Effects of some artificial diets on the feed utilization and growth of the fry of climbing perch, *Anabas testudineus* (Bloch, 1792)

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ABSTRACT: An experiment on culture of climbing perch (*Anabas testudineus*) in cement tanks using different protein level diets was conducted to find the appropriate feeding diets and their effects on the growth and survival of 15-days old koi (*Anabas testudineus)* fry in intensive culture of this fish. The experiment was carried out for a duration of 60 days with 4 treatments in 8 cement tanks each of size 12×6×1.5 feet. The initial length and weight were 14.5 ± 0.4 mm and 0.95 ± 0.05 g respectively. The feeds were applied twice a day at the rate of 10 %(initially) to 5% (later on) of the body weight of the fry/day. The results showed that the growth of fry varied significantly ($P<0.05$) with different diets. The highest growth and survival rate were found in the trial where fishes were fed on Sabinco feed containing 50.92% protein (on dry matter basis), followed by Feed-3 containing 30% protein. The poorest growth rate was shown by Feed- 1 (prepared by rice bran, wheat bran, fish meal and soybean meal) containing 20% protein. There was no significant difference in survival rates among the fry fed with Sabinco and prepared diets. The experiment suggests that Feed-2 (Sabinco Feed) can be recommended for the intensive culture of climbing perch. [Report and Opinion. 2010;2(2):3-28]. (ISSN: 1553-9873).

CHAPTER I

1. INTRODUCTION

Bangladesh like many other countries of the world is confronted with mutually aggravating problems of hunger and protein malnutrition. This problem can not be filled up through increasing agricultural production alone while the per capital availability of land in this country is only 0.33 acre. Since the scope for further expansion of agriculture is rather restricted, by activating the country's vast fisheries resources, an extraordinary blow can be struck against the existing problem of animal protein malnutrition. Recommended protein intake of a healthy person is 45 g/capita/day of which 15 g/capita/day animal protein is necessary. But present animal protein intake of our people is only 11 g/capita/day. This shortage in animal protein intake can be filled up increasing the aquaculture production through scientific culture.

Bangladesh is an agro-based riverine country enriched with vast fisheries resources. The total area of inland water is estimated about 43.4 lakh hectares. Inland water including rivers, beels, Kaptai Lake and floodplains comprise about 40.47 lakh hectares and closed waters including ponds and tanks, baors and coastal shrimps farm comprise about 2.92 lakh hectares (DOF, 1998). The republic has a 480 km long coastline and approximately 166 lakh hectares of seawater area. The nation's exclusive economy zone contains up to 200 nautical miles from the coastline. Thus the nation's total area of waters having fish production potential is of very great importance. Fisheries play a vital role in our national economy and contribute 5% to the GDP, 16.7% to the agricultural products and 8% to the export earnings (DOF, 1998). In 1996-1997, Bangladesh has earned 1457.41 crore taka by exporting 41549 metric tons of fish and fishery products to foreign countries (DOF, 1998). About 1.2 million people are engaged in subsistence fishing and activities related to the fish sector (DOF, 1998).

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.

In our country more or less in every house there are small ditches, which were dug for raising ground for the purpose of constructing houses. These ditches/mini ponds retain water for 5-6 months in a year. These water bodies either remain fallow or used for the purpose of jute rotating and in most cases are covered with aquatic vegetation. The farmers believe that these waters could not be utilized for production purpose because of their seasonal nature, but actually they hold tremendous potential for adopting intensive culture of species having shorter life cycle. Proper aquaculture as a whole and high density fish culture using supplementary formulated feed in particular is not very popular in Bangladesh. However, there is increasing interest in hardy fishes particularly those of air breathing fish farming in Bangladesh. Among various production inputs, the choice of fast growing species with desirable aquaculture traits is a pre-requisite for augmenting fish production in culture-based fisheries. Natural food based culture of major carp is still in practice in Bangladesh but carp culture could not be widely practiced in the shallow and seasonal ponds. In this regard, tilapia, koi fish (*A. testudrneus)* are an excellent fish for growing in the shallow and seasonal ponds in a country like Bangladesh (Hussain *et al.* 1989, Gupta 1992, Kohinoor *et al.* 1993, Akhteruzzaman *et al*. 1993, Gupta *et al*. 1994) because Bangladesh enjoys very suitable climatic and ecological conditions for culture of warm-water species.

MAEP (1995) has shown that pond size affects the production of major carps. Small ponds below 0.1 acre size may not be profitable for conventional carp polyculture. Mustafa and Brown (1984) reported that growth rate in small ponds was rapid than in large impounded ponds. They commented that small ponds were more productive and easily manageable in our country. Edible fish production per unit area, *A. tcstudineus* is more productive than most farm animals at the same level of intensification.

Air breathing fish in Bangladesh form an economically important group of fishes. *A. testudineus* is one of them. This fish is highly esteemed for its highly nourishing quality and prolonged freshness. This fish is very suitable for cultivation in ponds, reservoirs and rice fields. The cultivation of koi fish is becoming increasingly popular among the aqua culturists of Bangladesh. Because forth cultivation of *A. testudineus* fish swampy water may be used which is not feasible for carp culture.

The labyrinth fishes, Anabantoidie, derived their name for having a labyrinth like accessory - breathing organ on either side of the head. Two widely known Asian members of the groups are climbing perch (*A. testudineus)* and gourami *(Ospharonemus)* climbing perch, *A. testudineus* (Bloch), is a fresh water fish indigenous to South and Southeast Asia. It can thrive well in low dissolved oxygen (DO), different waters and it can migrate between ponds. Wild climbing perch (*A. testudineus)* is a popular fish in Asia with larger fish (over 60 g) fetching a high market price. However, fish of this size is not very abundant, though smaller specimens can easily be caught during the wet season. This has lead to carnivores for information on how to culture the fish, more specifically information or fetching of smaller fish.

This species has an advantage due to its air breathing ability and tolerance of adverse environmental conditions for being a candidate for fish culture. This fish is hardy and able to aestivate during the dry season. It can bury in the mud and passes into a vesting stage similar to that observed in the African lungfish. However, information on feed choice and nutritional requirements in relation with its growth rates is scarce.

It is found in fresh and brackish waters mostly in ponds, swamps and lakes of Bangladesh, India and Southeast Asian countries. In Bangladesh it is found in all districts. In Southeast Asian countries it is found in Pakistan, Nepal, Srilanka, Burma, Thailand, Indonesia, Singapore and China. It is commonly called as koi fish.

A. testndineus are caught from ponds, rivers and swamps. Larvae and young fry fed on phytoplankton and zooplankton, larvae fry and adults feed on crustaceans, worms, moluscs and insect, algae, soft higher plants and organic debris (Potogkam, 1972) *A. testudineus* has been described a predator, carnivore (Pandey *et al.* 1992). However gastric contents analysis of 204 specimens of *A. testudineus* showed that the stomach contained 19% crustaceans, 3.5% insects, 6% mollusk, 9.5% fishes, 47% plant debris and 1.6% semi - digested matter (Nargis and Hossain, 1987). Major food item in the gut were found to be more or less consistent irrespective to spatial and seasonal distribution in Bangladesh (Nargis and Hossain, 1987) indicating *A. testudineus* an omnivore. Relatively higher frequency of empty

stomached mature fish during the month of May indicates peak spawning activity.

Any fish cultured with artificial feeds need high percentage of protein in the diet for fast growth and better growth performance. However requirement of protein by a particular species of fish vary with others.

It has been established that protein is required by all animals for body maintenance and growth, and that the protein level needed for these functions varies with the species and culture environment (Munson *et al.* 1954, Phillips *et al.* 1957, Delong *et* al. 1958, Lovell, 1972). For fish, the optimum amount of protein in formulated feeds is important because either low or high levels of protein may lead to poor growth. As well, excess protein in fish diet may be wasteful and cause the diets to be unnecessarily expensive.

Therefore, attempts were taken to investigate the requirement of optimum protein level, growth performance in formulated diets for *A. testudineus* fish. For carrying out feeding trial under laboratory conditions, rearing facilities were created and *A. testudineus* fish has been selected as the experimental fish.

