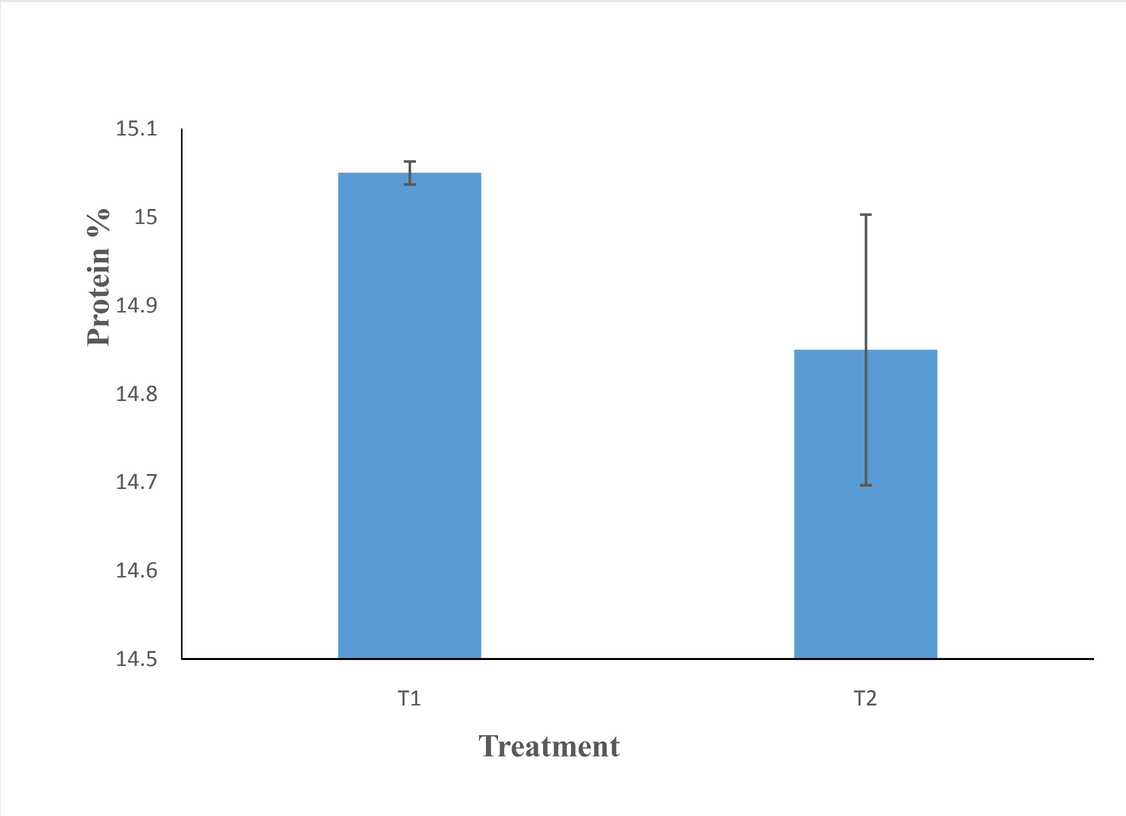


Figure 9: Protein content,(Mean± SEM) in gulsha fish cultured for fourth month with two different treatments.

3.3 Minerals analysis of fish sample

Calcium Content

In the present study, calcium content was found to be in the range of 112.32-115.6 mg/ 100 g of fish. From these observations highest value of calcium (115.6mg/100g of fish) was found in the fingerlings fed with rice bran andspirulina in T1 while the lowest value (112.32mg/100g of fish) is observed in those fed with commercial feed in T2 Figure 10.

Phosphorus Content

Phosphorus content was compiled in Figure 10 in which the highest amount (166.7mg/100 of fish) was found in fingerlings fed with rice bran and Spirulina while the fingerlings fed with commercial feed contain the lowest percentage (161.3mg/100 g of fish).

Potassium Content

Potassium content in gulsha fed with dilferent feed was depicted in Figure 10 in which the highest amount (207.23mg/100 of fish) was found in fingerlings fed with rice bran and Spirulina while the fingerlings fed with commercial feed contain the lowest amount (205.37mg/100 g of fish).