A. testudinens fish were used to determine optimum level of protein requirement in formulated diets for their better growth, survival rate, FCR etc. This species was selected for there ability to survive in relatively polluted water, requirement of less dissolved oxygen in culture water and highly nourishing quality. There were many published reports on protein requirement for fresh water and marine fishes. However data showing protein requirements in diet for *A. testudineus* are comparatively few.

Therefore, in the present study attempts were undertaken to determine the growth performance and feed utilization of koi fish (*A. testudineus)* after feeding and rearing trial in six different tanks constructed and kept in the laboratory.

1.1 Objectives

The aim of the feeding trial is to evaluate the formulated feed on comparative basis with natural and live food organism. The experiment was conducted with the following objectives:

- to explore a suitable artificial feed for *A. testudineus* fry to obtain its maximum survival and growth.
- to develop culture techniques through determination of effective feeding rate and locally available suitable feeds in on-station and on-farm condition.
- to estimate the proximate chemical composition of feed ingredients to be used for the formulation and development of quality fish feed.

1. 2 Species summary

Anabas testudineus, Bloch, 1792
Climbing Perch

Anabas testudineus (Bloch, 1792)	
Family:	Anabantidae (Climbing gouramies)
Order:	Perciformes (perch-likes)
Class:	Actinopterygii (ray-finned fishes)
Common name:	Climbing perch
Max. size:	25.0 cm TL (male/unsexed)
Environment:	demersal; potamodromous; freshwater; brackish; depth range - 0 m
Climate:	tropical; 22 – 30°C; 28°N - 10°S
Importance:	fisheries: commercial; aquaculture: commercial; aquarium: commercial
Resilience:	High, minimum population doubling time less than 15 months(Assuming tm=1; Fec=40,000-80,000)
Distribution:	Asia: India to Wallace line including China. May have been distributed in more areas than were commonly reported.
Morphology:	Dorsal spines (total): 16 - 20; Dorsal soft rays (total): 7 – 10; Anal spines: 9 – 11; Anal soft rays: 8 – 11. Color in life dark to pale greenish, very pale below, back dusky to olive; head with longitudinal stripes ventrally; posterior margin of opercle with a dark spot; iris golden reddish. Body form variable, affected by age and amount of food consumed. Scaled head with 4-5 rows between eye and rear margin of preoperculum. Scales large and regularly arranged, ciliate.

Scientific Name: *Anabas testudineus*

Other scientific names appearing in the literature of this species: Lutjanus scandens, L. testudo, Perca scandens, Sparus scdandens, S. testudineus, Amphiprion scansor, A. testudineus, Anabas macrocephalus, A. microcephalus, A. scandens, A. spinosus, A. trifoliatus, A. variegatus, Anthias testudineus, Cojus, and cobujius,

Common Name: Climbing perch, climbing bass, kawai, koi, coi, kai, kou

Distinguishing features

Typically climbing perch are gray to green in color, with one dark spot at the caudal base and another just behind the gill plate. The edges of their scales and fins are brightly colored (Axelrod et al., 1971; Sterba, 1983). The opercule and preopercule are both serrated. The single dorsal and anal are both long. Fin counts are as follows: Dorsal= XVI-XVIII, 8-10, Anal= VIII-XI, 9-11, and Pectoral= 14-15 (Talwar and Jhingran, 1991). The body is moderately deep, its depth 3 to 3.5 times in SL. There are 21-29 scales in a lateral series. The mouth is fairly large and the teeth are villiform (Talwar and Jhingran, 1991). The elaborate labarynth organ is in a cavity above the third or upper portion of the first branchial arch (Jayaram, 1981).

Climbing perch are exceptionally variable in form over different portions of their range. There may be more than one species currently under the name Anabas testudineus (Roberts, 1989).

Biology

Anabas testudineus possesses a pair of accessory respiratory organ which enable it to breathe from air and survive out of water for prolong periods. These organs are in the foam of spacious air-chamber on either side of the skull, communicating freely with the tucco-pharyngeal cavity on one side and opercula cavity on the other. The respiratory epithelium lining the air-chambers is highly vascular zed. Besides this, a characteristic labyrinthine organ is lodged in the air-chambers which are Rosetta like structures made up of a number of concentrically arranged sholl- like plates, with wavy edges. These are, in fact, outgrowths from the branchial arches and evolution of the accessory respiratory organs of Anabas attracted considerable attention of the scientists in the past. *A. testudineus* was first introduced to the scientific literature in a memoir written in the year 1797 by Daldorf, a lieutenant in the service of the Danish East India Company at Tranquebar. The fish derives its name "the climbing perch" from the widely held belief that it can climb on trees which is actually the result of faulty observation. Anabas in certainly found lying very often in the forked branches of the trees or inside the today containers hung on palm trees, but they do not reach there by themselves. They are, in fact, dropped or placed there by crows and kites which catch the stranded fishes in drying pools or during their catch

their overland travels for migrating from one pond to another.

A. testudineus is considered as a valuable item of diet for sick and convalescent. According to Liem (1987), the fish contains high values of physiologically available from and copper essentially needed for hemoglobin synthesis. In addition, it also contains easily digestible fat of very low melting point and good many of essential amino-acids.

Over their native range, climbing perch occur mainly in low lying swamps, marsh lands, lakes, canals, pools, small pits, and puddles (Jayaram, 1981; Talwar and Jhingran, 1991). They are renowned for their ability to migrate between ponds over land. Migration is most common at night and after rain storms (Liem, 1987). They use mainly their tail and spiny opercula for this purpose (Liem, 1987). Climbing perch have specialized labyrinth organs under their operculum which enable them to breathe atmospheric air (Roberts, 1989). In laboratory experiments, over crowding and starvation are the main stimuli triggering land migrations (Liem, 1987).

Salinity tolerance

Over their native range climbing perch occur both in fresh and brackish waters (Davenport and Matin, 1990).

Temperature tolerance

Cold temperatures are believed to be responsible for the eradication of the population formerly established in Manatee County, Florida (Davenport and Abdul Matin, 1990).

Maximum Size

This species may grow to 250 mm (Axelrod et al., 1971; Courtenay et al., 1974; Sterba, 1983; Talwar and Jhingran, 1991).

Distribution

This species naturally occurs in India, Pakistan, Bangladesh, Ceylon, Burma, Sri Lanka, Thailand, Cochin-China, Tongking, southern China, Philippines, Polynesia, and Malaya (Axelrod et al., 1971; Jayaram, 1981; Sen, 1985; Talwar and Jhingran, 1991).

Bionomics & life history

Reproduction
Sexuality

Mookerjee and Mazumdar (1946) observed that during the breeding season, the female puts on a light brown color on the body and the fins, particularly the pelvics which turn deep brown. Usually, it appears in March-April and gradually disappears after spawning. The coloration actually indicates the ripening of the ova. The mature males during the breeding season acquire glazy black coloration.
Mature males, at the time of breeding, acquire reddish blue on the body; particularly on coloration on the body but their fine do show reddish hue, though faint. The black spot at caudal peduncle becomes very prominent. In males, the spot takes the shape of a diamond showing sharp boundary whereas in females, it is oblong and somewhat diffused. Fully ripe females have prominently bulged abdomen due to which the ventral distance between the bases of two pectorals measures markedly more than in males. With the onset of the breading season, females exhibit prominent outgrowth at the vent in the form of genital papilla when gently pressed at the abdomen. Males show complete absence of such a structure.

Mating

Studies on ova-diameter and gonadosomatic index of the fish reveal that *A. testudineus* has a single specific spawning, implying that each individual spawns only once during the breeding season.

Anabas testudineus is monogamous observed that whenever tow males were put with a female to form a breeding set, one of the males proved dominant over the other. In each such set, within an hour after injection, agitation movement was noticed with the dominant male trying to chase away the other male with sharp pushes and bites. The second male, however was observed to contribute significantly in the overall spawning season. It only hastened the spawning process but actually involved itself at the later phases of mating.