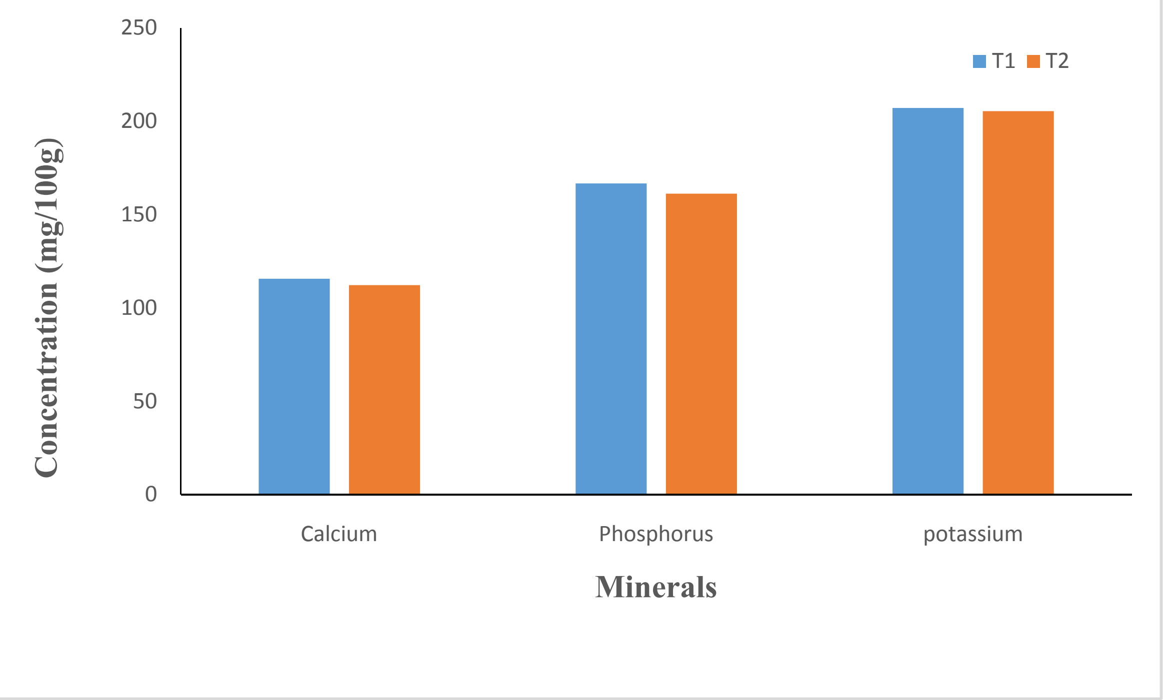


Figure 10: Ca, P and K content in gulsha fish cultured for fourth month with two different treatments.

Iron Content

Iron content in gulshafed with dilferent feed was depicted in Fig 11in which the highest amount (1.85mg/100 of fish) was found in fingerlings fed with commercial feed while the fingerlings fed with rice bran and Spirulina contain the lowest percentage (1.78mg/100 g of fish).

Magnesium Content

From these study highest value of magnesium (25.97mg/100g of fish) was found in the fingerlings fed with rice bran and spirulina in T1 while the lowest value (24.15mg/100g of fish) is observed in those fed with commercial feed in T2 Figure 11.

Manganese Content

Manganese content in gulshafed with different feed was depicted in Figure 11 in which the highest amount (0.23mg/100 of fish) was found in fingerlings fed with commercial feed while the fingerlings fed with rice bran andSpirulina contain the lowest percentage (0.18mg/100 g of fish).

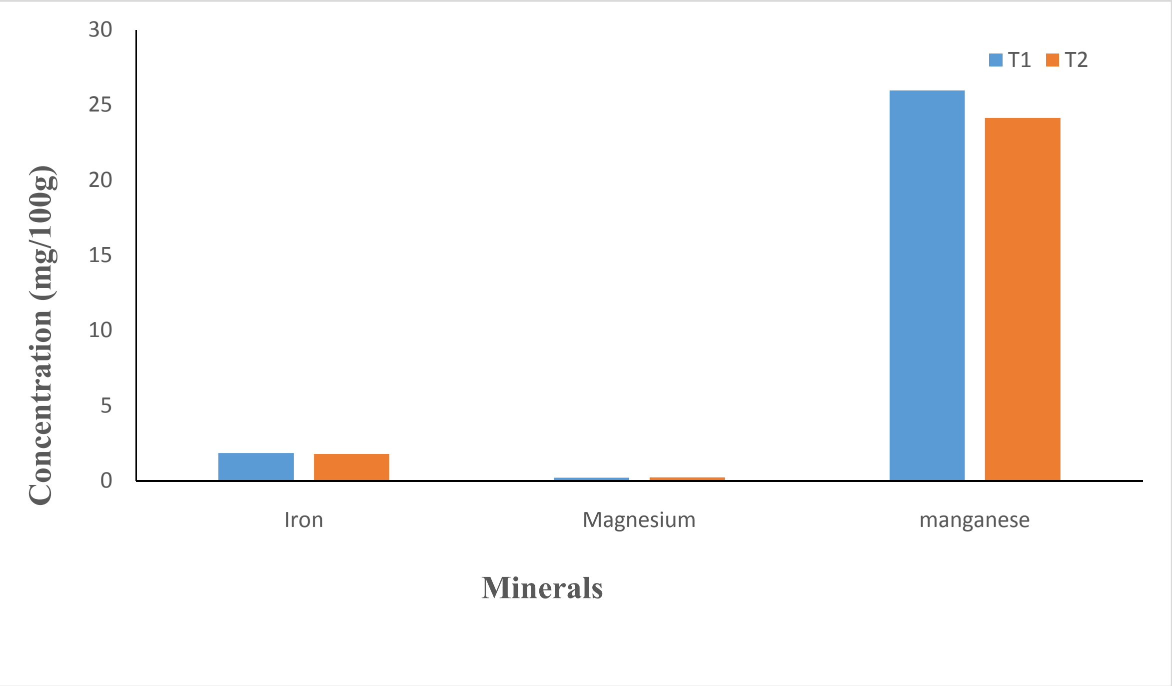


Figure 11: Fe, Mg and Mn content in gulsha fish cultured for fourth month with two different treatments.

Chapter 4

Discussion

Discussion

The present study descried the growth performance and the proximate contents (moisture, protein, lipid and ash) and essential minerals in gulsha fingerlings fed with different feed. As it is well known, fish constitutes the major share of animal foods in the Bangladeshi diet. Fish considered being a fair access to essential micronutrients in which our habitual diet remains to be grossly deficient. In present study gulsha was selected for analysis because of its availability and easy culture and also because of its great acceptance to the poor people of Bangladesh to meet their nutritional requirements. In this experiment different kinds of feeds are supplied to determine the effect of feeding regime on the growth performance of fish and also the proximate composition and minerals content of fish.