Fertilization

Fertilization is external. Unlike other Anabantoids, *A. testudineus* neither builds nest of bubbles for laying eggs nor it guards or care for the eggs.

Fecundity

Mookerjee and Mazumdar (1946) examined 3 specimens of almost same length/weight reared under laboratory and natural conditions to record the number

of ripe ova in the fish. The average number of ova worked out to be 4590.7 and 9934.6 for the specimens reared under laboratory and natural conditions respectively (Khan and Mukhopadhyay, 1972) made observations on the fecundity of the fish obtained from Port Canning in West Bengal and found the same to vary from 10002 to 36477 in the specimens measuring 99 to 169 mm in total length. The Log to Log relationship of fecundity to length and weight of the fish, worked out by them, are as under:

Log F = 1.9386 Log L + 0.1707 (r=0.7242)
Log F = 0.5877 Log W + 3.3517 (r=0.6856)

Anabas testudineus is notorious for migrating and breeding into prepared carp nurseries. This habit can be taken advantage of in breeding the fish in some of the sparable nurseries of a fish farm under inducement. This also helps in solving the problem of larval rearing to a great extent.

Behavior

Respiratory behavior

A. testudineus is an obligatory air-breather. Rao (1971) observed that *Anabas* gets asphyxiated sooner than Hoteropnoustes when not allowed access to the water surface. Mookerjee and Mazumdar (1946) observed that *A. testudineus* obtains 53.6% of its total oxygen supply from air and the rest (46.4%) from water. He also noted that the combined surface area of the labyrinthic organs and suprabranchial chambers in Anabas is greater than that of the gills which suggests that the fish has greater dependence upon air-breathing organs for respiration. The capacity of the smaller Anabas to survive for longer periods depending upon gill breathing alone than those of bigger ones may also be related up to certain extent to comparatively higher diffusing capacity of their gills in gaseous exchange. This is also evident from the fact that in smaller Anabas, the number of secondary lamellae/mm remains fairly high in comparison to the bigger ones Rao (1971). *A. testudineus* also activates. If its home pond dries up, it walks down to another body of water or burrows itself into the mud where it remains dormant through the dry season.

Feeding behavior

Mookerjee and Mazumdar (1946) reported that *A. testudineus* is seen to lay in wait and spring up to snatch grass-hoppers and other insects sitting on rice plants or weeds. Its eyes are capable of aerial vision. The fish is guided by its sight to prey upon its food. Rao (1971) stated Anabas is found in paddy fields where it feeds on paddy grains. In a way, it destroys paddy crop. Just before the crop is ready, the plant bonds. Anabas is then seen jumping out to bite the grain.

In laboratory the fish accepts almost anything form "idli" to cockroaches. When fed with paddy grains, it immediately swallows them, and then makes grinding sounds which are clearly audible form a distances and following this, it vomits out the husk. For this the fish processes and efficient grinding apparatus in the pharynx. It consists two sets of teeth, on the root of the pharynx is in three patches. An anterior T shaped patch consisting of a number of conical teeth. Behind this, there are two passage of teeth arranged side by side near the longitudinal arm of T shaped patch. In these two passage a number of teeth blunt surfaces remain crowded together so that a hard, uneven is formed very much resembling the face of a grinding stone. The ventral set of teeth forming the floor of the pharynx consisting of two passages. Both these passage are more or less triangular in shape and are so closely set that it appears as a single triangular patch. The sound that is audible when fish feeds paddy grains is evidently dear to the grinding of grains between these two sets of pharyngeal teeth. As a result of grinding the paddy grains are dehisced, husk is vomited and the ground particles are swallowed.

CHAPTER II

2. LITERATURE REVIEW

Shafi and Mustafa (1976) made observation on some aspects of the Biology of the Climbing perch, *(Anabas tesludineus)* in the city of Dhaka. The male and female fish showed an annual growth rate of 10 mm and 13.3 mm respectively. The length weight relationship in male and female was also determined. The sex ratio was apparently 1:1.

Nashida *et al.* (1986) worked with *Anahas testadineus* (Bloch) and observed a highly co-relation of the number of eggs spawned by the female *A. testudineus* with the size of the fish.

Nargis and Hossain (1987) investigated on the food and feeding habits of Koi fish *(Anabas testudineus)* and observed that small fish consume more food than that the large one. The mature fish fed less actively in May, due to spawning activity. The fish is an omnivore in nature.

Hussain *et al.* (1987) conducted an experiment on the semi -intensive culture of O. *niloticus* in two treatment viz. supplementary feeding treatment and

fertilizer treatment. He recorded 90% and 82% survival rate in supplementary feeding pond and fertilized pond respectively.

Guenero (1987) conducted an experiment on tilapia farming in Philippines and described a supplemental feed that composed of 20% ipil-ipil, 40% copra meal and 10% fishmeal. He advised that the feed to be administered for tilapia should be 10 to 20% of body weight.

Bairage *et al.* (1988) have made a comparison study between a few selected of Magur fry. They used Artemia, Salina, Naupli Zoo-plankton in alive and frozen from first four weeks of live of the fry of *Clarias batrachus* in aquarium. It was found that there was significant effects of the feed on the growth and survival of cat fish fry live. Artemia, Salina and Naupli gave the best result among the tested feeds.

Hussain (1988) stated that tilapia fry weighing between 0.5 to 1.0 g could be stocked in earthen pond at the rate of 25 to 30 ind/m2 and nursed for 40 to 60 days feeding with a mixture of SO% rice bran, 40% moc and 10% dried duckweed *(Lemna sp.)* at 8-10% of body weight installed twice or thrice daily. The attained final weight of fry was 5 - 10 gm within 40ty days.

Begum *et al.* (1988) have made study from the effect of various feeds on the hatching of *Cyprinus carpio* in aquarium with reference to temperature. Under laboratory condition a feeding experiment of *Cyprinus carpio* (Linn.) hatching was carried out in glass aquarium for a period of 30 days. They studied on the growth and survival rate of the experimental hatching were studied on seven different feeds of both artificial and natural origin. Growth rate and survival rate showed different pattern depending on the nature of ingredients of the formulated feeds. However know fetal effect was required due to temperature during study (10°C).

Nargis and Hossain (1988) made observations on the fecundity and sex ratio of the Climbing perch, *A. testudineus* and they found fecundity of *A. testudineus* to vary from 3739 to 72562 egg with mean 31171 ± 186.69 eggs. The number of egg was found to increase linearly with the increase of body length, ovary length, body weight, ovary weight and the standard length of the body. Male female ratio seemed to be - 33.97: 66.03.

Bhuiyan *et al.* (1989) have conducted a survey on the potential fish feed ingredients of Bangladesh on the basis of their availability and biochemical composition for suitable fish feed. A nation wide survey was conduced to identify potential fish seed ingredients. The survey covered materials that are being traditionally used and also non-conventional items such as kitchen waste, processed waste from the food/fish industry, aquatic weed etc. results of the survey indicate high potential of most of the enlisted conventional and non-conventional ingredients that can be economically used for the manufacture of quality fish feed. The most wanted protein percentage found was encouraging and can be summarized in these ways, less than 10% protein in 24 ingredients, 10-30% in 42 ingredients, 30-50% in 8 ingredients and more than 50% in 9 ingredients. As many as 35% ingredients were potential enough to be classified as protein supplement. Ingredients classifiable as energy supplement numbered 28. Twenty four ingredients were classified to be roughages. Most of the enlisted ingredients are abundantly available in the country and prices affordable to aqua farmers.

Getachew (1989) conducted an experiment on stomach pH, feeding rhythm and ingestion rate, in O. *niloticus* and reported that the fish ingested 11.5% feed of their body weight daily when average temperature was 21.5°C.

Hossain *et al.* (1989) have studied on the effects of artificial feeds on the growth of *I,abeo rohita (Ham.), Cirrhina mrigala* (Ham.) and *Hypopthalmichthys molitrix* (Vel.) in composite culture. The results of the study on the effects of different artificial feeds on the growth of *Labeo rohita (Ham.), Cirrhina mrigala* (Ham.) and *Hypothalmichthys molitrix* (Vel.) in river fertilized mini-ponds are presented. All the specie showed high growth rate with artificial feeding. Protein rich artificial feeds containing wheat bran, fish meal and mustard oil cake gave maximum growth rate. Wheat bran was better than the rice bran.