Growth performances in terms of total length (TL), body weight (BW), specific growth rate (SGR), feed conversion ratio (FCR), average daily gain (ADG) and condition factor (K) were recorded between two types of treatment.

Condition factor (k) showed the less variation and good performance at T1. Where K increased according to the treatment but at T1 the k value (K= 0.93810) was higher than treatment T2. The lower value of the k factor (K=0.6063) was found at T1. Besra (1997) observed K value nearly 1.0 in *Anabas testudineus*Rahman et al.(1997) in a study on the survival and growth of eat fish giving selected supplemental feeds got the values of condition factor between0.51 to 0.8.

From this study, gulsha fingerlings showed the similar or less variation among average daily gain (ADG). The maximum ADG value was found at T1 and the minimum ADG value found at T2 and the others were moderate value showing in ADG Figure 2. 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. 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, predatory fish, weed fish etc. Moreover ADG value relies on size of fish, sex, age, physiological condition and so on.

In this present study specific growth rate (SGR) was found in different variation. The maximum value of SGR found at T2 during rearing period of gulsha fingerlings while the minimum value of SGR found at T1 his finding resembles the Medawars (1945) fifth law “the specific growth rate declines more and more slowly as the organism increases in age” at the various conditions. Minot (1990) reported 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.

In this study, feed conversion ratio (FCR) of gulsha fingerlings was different at the two treatment. Moreover, the highest value of FCR was found at treatment T2 and the lowest one at T1. The efficiency feed conversion ratio depends on many factors but the best response is probably strongly related to optimize the environment to approximate that to which the fish is accustomed found that growth rate, feed intake and feed conversion efficiency had the highest values at treatment T2.

The values of survival rate highest in treatment T2 and lowest in treatment T1 after fourth month culture period. Survival rate depends on several climatic factors including temperature, salinity, DO, light intensity, water current and other various factors such as availability of feed, stocking density, predatory fish, weed fish etc.

During study period moisture content was found to be in the range of 76.30 – 78.80%. From this it was observed that moisture content in gulsha fingerlings fed with commercial feed was highest (78.69%) than the fingerlings fed with rice bran and spirulina77.28%). In a study (Stansby et al. 1963), moisture content for fresh water fish was reported to be in the range of 72.1-83.6 %, with a mean of 77.64% and the observations were nearly to the present study. 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% which is also nearly similar to the present study. Desrosier et al. (1977) in another study dealing with the amount of moisture, fat and protein in fish reported that general fish contains 70-80% moisture.Moisture content of fish depends on some factors such asseasons, sex, age etc.Thus, moisture content in present study is in a good agreement with the values reported in all these previous studies (Siddiqui and Chowdhury, 1996); BARC 1983; Desrosier et al.1977, Stansby et al. 1963;Rubbi et al. 1987).

Venkataraman and chari (1951) made a thorough study on the seasonal variation in chemical composition of Mackeraland found that moisture and fat were subject to seasonal variation. Mohsin et al. (1990) in a study with *Cirrhinusmrigal*, stated that nutrient content varied due to the variation of habitat and physiological condition.

The depleted fish (after spawning) contained higher amount of moisture and lower amount of fat and protein than the spawning ones. In another study (Mohsin, 1994), worked on Cirrhimusmrigala (Hamilton,1822) and observed that larger fish contain lower amount of moisture than those of the smaller ones.

In the present study, lipid content was found to be in the rage of (4.616-2.09) %. The highest percentage was found in fish fed with rice bran and spirulina (4.17%) while the lowest percentage was present in fish fed with commercial feed (1.57%).Rubbi et al. (1987) reported the lipid content of 27 species of freshwater fish in Bangladesh. In their study, fat content varied from 0.89-15.l 1%. Thus fat content, in the earlier studies, were higher than the present findings. Lipid portion of fish comprises of a complex mixture of glycerides, polar lipids and lesser components (sterol, sterol ester, free fatty acids etc.) the portion of each again is dependent on species and seasons (Woyewode et al. 1986). Total lipid concentration differs due to size of fish, portion of fish, time of fish catch, water temperature as well as season (Quazi, 1989).

It is evident from the study, percentage of crude protein for gulsha species under study was compiled in Figure 9. In present study protein content was found to be in the range of 14.85-15.05%.The highest content being present Treatment 1, while the lowest being present in Treatment 2. In a previous study (Dresosier, 1977) protein content in fish was reported to be in a range of 13-20%. In another study (INFS, 1980) protein content of freshwater fish was demonstrated to be 15-18%. Govindan (1985) also demonstrated a range of 9-25% protein for freshwater and marine fish. All the previous studies suggest a wide range for protein to be present in general fish. Protein content of fish, as revealed in present is in good agreement with the valued reported in these previous studies. Protein content is subjected to the variation of factors such as size, sex, habitat, physiological condition and also season (Mohsin et al. 1990, Borgstrom, 1961, Khuda et a1.1962).