Ray and Patra (1989) conducted a laboratory feeding trial comprising 4 isonitrogenous diets containing 35% protein. The feed were formulated using soybean meal + mustard oil cake (Diet 1), groundnut oil cake + fish meal (Diet 2), ground nut oil cake + goat blood (Diet 3) and ground nut oil cake + animal carcass waste (Diet 4) as protein sources (Table 3). Fish having an initial weight of 10 g grew within 2 months up to 19g, 21g, 22g and 28g in response to dietary treatments 1 -4, respectively. Food conversion ration (FCR) ranged from 1.93 (Diet 1) to 1.24 (Diet 4) and protein efficiency ratio (PER) ranged from 1.5 - 2.3.

Sangrattanakhul (1989) concluded that the diet containing 37% protein was a reasonable formulation to be used for *Anabas* in terms of feed economics, PER, FCR and survival rate.

Watanable *et al.* (1990) studied the growth, survival and feed conversion ratio of Florida red tilapia of 8.78 gm average weight and stocked at densities of 100, 200 and 300/m^3 for a period of 84 days. Fish were fed on commercially prepared diet containing 28% or 32% protein. Final mean weights were higher for fish fed the diet with 28% protein (average = 176.8 g) than those fed with 32% protein (average = 166.4 g) under all density. The average daily gain was 1.94 g and average SGR was 3.54.

Kohinoor *et al.* (1991) made observations on the induced breeding of Koi fish (*A. testudineus)* in Bangladesh. A total 27 female *A. testudineus* were induced breed from March to June. Female fish were given simple injection of 8-12 mg PG/Kg body wt and male fish were given only 4 mg PG/kg body wt. Ovulation occurred in all injected females. Spawning occurred within 7-8 hours at the ambient temperature of 27-30°c.

Ahmed *et al.* (1991) worked on the effects of aeration on growth and survival of *Clarias gariepinus* larvae under culture. *Clarias gariepinus* larvae of 8 day old were reared in six fiber glass tanks under continuous aeration facilities and without aeration. The level of dissolve oxygen concentration was 4.5 -6.0 mg /L in the aerated tanks and .3 - .7 mg/L in the non aerated tanks. An increased growth rate and survival percentage of catfish larvae were recorded from the tank provided with aeration when compared with the non aerated ones.

Sarder *et al.* (1991) investigated on the effects of stocking density on the growth of African catfish C*larias gariepinus* fry. They observed growth of 7 day old C. *gariepinus* larvae (10.08 mg and 8.06 mm) were increased in the treatment with lower stocking density (250fry/tray) over an experimental period of *20* days. Similarly, percentage of growth was also higher in the larvae of above treatment when compared with the treatments stocked with higher densities (500 and 1000 fry/tray). However, highest mortality percentage was recorded from the treatment stocked with the highest density of catfish larvae and the lowest from the treatment stocked with 500fry/tray.

Mollah (1991) investigated on the effects of stocking density on growth and survival of catfish *(Clarias batrachns)* larvae. They observed on the basis of experiments with 7-day old *C. batrachus* larvae of initial total length 9.9t 0.8 mm at 5 different stocking densities for *28* days. It is recommended that a stocking density of 12 $fish^{-1}$ will be economic although the growth rate would be a little slower compared to those in lower stocking densities tested on the survival rate of the larvae until 21 days.

Nargis and Hossain (1992) worked on reproductive periodicity of climbing perch. They collected 211 specimens of *Anabas testudineus* during 13 months (May 80 - May 81). They determined sexual periodicity with different methods and result showed that the species has one annual breeding season.

Doolgindachabapom (1994) investigated a series of feeding trials was conducted to evaluate the effect of dietary protein level and stocking density on growth and mortality rates of *Anabas* fry weighing 1- 2 g. The results showed no significant differences in terms of growth and mortality rates, feed conversion ratio (FCR) and protein efficiency ratio (PER) among treatments; 30.6%, 36.7%, 41.1% and 47.3% protein levels. However, when fish were fed diets containing 21.7%, 26.4%, *31.8%* and 35.7% protein, there were significant differences between some of the treatments in terms of growth rate, FCR and PER. A third feeding trial with 30.6%, 36.7%, 41.1 % and 47.3% protein levels showed no significant differences between treatments.

Misra (1994) studied on the thyroid and internal cells *of Anabas testudineus* during breeding and non breeding seasons. Seasonal activity of the thyroid and internal cells of *Anabas testudineus* appears to be related to the annual ovarian cycle according to their study. The thyroid and internal cells show hyperactivity during breeding (May - July) and inactivity in the non -breeding (August - April) periods.

Leboute *et al.* (1994) conducted a trial with all male Nile tilapia fry, *O. niloticus* to evaluate their performance in cages, stocking with 4 different stocking densities (fish/m^3): 40, 60, 80 and 100 fish/m' was the best stocking density. After 5 months, mean body weights gain were 140.50, 84.10, 79.80 and 71.00 at densities of 40, 60, 80 and 100 fish/m' respectively. Mean survival rate was 93%.

Doolgindachabapom (1994) recommended that the feed containing 30.6% protein as the best feed formula in terms of growth and mortality for *Anabas*

fry, though fish fed 27% protein feed showed the best performance. The optimum stocking density was 20 fish larval 1-1.

Doolgindachabapom (1994) reported induced breeding of *Anabas testudineus* by means of hormone injection. Female *Anabas testudineus* were injected with common carp *(Cyprinus carpio)* pituitary gland. The whole pituitary gland, removed from 200 g carp, was used to induce female and male *Anabas testudineus* weighing 100 and 80 g, respectively. Females were injected intramuscularly below the dorsal fin. After injecting 2 doses, females were transferred to breeding tanks containing 30 cm of water along with 2 males. Females spawned approximately 12 hours after injection. The adults were removed from spawning tanks after fertilizing eggs and the depth water increase up to 50 cm. The fertilized eggs hatched within 18-20 hours at 25-27 ° C. Yolk sac was absorbed after 3-4 days.

Nargis and Hossain (1995) studied on the longevity of *Anabas testudineus* without taking surface air. They found the longevity of the fish under water without taking air from above water ranged from 55 to 385 minutes.

Kohinoor *et al.* (1995) investigated on the food size preferences of the Climbing perch, *Anabas tesludineus* (Bloch) and African catfish, *Clarias gariepinus* Burchell Larvae. They found a linear relationship between mouth size and total length of fish. The mouth size exhibited a close relationship to the size of their natural food. Anabas having a small mouth was found to ingest only rotifers while *Clarias* having a relatively big mouth size was found to take most of the zooplanktons, though they were found to eat only rotifers.

Alam *et al.* (1995) investigated on the effect of stocking density on the growth and survival of magur (*Clarias batrachus* Linn.). The experiment was made for period of 4 months, starting from 1st September to 31st December 1987 in the Fisheries Research Institute, Mymensingh, Bangladesh. The study was carried out according to 3x3 randomized block design. Three stocking density, viz., 2,500/ha, 5,000/ha and 10,000/ha were employed. In the experiment it was revealed that decreased stocking density favors increased growth rate and vice versa. Growth rate retarded gradually in respect of length and weight. On the other hand, the stocking density had a significant influence upon length and weight ($p<0.01$) but survival percentage remain same.

Saha *et al*. (1995) reported that the daily weight gain of *Labeo* rohita was 1.6 in closed loop re-circulatory system for fish production at central Institute of Fresh Water Aquaculture (CIFA), Bhubaneswar. The stocking biomass was, 8.75-kg/ m' with *L,abeo* rohita of 219.5 g size.

Faruq *et al.* (1996) made observations on the fecundity of *Clarias batrachus* (Linn.) and the relationship of fecundity with length and weight. The fecundity of *Clarias batrachus* was from 3028 eggs (total length 18.1 cm) to 9064 eggs (total length 26.2 cm). Average no. of ova per gram body weight found to be 57, no. of ova per gram ovary weight 362, and ova diameter 810 [tm. The regression equation *of* total length and fecundity relation ship were $y = -3249.66 + 448.89x$ with $r = 0.84$.

Khan, (1996) studied the growth performance and production potential of GIFT tilapia in cages under pond condition. The pond was situated on the northern side of Fisheries Faculty, BAU, Mymensingh and the experiment was undertaken over a period of 60 days from August to October. The initial length and weight were 8.18 cm and 10.29 g. After the period *of* study, the highest net gain in length and weight were 3.11 cm and 18.82 g and percentage net gain in length and weight were 38.02% and 182.89% respectively. The highest ADG (weight) and SGR were 0.313 and 1.70.

Mashiha Akter *et al.* (1997) worked on the Helminth parasite *of Anabas testudineus* (Bloch) in Bangladesh. One trematode and seven nematodes were collected from 90 out of 125 host fish. All nematodes are reported for the first time in the Climbing perch.

Ahmed *et al.* (1997) worked on the culture feasibility of African catfish *(Clarias gariepinus* Linn.) fry in glass tank and synthetic hapa system using supplemental diets. Effects of different feeds on growth and survival of African cat fish fry (10 days old) were determined in glass tanks for a period of 42 days. Two tanks and two ponds were taken for the experiment. One tank was named as tank *tubifex* and another tank was named as tank sabinco. One pond as pond tubifex and the other pond as pond sabinco. Live *tubifex* (protein 64.48%) was supplied to tank *tubifex* and pond *tubifex* and sabinco was supplied to the other two treatments. Growth of cat fish fry in *tubifex* pond in terms of both length and weight were significantly higher than those of the other treatments. !n terms of growth performance and survival rate *tubifex* pond showed better result.

Hossain and Shikha (1997) worked on the apparent protein digestibility (APD) of locally available feed ingredients such as leucaene, water hyacinth, wheat bran, rice bran and duck weed by the African catfish *(Clarias gariepinus)* at 30% inclusion level using a fish meal and soybean leased feed as the reference diet. The result showed that the APD of reference diet was fairly high (88.62%). The APD values of other test diets ranged between 80.34 to 83.52%. The APD in rice bran was 71.54 followed by water hyacinth (68.52%), duck weed (66.08%), wheat bran and leucaena. From the protein digestion coefficients obtained in this study, it is suggested that the above ingredients are suitable for inclusion in the diet of *Clarias gariepinus* at lower levels.

Kuddus *et al.* (1997) made observations on the length frequency distribution, length-girth relationship and fishing of singfish. Length-frequency data indicated that singfish grows for 4-5 years. In the first year, the fish grows to 14 cm and in the second year to *19* cm in total length. At the same age, males grow smaller than the females. Pond exhaustion was found as the most effective and widely used method for singfish fishing.

Rahman *et al.* (1997) conducted feeding experiments for 21 days to study the effect of live food (*tubifex*) and three prepared supplemental feeds on the growth and survival of 13 days magur (C. *batrachus)* fry. It was observed that the growth of fry varied significantly with different diets. The best growth was shown by the fry fed with *tubifex* followed by those fed with the diet containing yeast (30%), milk powder (30%) and chicken egg (30%). The poorest growth rate was given by the fry fed on yeast (45%) and fish meal (45%). There was no significant difference in survival rates and condition factors among the fry fed with live food and prepared feeds.

Sultana *et al.* (1997) conducted and experiment on comparative growth studies of Genetically Improved Farmed Tilapia (GIFT) and existing strain of Nile tilapia (O. nilolicus) in nursery system, at the freshwater station, Mymensingh. The nursery trials of GIFT and existing strains were conducted in hapas for two months. The initial mean weight of GIFT and existing strains of tilapia were 1.03 g and 1.12 g and the mean initial length were 3.75 cm and 3.79 cm respectively. The stocking density for both the strains was maintained at 150 fish /M3. Supplementary feed with 31.29% protein level consisting of rice bran (2S %), moc (1S %), wheat flour (30%) and fish meal (30%) was given at the rate of 8% of estimated body weight. After 2 months the final cumulative mean weight of GIFT and existing were 8.38 g and 5.51 g and the mean final length were 7.59 cm and 6.44 cm respectively. The net gain for weight of GIFT and existing strains of tilapia were 666% and 368% and the mean survival rate 95.75% and 81.25%, respectively. The GIFT strain showed significantly ($p<O.OS$) higher net gain in weight and also higher ($p<0.01$) survival rate than the existing strain.

Shahjahan (1997) conducted an experiment on culture of GIFT tilapia in cages for a period of 90 days from August to November in fishponds of Faculty of Fisheries, BAU, Mymensingh. The GIFT tilapia were stocked at a rate of 300 fish/m' in cages. The fish were supplied with 3 different supplemental feeds such as diet-1 (70% whet bran + 30% moc), diet 2 (kochu plants SO% +wheat bran 25% + moc 25%) and diet -3 (kochu plants 70% + fish meal 10% + wheat bran 10% + moc 10%). Fishes fed with diet 2 gave the best growth followed by diet - 1 and diet - 3. The net gain in length were 5.65 cm, 6.3 cm and 5.2 cm in length and 13.90 g, 15.80 g and 12.70 9 in weight for diet 1,2 and 3 respectively. The percentages of net gain were 212.28%, 228.97% and 198.76%, the ADG were 0.154 g, 0.175 g and 0.141 and the SGR were1.93, 2.04 and 1.83 for diet 1, 2 and 3 respectively. The production of fishes per cubic meter were 5.1 kg, 5.6 kg and 4.7 kg for diet 1,2and 3 respectively.

Sarker (1998) studied on a comparative growth and survival of sarputi and GIFT tilapia in relation to physico-chemical factors in the pond complex at the Faculty of Fisheries, BAU, Mymensingh for a period of 4 months from July to November, 1997. The initial length and weight of GIFT tilapia were 4.9 cm and 4.2 mg. The fishes were stocked in monoculture and polyculture (GIFT tilapia + Thai sarputi) system. After the study period (120 days) the net increase in length and weight recorded in GIFT tilapia were found to be 11.4 cm and 72.79 mg in monoculture system and 9.5 cm and 48.0 mg in polyculture system. GIFT tilapia was found to increase 232.7% in length and 1730.9% in weight in monoculture system. In polyculture system the fish increase 193.9% in length and 1170.7% in weight. The survival rate of GIFT tilapia varied between 69.5 to 72%.

Ali *et* al. (1999) evaluated the growth and economic performance of Nile tilapia, O. *niloticus* at BARI Regional Station, Habigonj. Fish reared in the irrigated boro rice field with different fertilizer levels. Results indicated that D. *niloticus* could successfully be reared in the irrigated boro rice field with recommended fertilizer level. Fish was

harvested at the end of boro season. The fish attained an average length of 5.88cm and average weight of 26.47 g in experiment I and average length of 8.9 cm and weight of 45.04 g in experiment II. The ADG were 0.053cm and 0.072 cm in length and 0.236 g and 0.361 g in weight respectively.

Kohinoor *et al.* (1999) studied the growth of red tilapia and nile tilapia (O. *niloticus)* in 6 ponds of fresh water station, Mymensingh, for a period of 6 months. Three ponds were stocked with fingerlings of *O. niloticus* (average weight 11.4 g), other 3 ponds were stocked with red tilapia (average weight 10.72 g) at a density of 20,000 fingerlings /ha. Supplementary feed consisting of rice bran was given daily at 4-6% of standing biomass. Ponds were fertilized at fortnightly intervals. After 6 months of rearing, nile tilapia attained an average weight of 125 g as compared 151 g attained by red tilapia. The ADG ranged from 0.40 to 1.37 g in case of red tilapia and 0.37 to 1.20 g in case of nile tilapia.