It was observed from the present study, the range for ash content in different gulsha fingerlings was 2.92-3.82%.The highest percentage was found in fish fed with rice bran and spirulina (3.45%) while the lowest percentage was present in fish fed with commercial feed (3.15%). Ash contains kinds of minerals which play important role in body structure for each organism including calcium, magnesium, phosphorus, iron, zinc and so on. 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.

Calcium content ranged considerably from 112.32-115.6 mg/ 100 g. These results are within the range of fish and seafood reported elsewhere (FAO/ INFOODS, 2013). As would be expected, calcium content was much higher in species in which bones are commonly consumed and included in the edible parts.Calcium deficiency nationally has not been evaluated, however, it has been implicated in the development of rickets, estimated to affect 550,000 children in 2008 (Craviariet al. 2008; Fischer et al.1999; ICDDRB, 2009), and in a study in two rural sub-districts of Bangladesh, it was estimated no women or young children had diets adequate in calcium, attributable to low food intake and low dietary diversity (Arsenault et al. 2013). In developed countries, dairy products tend to be the primary source of dietary calcium; however, this is not the case in Bangladesh where frequency of dairy consumption is very low (Belton et al. 2014).

Iron content varied considerably with a range from 1.85 to 1.78 mg/100. These results show a greater range in iron content compared to a values reported in the global FAO/INFOODS database on fish and shellfish (excluding molluscs) (FAO/INFOODS, 2013). This may be partly attributable to sampling variability, methodological differences in analysis of iron content, or may reflect real differences in the accumulation of iron in this species based on differing environmental conditions. The true nature and magnitude of these differences should be further investigated. Overall, the data presented here indicate that this species may contribute significantly to dietary iron intakes in Bangladesh which is of high bioavailability as an animal-source food (FAO and WHO, 2004). This may have important policy implications given the public health significance of iron deficiency in Bangladesh, with prevalence recently estimated at 10.7% in preschool aged children and 7.1% in adult women (ICDDRB, 2013), and the well documented negative effects of deficiency on physical and cognitive development,pregnancy outcomes, morbidity and mortality.

Phosphorus content ranged from 166.7 to 161.3mg/100 g, with higher composition in fish species with bones included in edible parts, also consistent with values reported elsewhere (FAO/INFOODS, 2013). The ranges of magnesium is 25.97to 24.15 mg/100 g, and potassium (207.23 to 205.37 mg/100 g) content were broadly consistent with ranges for other fish and seafood reported elsewhere (FAO/INFOODS, 2013). Manganese content ranged from 0.23 to 0.18 mg/100 g and is higher than results reported elsewhere (FAO/INFOODS, 2013), which may be related to water pollution (Tornqvist et al. 2011).

In Bangladesh, only 6 percent of the daily food intake is animal food, of which fish accounts for about 50 percent. Rice is the mainstay, making up 60 percent of the daily food intake. However, many nutrients such as calcium, iron, zinc, phosphorus are not adequate in rice and have to be got from other sources. Fish-big or small, to this effect, has the potential to contribute positively to the diets of the rural and urban people in Bangladesh, and thus can improve our diet-quality.

Chapter 5

Conclusion and Recommendations

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5.1 Conclusion

In this experiment, the significant effects of different feeds on growth performance and nutrient content of gulsha (*Mystuscavasius*) were investigated. Growth performance of gulsha can cope with the different types of physiochemical parameters such as temperature, pH, D0, conductivity, total dissolved solids, light intensity and so on. From the experiment conducted it was observed that the length and weight of gulsha fish varied with different feeds. The best growth performance was observed in rice bran and Spirulina. Rice bran and Spirulina has the best proximate composition for human nutritional interest and thus it was found to be the better option to produce healthy fish for human use. Culture of gulsha with different compound feed utilization in small ponds to improve growth and to enhance the nutrient contents of producing fish, for the well-being of consumers.

5.2 Recommendations

- Present study is laboratory based, so field level studies are required to identify the actual effect of rice bran and spirulina on gulsha.
- To enhance the production of gulsha further research should be undertake to identify the mechanisms which are associated with the regulation of response to rice bran and spirulina.
- Research should be carried out to check the potentiality of different types of feeds.

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Appendices

Appendix 1

Table 1: Condition Factor, K (Mean ± SEM) at Fourth Month Rearing Period.

Treatment	Rearing period	Condition Factor (%)

T1	Zero Month	.927±0.09
	First Month	.844±.08
	Second Month	.787±0.02
	Third Month	.788±0.02
	Fourth Month	.938±0.09
T2	Zero Month	.607±0.23
	First Month	.806±0.02
	Second Month	.784±0.02
	Third Month	.756±0.02
	Fourth Month	.796±0.02

Condition factor (K)

Case Summaries[a]

			Zero_Month	First_Month	Second_Month	Third_Month	Fourth_Month
Treatment	Rice Bran + Spirulina	1	.57870	.73971	.85938	.77536	1.83004
		2	.73525	.93491	.87791	.82400	1.02179
		3	.61218	.76305	.88043	.86400	1.08507
		4	.57064	.72800	.85600	.52292	.43821
		5	.76542	1.42188	.75387	.81142	.56896
		6	.72489	1.28280	.70549	.98627	.68433
		7	.53303	.21250	.73525	.71916	.52770
		8	.67123	1.19486	.78125	.63110	.96292
		9	.75128	.58560	.73525	.83217	2.22951
		10	.57317	1.97568	.55200	.64746	1.12271
		11	1.30208	.89063	.76818	.88000	1.11177

	12		.77904	.73438	.98145	.84800	.95260
	13		.46096	.66941	.69600	.76140	.67919
	14		1.51052	.63649	.76800	.85272	.91204
	15		1.82216	.73438	.96573	.95473	.49203
	16		1.33333	.78981	.76818	.73215	.80603
	17		.77289	.63649	.78400	.62509	.68520
	18		1.11948	.70187	.77915	1.01710	.84999
	19		1.26350	.63649	.73202	.71120	.88201
	20		1.67930	.61643	.76935	.77536	.92022
	Total	N	20	20	20	20	20
		Mean	.9279537	.8442669	.7874436	.7885799	.9381157
		Median	.7583482	.7343750	.7687610	.7933884	.8970254
		Std. Error of Mean	.09254427	.08422717	.02163425	.02816186	.09645387
		Sum	18.55907	16.88534	15.74887	15.77160	18.76231
		Minimum	.46096	.21250	.55200	.52292	.43821
		Maximum	1.82216	1.97568	.98145	1.01710	2.22951
		Std. Deviation	.41387056	.37667534	.09675132	.12594365	.43135482
Commercial feed	1		.72547	.94376	.73469	.59354	.69657
	2		.54673	.66661	.97971	.66204	.76334
	3		.38400	.85938	.58333	.77536	.69013
	4		.52870	.83965	.73328	.89557	.83707
	5		.68587	1.00958	.66400	.69907	.79630
	6		.63749	.87963	.85470	.96012	.84632

	7		.57870	.96588	.67318	.60082	.75131
	8		.72489	.75017	.73428	.71200	.72169
	9		.65306	1.05024	1.00620	.73124	.79630
	10		.60421	.51312	.69722	.68056	.94966
	11		.59227	.91360	.79339	.74537	.86111
	12		.37037	.68056	.83707	.74436	.80473
	13		.63749	.86384	1.04000	.76677	.95162
	14		.76091	.78400	.69444	.81944	.91033
	15		.72943	.80556	.78289	.77566	.67919
	16		.65844	.73600	.72222	.84776	.69704
	17		.56587	.71525	.76800	.65337	.82567
	18		.68587	.79260	.82344	.73036	.88261
	19		.52870	.65515	.72800	.88000	.82344
	20		.52827	.71525	.83705	.86055	.63717
	Total	N	20	20	20	20	20
		Mean	.6063369	.8069907	.7843557	.7566975	.7960806
		Median	.6208494	.7990781	.7513469	.7448643	.8005150
		Std. Error of Mean	.02385281	.02985919	.02629131	.02194410	.02032079
		Sum	12.12674	16.13981	15.68711	15.13395	15.92161
		Minimum	.37037	.51312	.58333	.59354	.63717
		Maximum	.76091	1.05024	1.04000	.96012	.95162
		Std. Deviation	.10667300	.13353434	.11757833	.09813698	.09087733
Total	N		40	40	40	40	40

	Mean	.7671453	.8256288	.7858996	.7726387	.8670981
	Median	.6648350	.7566128	.7681756	.7710623	.8245531
	Std. Error of Mean	.05373883	.04420591	.01680620	.01780459	.04996106
	Sum	30.68581	33.02515	31.43598	30.90555	34.68393
	Minimum	.37037	.21250	.55200	.52292	.43821
	Maximum	1.82216	1.97568	1.04000	1.01710	2.22951
	Std. Deviation	.33987422	.27958275	.10629175	.11260614	.31598147

a. Limited to first 1000 cases.

Appendix 2

Table 2: Average Daily Gain, ADG (Mean ± SEM) at Fourth Month Rearing Period.

Treatment	Rearing period	ADG (%)
T1	First Month	.006±.003
	Second Month	.392±0.088
	Third Month	.382±0.089
	Fourth Month	.527±0.148
T2	First Month	.020±0.003
	Second Month	.014±0.004
	Third Month	.001±0.003

	Fourth Month	.005±0.0036

Average daily gain (ADG)

Case Summaries[a]

				One_Month	Second_Month	Third_Month	Fourth_Month
Treatment	Rice bran + Spirulina	1		.02133	.85938	.77536	1.83004
		2		.00800	.87791	.82400	1.02179
		3		.01433	.88043	.86400	1.08507
		4		.02467	.85600	.52292	.43821
		5		.01633	.75387	.81142	.56896
		6		.00400	.70549	.98627	.68433
		7		.00400	.73525	.71916	.52770
		8		.02033	.78125	.63110	.96292
		9		-.00700	.73525	.83217	2.22951
		10		.01233	.55200	.64746	1.12271
		11		.01300	.00433	.01333	.00667
		12		.00933	.03033	-.01067	.01467
		13		.01567	.00867	.00467	.00400
		14		.00433	.01267	-.00433	.03800
		15		.00233	.01567	-.00233	.00300
		16		.01533	-.00400	.01300	.00367
		17		-.04067	.01333	.00200	.00333
		18		.00267	.00633	.00933	.00033
		19		-.00200	.00600	.00800	.00567
		20		-.00267	.02133	.00033	.00267
		Total	N	20	20	20	20
			Mean	.0067833	.3925749	.3823597	.5276617
			Median	.0086667	.2911667	.2681242	.2381071
			Std. Error of Mean	.00314515	.08884158	.08937071	.14889630
			Sum	.13567	7.85150	7.64719	10.55323
			Minimum	-.04067	-.00400	-.01067	.00033
			Maximum	.02467	.88043	.98627	2.22951
			Std. Deviation	.01406554	.39731161	.39967799	.66588448
	Commercial Feed	1		.01200	.05533	-.02967	-.01133