Leena *et al.* (2000) worked on the carp growth hormone on lipid metabolism in a teleost, *Anabas testudineus* (Bloch). Three doses of CGH were given as intra peritoneal injections (0. 1, 0.2 and 0.5 mμg/gm/body weight/day) for 10 days. CGH stimulated the activities of two lipogenic enzyme while inhibiting malic enzyme activity. Results indicate a lipogenic action of CGH in *Anabas testndineus* and pattern of lipid mobilization or deposition depends on the concentration of hormone injected.

Wijeyaratne and Perera (2000) studied on the food and feeding habits of five indigenous fish species namely *Iaroplus maculatus, Rasbora daniconius Anabas testudinens* and *Mystus vittatus* co-occurring in minor irrigation reservoirs in Sri Lanka where aquatic macrophytes are abundant. They observed that the smaller species feed on aquatic macrophytes and decaying plant matter larger individuals are used as food fish and the small ones have a high demand in the ornamental fish industry.

Sekino and Hara (2000) of Japan worked on seven natural populations of Climbing perch, *Anabas testudineus* collected from the central, eastern and peninsulararеas in Thailand. Allozymic analysis was carried out to examine genetic relationships among geographic populations. Significant differences in allelic frequencies were observed in all pairs of populations at one or more location. Six locations showed the regional characteristic in accordance with allelic compositions.

Santhakumar *et al (2000)* made observations on the effect of monocrotophos on the optomotor behavior of an air breathing fish, *Anabas testudineus* (Bloch). Behavioral

Changes were studied by exposing fish *Anahas testudineus* for 21 days to three sub lethal concentration of monocrotophos -1.9, 4.75 and 9.5 mg/L. Fish behavior were measured One was hypoactive and another was lethargy.

Santhakumar and Balaji (2000) made observations on the acute toxicity of insecticide monocrotophos to the fresh water fish *Anabas testudineus* using static bio assay method. The 24, 48, 72, and 96 hours LC50 were found to be 22.65, 21.2, 14.75 and 19 respectively. The calculated safe concentration of monocrotophos was 0.19 ppm. Decrease in opercular movement, loss of equilibrium, increase in surfacing behavior, change in body color, increase in mucus secretion all over the body, irregular in mucus secretion all over the body, irregular swimming activity and aggressiveness were observed in fish on exposure to monocrotophos.

Santhakumar *et a1.* (2000) studied on the pathological effects of monocrotophos on the brain by exposing the fish to sub lethal concentrations-1.9, 4.75 and 9.5 ppm for 21 days. The histopathological changes were found to be dose dependent. The pesticide produces rupture of cortex, atrophy of molecular and granular bys, vascular dilation, nuclear pyknosis, fibrosis, vacuolation, cerebral odema and interzonal detachment. Such change may affect the proper survival of the fish in its aquatic environments.

AL-Harbi and Siddiqui (2000) investigated on the effects of feed input and stocking density (1, 5, 10 and 15 kg m') of hybrid tilapia *(Oreochromis niloticus x O. anrerrs)* on growth and water quality each density treatment was replicated three times, and stocking densities were reported by removing fish at 14-day intervals. Fish were fed a 34% protein feed twice daily. Both feeding rates (% body weight day^{-}') and percentage increase in biomass decreased significantly with increasing stocking density, but feed conversion efficiency was not significantly affected. Ammonia nitrogen, nitrate, nitrogen and total phosphorus increased while DO decreased as the stocking density of fish and feed input increased.

Masiha *et al.* (2001) studied on the histopathology of climbing perch, *(Anabas testudineus* Bloch) associated with tissue penetrating nematodes. They identified seven species of nematodes recovered

from the stomach, intestine and from body cavity, histopathology shows that *Teylamema spp*. are associated with the development of fish tuberculosis.

Leena and Oommen (2001) made observations on growth hormone and prolactin action in a teleost *Anabas testudineus* (Bloch). They observed differences on the effect of morning versus evening injections of growth hormone and prolactin in a teleost *Anabas testudineus.* Early morning administration of growth hormone increases malic enzyme, glucose -6- phosphate dehydrogenase and isocitrate dehydrogenase activities while evening administration of growth hormone does not affect these enzymes. The results reveal that a given dose of hormone may provide a different message to the target tissues at different period of the day.

Santhakumar *et al.* (2001) investigated on the gill lesions in the perch, *Anabas testudineus* exposed to monocrotophos. Histopathological effects of sub lethal doses of monocrotophos on the gills have been studied by exposing the fish for a period ranging from ten to twenty days. The extent of damage of gills was dependant on the dose and duration of exposure.

CHAPTER III

3. MATERIALS AND METHODS

The experiment was conducted in 8 cemented tanks of equal size (12×6×1.5 feet) for a period of 60 days at Tongi Fish Seed Multiplication Farm, Gazipur. Four different feeds were used in this experiment to observe their effects on growth and survival of *A. testudenius*

3.1 Fry source

The fry of *A. testudenius* used in this experiment were obtained from a private hatchery named Reliance Aqua Farms situated at Bailor, Trishal, Mymensingh. Fry's were carried to the study area under well oxygenated conditions

3.2 Experimental design

Eight cemented tanks of 12×6×1.5 feet each were used in this experiment. The tanks were divided into four treatments namely treatment I, II, III and IV each having two replications. Four different feeds namely Feed I, Feed II, Feed III and Feed IV were applied to the treatment I, II, III and IV respectively. 15 days old 100 fry of *A. testudenius* of almost equal sizes were stocked in each of the tank.

3.3 Preparation of the tanks and release of fry

Cement tanks with 2-4m3 water volume was used for nursing Koi fry. The tank was filled with well filtered water approximately one week before stocking. Water outlet of tanks was covered with proper mesh size net. The mesh size was changed (increased) during fish rearing to facilitate washing out the feces and waste food. The proper mesh size was 1.0-1.5 mm at beginning of tank rearing. After the preparation of the tank the collected fingerlings were gently acclimatized with the tank water and released carefully.

3.4 Management of the tanks & fry

For maintenance of good health and growth of fry, frequently cleaning of tanks and feeding of fry are necessary. Further frequent prophylactic treatments of tank are necessary. Accordingly, all the tanks were rubbed daily for removing bacteria accumulated on inner surface of rearing tanks. Feces, waste particles of food and dead bodies of fish were siphoned at regular interval.

From the 5^{th} or 6^{th} day of rearing antibiotic treatment of was used. The most efficient antibiotic is Terramycin.Terramycin is suitable for prophylactic treatment also. Four-six tablets of terramycin (2-3 g) produced for veterinary treatment can control the outbreak of bacterial disease and were used in the experiment accordingly. Moreover, when there was deterioration in water quality 10-20% water was replaced. Chemical treatment of fry was not necessary throughout the experiment.

3.5 Collection of feed ingredients

Sabinco feed, fish meal, Soybean meal, rice bran, wheat bran and vitamin pre-mix were collected from local fish and poultry feed traders of Gazipur town. Poultry viscera were collected from local Kacha Bazar. Rodovit GSS of Rhone Poolenc was used as vitamin pre-mix.

3.6 Feed formulation and preparation

3.6.1 Fish feed manufacturing

The following factors are to be considered during fish feed manufacturing:

Physical inspection

Preliminary physical inspection of raw materials to be used as ingredient: Physical inspection includes:

- evidence of wetting; mold growth confirms water damage;
- presence of scrap metals, stones, dirt or other non-biological contaminants;
- presence of insects.

Biochemical analysis

"Weende" analysis for proximate composition of both raw material and finished diet. It includes:

- moisture, ash, crude fiber, crude lipid, nitrogen free extract, non-protein nitrogen;
- acid insoluble ash (for materials having high ash content);
- salt;
- calcium and phosphorus;
- amino acid spectrum;
- determination of toxic substances in the ingredients, like urease, gossypol, aflatoxins;
- determination of total sugar using molasses.

Formulation and manufacture of fish diet

Depending on the chemical composition and caloric values of the different ingredients dietary formulae will be prepared. Physical properties that are to be considered include:

- size;
- flavor and odor;
- texture;
- color;
- density (sinking rate);
- dehydration capacity;
- water stability.

Diets in the form of meal, paste and cakes will be tried; however at the later stages pellets (soft) will be prepared. Preparation of flake diets may also be considered.

3.6.2 Requisites

a) Materials

The following materials were required to carry out the whole experiment as per schedule:

- 8 cemented tanks each of size 12×6×1.5 feet;
- A total of 800 (10 % mortality) fry of *A. testudenius* of about 0.28 g each.

b) Equipment

- Electrical balance;
- Thermosthatically controlled oven;
- Kjeldahl apparatus;
- Muffle furnace;
- Porcelain crucibles;
- Desiccator;
- Soxhelt apparatus;
- Heating units for fibre determinations;
- Glassware (volumetric flask, Erlenmeyer, beakers, burettes, pipettes);
- Homogenizer.

c) Chemicals

Different kinds of chemicals will be required to carry out the proposed experiment for complete proximate analysis, such as H2SO4, HCl, NaS203, NaOH, isopropanol, boric acid, mercury oxide, potassium sulphate, Ba(OH)2, and some others.

These personnel work under the direct supervision of the project co-investigator throughout the study period. Their availability should be ascertained to the project investigator as per work schedule.

3.6.3 Feeding trials

Feeding trials are conducted in pond series, depending on the available facilities at FARS. Feeding will be done for each kind of fish separately. The following are to be recorded:

- species of fish;
- type of fish;
- sex;
- age;

- number of animals in treatment;
- physiological state;
- percent of test ingredient in ration;
- length of trial;
- daily dry matter consumed.

This information is to be used for studying some of the following parameters depending on the importance and existing facilities in the laboratory:

- measuring indexes of growth and food intake;
- indexes of food utilization;
- general relationship between food and growth;
- growth efficiency;
- body size, food and growth and different rations;
- factors influencing the use of food for growth;
- effect of temperature on food-growth relations;
- effect of food quality on growth;
- effect of crowding.

3.6.4 Preparation of diet/feed

Table 1: Composition of the test feeds (%)

Ingredients	Feed - I	Feed II(Sabinco)	Feed - III	Feed - IV
Fish meal	18.0	40	30.0	22.0
Soybean meal	8.0	25	15.0	11.0
Wheat bran	30.0	15	21.0	20.0
Rice bran	43.0	19	31.0	44.0
Poultry viscera	-	-	2.0	2.0
Vitamin & Mineral mixture	1	1	1.0	1.0

The selected ingredients as mentioned above will be used for the preparation of three different isocaloric fish feeds, denoted by Feed I, III and IV according to the treatment groups, by mixing the ingredients in such a manner so as to give crude protein values of 20, 30 and 24.7 % respectively. The amounts of ingredients needed to prepare 1 kg of feed should be calculated from their proximate chemical composition and adjusted in such a manner that all the feeds contain nearly the same amount of energy per kg of feed. The feed should be made into bite size pellets by adding starch solution or liquid from boiled rice and dried in an oven at 40° C for 2 days; or be extracted as pellets from a pelleting machine and stored, sealed and frozen until used. The pelleted feeds have many advantages over the conventional feeding system, since it keeps down wastage of feed to a minimum and further also provides a good way of effective checking food utilization by the cultured fish (Jeychandran & Paulraj, 1977). These pellets were spread out on polythene paper and allowed to sundry for 4-6 hrs. Then the dried feed was stored in an air tight plastic bag. In case of feed D, before preparation of feed the chopped poultry viscera was boiled and mixed with food ingredients.

3.7 Proximate analysis of the test feed

Proximate composition of the test of feeds were determined following the standard methods given by Association of official Analytical Chemists (AOAC, 1980) in the Nutrition Laboratory of Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. The proximate composition of different feeds is shown in Table-2

Table 2: Proximate analysis (% dry matter basis) of test feed

Parameters	Feed - I	Feed – II	Feed - III	Feed - IV
Dry matter	86.70	88.90	87.78	89.62
Crude protein	20.60	50.92	30.10	24.7
Crude lipid	11.61	8.35	10.40	12.01
Ash	12.30	16.36	15.30	13.80
Moisture	13.30	11.10	12.22	10.38
NFE*	31.80	24.36	9.9	20.3
Energy (Kcal/100g)	419.25	580.6	472	470. 5

- Nitrogen Free Extract calculated as: 100 - %(Moisture Protein + Lipid + Ash + Crude fiber).

3.8 Methods of feeding

There are several physical and chemical requirements for artificial dry feeds. The feed must have the correct particle size (0.35–0.50 mm for fry of 50–100 mg; 0.50–0.75 mm for 100–250 mg fish; 0.75–1.25 mm for 250 mg-1 g fish). The fry must be able to recognize the feed chemically and optically. The feed particles must be water-stable to restrict nutrient leaching. The feed must have a low moisture content (<10%) to allow good storage and the complete range of nutrients required for fry must be present in each particle.

After acclimatization of the released fish in the tank the *A. testudineus* fry were fed with Sabinco food. and three other prepared supplemental feeds at the rate of 10%- 5% of total body weight of stocked fry twice daily up to satiation at 09:00 and 17:00 hrs. In the early stage the feed was diluted with small amount of water and then it was applied in the tank and later on it was spread directly. The fry were considered to be satiated when they stopped feed up taking or searching for food. After half an hour of feed supply the uneaten food particles and feces were removed by siphoning. The dead fishes were removed as soon as they were detected.

3.9 Sampling & health monitoring

Sampling was done at 7 days interval and random samples of 20 *A. testudineus* fry were caught by glass nylon hapa from all the treatments. The fry were checked once a week and inspected for signs of malnutrition or disease. Dead fishes were immediately removed from the tank. At high stocking densities malnutrition can manifest itself and disease can spread rapidly. Inefficient feeding practices also place the fish under stress which can result in parasitic infections, particularly monogonean trematode and/or *Trichodina* infections. However, during this stage, sibling cannibalism was probably the greatest cause of mortality.

Total length and weight were recorded using a graph paper attached Petri dish and a Metler AJ 100 digital balance respectively. After careful measurement the fry were released in the respective place.

3.10 Evaluation of the water quality parameter

Water quality parameters such as temperature, pH and dissolved oxygen were monitored every 7 days of interval during the experimental period. Temperature was recorded using a Celsius Thermometer; dissolved oxygen and pH were measured directly by a portable digital DO meter (WPA OX 20) and a portable digital pH meter (WPA CD 70).

3.11 Growth parameters

The following parameters were used to evaluate the growth:

Survival rate, weight gain (WG), specific growth rate (SGR), food conversion rate (FCR), were calculated using the as following formulas:

Survival rate (%) = 100* (final number of fish/initial number of fish)

Mean daily weight gain (MWG) (g/day) = (Wf-Wi)/Δt

Specific growth rate (SGR) (%/day) = 100*(LnWf-LnWi)/Δt

Feed conversion ratio (FCR) = Fd/(Wf - Wi)
Where**:** **Wf:** final weight
Wi: initial
Δt: duration (days)

Fd: feed used Data collected were subjected to one-way ANOVA analysis, and then to Duncan multiple range test ($p<0.05$) to compare differences in treatments using SPSS software.

3.12 Survival rate

Survival rate was monitored and recorded once a week. After 60 days all the tanks were dewatered and all the fish were counted to find out the final survival rate of respective treatment.

3.13 Statistical analysis

All the structured designs and data were analyzed using a one-way ANOVA. This included significant results ($P<0.05$) were taken as rejection of the null hypothesis-significant differences between the treatments. Significant results were further analyzed using Duncan's Multiple Range Test in order to determine ranking and significant differences between treatment means. These results are displayed as superscripts against each respective value. All statistical analyses were carried out using the computer package Stat graphics (version 7.0).