	2		.00767	.03667	-.00667	-.00533
	3		.01633	.02367	.00100	.02933
	4		.00567	.02867	.00900	.01367
	5		.01800	-.00100	.02267	.00700
	6		.03833	.00133	-.01967	-.00533
	7		.01500	.01967	.01767	-.01333
	8		-.00267	.04667	-.02867	.02767
	9		.00700	.01033	.00567	.02500
	10		.00133	.03133	.01033	.00367
	11		.04067	-.00667	.00967	.00833
	12		.04567	.01433	.00800	.00233
	13		.04700	-.02867	.00400	.02467
	14		.00633	.01733	.00900	.02433
	15		.03433	.01367	.00267	-.03667
	16		.01067	.02133	.00033	-.00700
	17		.02667	-.00767	.00833	.00800
	18		.02867	.00633	.00433	.00167
	19		.03000	-.00600	.00633	.00900
	20		.02467	.00933	-.00867	-.00100
	Total	N	20	20	20	20
		Mean	.0206667	.0143000	.0012833	.0052333
		Median	.0171667	.0140000	.0050000	.0053333
		Std. Error of Mean	.00338651	.00446925	.00308309	.00367536
		Sum	.41333	.28600	.02567	.10467
		Minimum	-.00267	-.02867	-.02967	-.03667
		Maximum	.04700	.05533	.02267	.02933
		Std. Deviation	.01514491	.01998710	.01378798	.01643669
Total	N		40	40	40	40
	Mean		.0137250	.2034374	.1918215	.2664475
	Median		.0126667	.0185000	.0081667	.0081667
	Std. Error of Mean		.00253747	.05333603	.05365435	.08457696
	Sum		.54900	8.13750	7.67286	10.65790
	Minimum		-.04067	-.02867	-.02967	-.03667
	Maximum		.04700	.88043	.98627	2.22951
	Std. Deviation		.01604834	.33732670	.33933991	.53491163

a. Limited to first 1000 cases.

Appendix 3

Table 3: Specific Growth Rate, (SGR) (Mean ± SEM) at Fourth Month Rearing Period.

Treatment	Rearing period	SGR (%)
T1	First Month	1.636±0.604
	Second Month	1.347±0.347
	Third Month	.750±0.230
	Fourth Month	.648±0.250
T2	First Month	3.396±0.539
	Second Month	1.580±0.460
	Third Month	.160±0.217
	Fourth Month	.327±0.239

Specific Growth Rate (SGR)

Case Summaries[a]

			One_Month	Second_Month	Third_Month	Four_Month
Treatment	Rice bran + Spirulina	1	1.45106	.12822	2.43396	1.18588
		2	.74381	1.37615	1.66330	1.93273
		3	1.65479	5.61596	.89105	-1.74416
		4	-.05013	1.57928	-.62034	2.09536
		5	-3.16013	3.36221	1.28554	1.14057
		6	7.32408	-.89778	2.84160	1.29071

		7		1.02056	-.42944	.84234	1.67023
		8		3.61115	.24703	1.44545	.35120
		9		5.59215	.53990	-.68974	.11301
		10		2.57730	.50860	.80612	-1.74416
		11		3.84227	.68481	1.50662	.55685
		12		3.01903	3.59035	-.87938	1.15732
		13		4.90606	1.18345	.49737	.37422
		14		.84594	1.67968	-.48503	2.88121
		15		.53756	2.31049	-.25796	.32813
		16		2.74400	-.52741	1.47618	.32048
		17		-3.77505	1.74841	.19808	.30603
		18		.55685	1.03812	1.10813	.03350
		19		-.32813	.90097	.91479	.52335
		20		-.39261	2.31049	.02594	.20056
		Total	N	20	20	20	20
			Mean	1.6360274	1.3474749	.7502018	.6486508
			Median	1.2358083	1.1107840	.8666952	.4487851
			Std. Error of Mean	.60436365	.34799594	.23022527	.25028561
			Sum	32.72055	26.94950	15.00404	12.97302
			Minimum	-3.77505	-.89778	-.87938	-1.74416
			Maximum	7.32408	5.61596	2.84160	2.88121
			Std. Deviation	2.70279639	1.55628514	1.02959869	1.11931126
	Commercial Feed	1		1.80775	3.58361	-1.45226	-.77979
		2		1.89698	3.74486	-.43635	-.39553
		3		7.38525	2.76316	.07843	1.73362
		4		2.13027	4.06834	.66642	.81026
		5		3.29537	-.11836	1.99480	.43405
		6		3.09845	.06945	-1.20861	-.42050
		7		6.30283	2.49402	1.29149	-.92544
		8		-.68617	5.36479	-2.25383	2.19619
		9		1.86539	1.63402	.64228	1.90928
		10		.66890	5.54183	.78947	.24054
		11		5.40894	-.47026	.66201	.48114
		12		8.95949	.85530	.39651	.10729
		13		3.52597	-1.69248	.29431	1.39817
		14		.71840	1.41889	.55171	1.15104
		15		2.98792	.70528	.12178	-2.26595

	16		1.42481	1.76022	.02130	-.47864
	17		3.71854	-.71592	.77147	.60314
	18		4.34983	.49765	.30218	.10930
	19		5.82303	-.60163	.63207	.73167
	20		3.24154	.70436	-.64881	-.08369
	Total	N	20	20	20	20
		Mean	3.3961742	1.5803576	.1608186	.3278079
		Median	3.1699949	1.1370988	.3493439	.3372951
		Std. Error of Mean	.53912496	.46040154	.21742905	.23968119
		Sum	67.92348	31.60715	3.21637	6.55616
		Minimum	-.68617	-1.69248	-2.25383	-2.26595
		Maximum	8.95949	5.54183	1.99480	2.19619
		Std. Deviation	2.41104013	2.05897828	.97237228	1.07188687
Total	N		40	40	40	40
	Mean		2.5161008	1.4639163	.4555102	.4882293
	Median		2.3537831	1.1107840	.5918922	.4041366
	Std. Error of Mean		.42383128	.28544750	.16325961	.17295246
	Sum		100.64403	58.55665	18.22041	19.52917
	Minimum		-3.77505	-1.69248	-2.25383	-2.26595
	Maximum		8.95949	5.61596	2.84160	2.88121
	Std. Deviation		2.68054435	1.80532850	1.03254442	1.09384742

a. Limited to first 1000 cases.

Appendix 4

Table 4: Feed Conversion Ratio, FCR (Mean ± SEM) at Fourth Month Rearing Period**.**

Treatment	Rearing period	FCR
	First Month	.479±0.326

T1	Second Month	.119±0.806
	Third Month	.551±0.450
	Fourth Month	.635±0.295
T2	First Month	.075±0.539
	Second Month	.126±0.460
	Third Month	.964±0.217
	Fourth Month	.124±0.239

Feed Conversion Ratio (FCR)

Case Summaries[a]

			First_month	Second_Month	Third_Month	Fourth_Month
Treatment	Rice bran + Spirulina	1	.01250	.15652	.10818	.14043
		2	.27917	.01822	.22842	.07636
		3	.05116	.13200	-.41222	-.14727
		4	.02297	.04593	.14875	.06857
		5	.08571	-.33882	.05203	.14706
		6	.35833	-.66182	.24348	.12689
		7	.10833	1.04000	.12895	.09224
		8	.05574	.45500	-.37450	.54000
		9	-.37143	.48533	.25586	1.74000
		10	.09189	.15714	.44692	-.14727
		11	.02549	.35077	.12564	.33000
		12	.22000	.04132	1.14333	.14455
		13	.51538	.18769	.17750	.50500
		14	.03115	.12211	.22167	.04368
		15	6.50000	.08000	8.96000	.58000
		16	.04359	-.54667	.39118	.59455
		17	.02273	.11600	-.43750	.62400
		18	.03864	.21895	-.67455	5.94000

	19		.04130	.25778	.19385	.35294
	20		1.46667	.08000	.09705	.96750
	Total	N	20	20	20	20
		Mean	.4799666	.1198722	.5512016	.6359607
		Median	.0534502	.1270526	.1631250	.2385294
		Std. Error of Mean	.32643707	.08067187	.45071642	.29540497
		Minimum	-.37143	-.66182	-.67455	-.14727
		Maximum	6.50000	1.04000	8.96000	5.94000
		Sum	9.59933	2.39744	11.02403	12.71921
		Std. Deviation	1.45987096	.36077559	2.01566511	1.32109117
Commercial Feed	1		.13889	.04145	-.57050	-.53625
	2		.13043	.03855	2.94000	.08795
	3		.01224	.06197	.31630	.21805
	4		.11176	.03349	.08544	.43143
	5		.05926	-2.29333	-.23017	-.50625
	6		.06522	3.80000	.14792	-.24750
	7		.01778	.07186	-.14244	.06434
	8		-.53750	.02000	.32941	.07760
	9		.13333	.12645	.26194	.80182
	10		.45000	.01872	.38733	.26727
	11		.02459	.15077	.31862	.38640
	12		.00730	.33951	.55417	1.83429
	13		.05319	.11500	.75833	.11514
	14		.41579	-.41391	.38889	.14548
	15		.06893	.49684	1.88125	-.12164
	16		.18750	-.48444	10.92000	-.44857
	17		.04875	.34000	.26880	.30250
	18		.03721	.38000	.73769	1.80000
	19		.02111	-.75077	.33526	.24444
	20		.06081	.44727	-.39577	-2.42000
	Total	N	20	20	20	20
		Mean	.0753302	.1269714	.9646239	.1248249
		Median	.0600350	.0669181	.3240162	.1303073
		Std. Error of Mean	.04220556	.23639178	.55177854	.19379455
		Minimum	-.53750	-2.29333	-.57050	-2.42000

		Maximum	.45000	3.80000	10.92000	1.83429
		Sum	1.50660	2.53943	19.29248	2.49650
		Std. Deviation	.18874901	1.05717619	2.46762866	.86667559
Total	N		40	40	40	40
	Mean		.2776484	.1234218	.7579127	.3803928
	Median		.0574985	.1155000	.2359497	.1462691
	Std. Error of Mean		.16565225	.12327875	.35318970	.17910812
	Minimum		-.53750	-2.29333	-.67455	-2.42000
	Maximum		6.50000	3.80000	10.92000	5.94000
	Sum		11.10594	4.93687	30.31651	15.21571
	Std. Deviation		1.04767685	.77968327	2.23376779	1.13277919

a. Limited to first 100 cases.

Appendix 5

Table 5: Survival rate of Gulsha fish at fourth month rearing period

Treatment	Rearing period	**Survival Rate (%)**
T1	First Month	95.0
	Second Month	96.5
	Third Month	96.0
	Fourth Month	96.7
T2	First Month	92.8
	Second Month	94.65
	Third Month	96.75

	Fourth Month	97.35

Survival rate

Case Summaries[a]

				First_month	Second_month	Third_month	Fourth_mor
Treatment	Rice bran + Spirulina	1		95.60	96.00	96.20	9
		2		94.50	97.00	95.80	9
		Total	N	2	2	2	
			Mean	95.0500	96.5000	96.0000	96.7
	Commercial feed	1		91.60	94.00	96.40	9
		2		94.00	95.30	97.10	9
		Total	N	2	2	2	
			Mean	92.8000	94.6500	96.7500	97.3
	Total	N		4	4	4	
		Mean		93.9250	95.5750	96.3750	97.0

Appendix 6

Table 6: Proximate composition (Mean±SEM) at fourth month.

Proximate composition	Treatment T1	Treatment T2
Moisture	77.28± 0.3036	78.69± 0.179
Ash	3.45± 0.128	3.15± 0.063
Lipid	4.17± 0.265	3.03± 0.180
Protein	15.05± 0.013	14.85± 0.153

Moisture content

Case Summaries[a]

			Moisture
Tretment	Rice bran + Spirulina	1	77.70

		2		76.30
		3		76.80
		4		77.67
		5		78.33
		6		76.90
		Total	N	6
			Mean	77.2833
			Std. Error of Mean	.30365
			Std. Deviation	.74379
			Median	77.2850
	Commercial Feed	1		78.80
		2		79.30
		3		78.30
		4		78.35
		5		79.10
		6		78.31
		Total	N	6
			Mean	78.6933
			Std. Error of Mean	.17929
			Std. Deviation	.43917
			Median	78.5750
	Total	N		12
		Mean		77.9883
		Std. Error of Mean		.27101
		Std. Deviation		.93880
		Median		78.3050

a. Limited to first 100 cases.

Ash content

Case Summaries[a]

			ash
Treatment	Rice bran + Spirulina	1	3.40453
		2	3.47446
		3	3.56923
		4	3.67432

		5		3.40987
		6		3.43532
			N	6
			Mean	3.4946210
		Total	Median	3.4548877
			Std. Error of Mean	.04361985
			Std. Deviation	.10684636
		1		3.05769
		2		3.01456
		3		2.97843
		4		3.31429
		5		3.33366
	Commercial feed	6		3.22277
			N	6
			Mean	3.1535680
		Total	Median	3.1402323
			Std. Error of Mean	.06383340
			Std. Deviation	.15635927
		N		12
		Mean		3.3240945
	Total	Median		3.3690967
		Std. Error of Mean		.06326192
		Std. Deviation		.21914572

a. Limited to first 100 cases.

7. Lipid content

Case Summaries[a]

				Lipid
		1		4.148
		2		4.103
		3		4.269
		4		4.616
		5		4.414
Treatment	Rice bran + Spirulina	6		4.292
			Mean	4.30690
			Median	4.28060
		Total	Std. Error of Mean	.076409
			Minimum	4.103

			Maximum	4.616
			Std. Deviation	.187162
			Range	.512
			Sum	25.841
	Commercial feed	1		2.078
		2		2.205
		3		2.162
		4		2.010
		5		2.053
		6		2.526
		Total	Mean	2.17227
			Median	2.12000
			Std. Error of Mean	.076471
			Minimum	2.010
			Maximum	2.526
			Std. Deviation	.187315
			Range	.515
			Sum	13.034
	Total	Mean		3.23958
		Median		3.31440
		Std. Error of Mean		.325909
		Minimum		2.010
		Maximum		4.616
		Std. Deviation		1.128980
		Range		2.605
		Sum		38.875

a. Limited to first 100 cases.

8. Protein content

Case Summaries[a]

			Protein
Treatment	Rice bran + Spirulina	1	15.03
		2	15.08
		3	15.09
		4	15.01
		5	15.02
		6	15.07

		Total	N	6
			Mean	15.0500
			Median	15.0500
			Std. Error of Mean	.01390
			Std. Deviation	.03406
			Minimum	15.01
			Maximum	15.09
			Sum	90.30
	Commercial feed	1		14.09
		2		15.00
		3		15.00
		4		15.04
		5		15.01
		6		15.01
		Total	N	6
			Mean	14.8583
			Median	15.0050
			Std. Error of Mean	.15378
			Std. Deviation	.37669
			Minimum	14.09
			Maximum	15.04
			Sum	89.15
	Total	N		12
		Mean		14.9542
		Median		15.0150
		Std. Error of Mean		.07908
		Std. Deviation		.27394
		Minimum		14.09
		Maximum		15.09
		Sum		179.45

a. Limited to first 100 cases.

Appendix 7

Table 7: Mineral contents (mg/100g) in gulsha fish cultured for fourth month with two different treatments.

minerals	Treatment 1	Treatment 2
Ca	115.6	112.32
K	207.23	205.37
P	166.7	161.3
Fe	1.85	1.78
Mn	0.18	0.23
Mg	25.97	24.15