CHAPTER IV

4. RESULTS

4.1 Growth

The initial average length of fry of *A. testudineus* increased from 14 mm to 40 mm, 15 mm to 66 mm, 14.5 mm to 56 mm and 14.2 mm to 51 mm in T_1, T_2, T_3 and T4 treatments respectively after the experimental period. The initial average weight of *A. testudineus* fry increased from 0.9 g to 3.5 g, 1.0 g to 7.3 g, 0.95 g to 5.82 g and 0.92 g to 5.4 g in the treatments T_1, T_2, T_3 and T4 respectively. The highest and lowest average final length was found in T_2, and T_1 respectively. Again the highest and lowest average final weight was also found in the same treatments respectively. The highest survival rate was found in T_2. The condition factor was 0.83, 0.83, 0.78 and 0.71 in T_1, T_2, T_3, and T4 respectively.

4.2 Survival

The survival rates were recorded 74, 85, 81 & 79 % in the treatments I, II, III & IV respectively**.** However, these differences were not significant ($P>0.05$) among treatments of each experiment.

The differences in results amongst replications of a treatment were invariably small. Consequently, all values for a treatment were determined by combining the results of replications and averaging them.

Table 3: Growth parameters and survival of *A. testudenius* fed on different test feeds

Parameters	T_1	T_2	T3	T_4
Initial length (cm)	14	15	14.5	14.2
Final length (cm)	40	66	56	51
Initial weight (g)	0.9	1.0	0.95	0.92
Final weight (g)	3.5	7.3	5.82	5.4
Condition factor	0.83	0.83	0.78	0.71
FCR	4.63	2.88	3.50	3.92
Survival rate (%)	74	85	81	79
Specific growth rate (% day/fish)	0.98	1.43	1.30	1.26

Means in the same row with different superscripts are significantly different ($P<0.05$).

4.3 Water quality parameter

The physico-chemical parameters of water ponds under different treatments during the trial are shown table-4 Temperature pH and DO of water in ponds under different treatments ranged between 28.8 and 29.8 ^{0}C, 7.6 and 8.9 and 5, 5 and 6.2 mg/l.

Table 4: Water quality parameters of the pond during the experiment of different test feeds

Parameters	T_1	T_2	T_3	T_4
Average temp. (^{0}C)	29.6	28.8	29.2	29.8
Average pH	7.9	8.1	7.6	7.8
Average DO (mg/l)	5.7	6.2	5.9	5.5

Table 5: Growth of *A. testudineus* fry in cement tanks from September 12 to November 15, 2005. (replications averaged).

Feed (Treatments)	Sample day									
	1		10		25		45		60	
	Length (mm)	Weight (gm)	Length (mm)	Weight (gm)	Length (mm)	Weight (gm)	Length (mm)	Weight (gm)	Length (mm)	Weight (gm)
T_1	14	0.9	18	1.35	24	1.92	34	2.8	40	3.51
T_2	15	1.0	24	1.9	35	3.52	53	5.89	66	7.3
T3	14.5	0.95	21	1.75	30	2.8	45	4.73	56	5.82
T4	14.2	0.92	20	1.65	28	2.58	42	4.44	51	5.4

Table 6: Total number of fish and survival rate from September 12 to November 15, 2005 by treatment (replications combined) where "No." = number of fish and "S.R." = survival rate (%).

Feed (Treatments)	Sample day									
	1		10		25		45		60	
	No.	S.R.	No.	S.R.	No.	S.R.	No.	S.R.	No.	S.R.
T_1	100	100	92	92	84	84	78	78	74	74
T_2	100	100	95	95	91	91	87	87	85	85
T3	100	100	91	91	87	87	83	83	81	81
T4	100	100	89	89	85	85	81	81	79	79

Table 7: Biomass (total weight of fish) of *A. testudineus* fry from September 12 to November 15, 2005, in gram in cement tanks (replications averaged).

Feed (Treatments)	Grams				
	day 1	day 10	day 25	day 45	day 60
T_1	90	124.2	161.28	218.4	259.74
T_2	100	180.5	320.32	512.43	620.5
T3	95	159.25	243.6	392.59	471.42
T4	92	146.85	219.3	359.64	426.6

4.1.4 ANOVA Result

ANOVA is done with a view to finding out any significant difference among the SGR%, Feed efficiency and Survival (%) of *A. testudineus* fry on Sabinco Feed and three types of formulated Feed in four pairs of experimental tanks with replications. The F value from the result of SGR (%), Feed efficiency and Survival have been found to be 5.63, 310.94 and 2.8. SGR (%) and Feed efficiency are significant at 5% level but Survival is non-significant.

Duncan's New Multiple Range Test is done with a view to finding out which fish feed will be the best in bringing growth and increasing weight, length etc. with proportional amount of nutrients.

Table 8: ANOVA summary under different replications and experimental tanks to find out significance in SGR% fed on formulated fish feed

Source	SS	df	MS	F
Treatment (between groups)	1.8554	3	0.62	5.63*
Error	0.4523	4	0.11	
Total	2.3077	7		

* Significant at 5% level

Table 9: ANOVA summary under different replications and experimental tanks to find out significant difference in feed efficiency fed on formulated fish feed

Source	SS	df	MS	F
Treatment (between groups)	1492.54	3	497.51	310.94*
Error	6.40	4	1.60	
Total	1498.94	7		

* Significant at 5% level

Table 10: ANOVA summary under different replications and experimental tanks to find out significant difference in survival (%) fed on formulated fish feed

Source	SS	df	MS	F
Treatment (between groups)	1.2655	3	0.42	2.8**NS**
Error	0.5921	4	0.15	
Total	1.8576	7		

NS: Non-significant

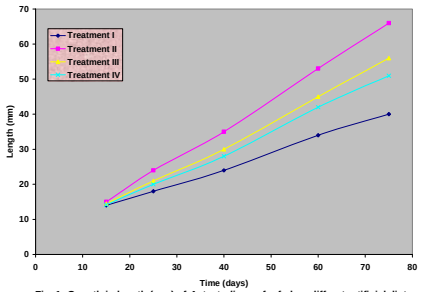

Fig. 1: Growth in length (mm) of *A. testudineus* fry fed on diffrent artificial diets for 60 days

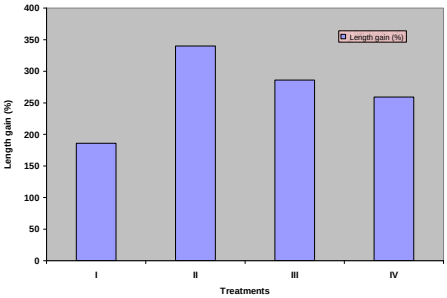

Fig. 3: Percentage increase in length of *A. testudineus* fry fed different experimental diets for 60 days

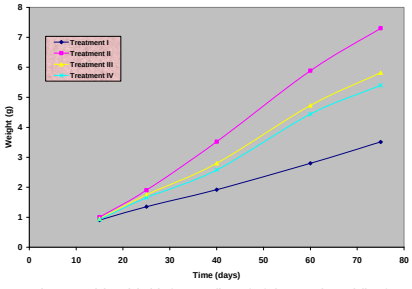

Fig. 2: Growth in weight (g) of *A. testudineus* fry fed on experimental diets for 60 days

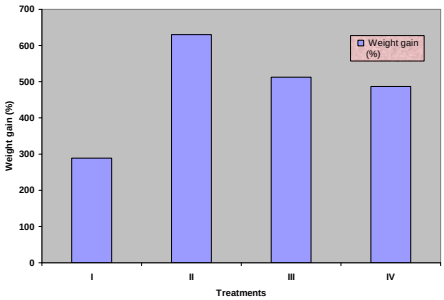

Fig. 4: Percentage increase in weight gain of *A. testudineus* fry fed different experimental diets for 60 days

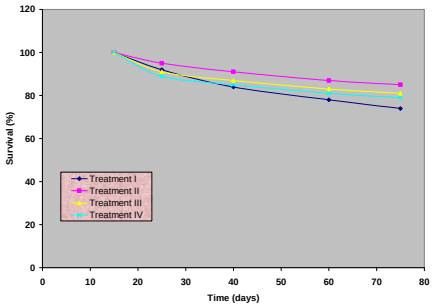

Fig. 5: Survival (%) of *A. testudineus* fry fed on different experimental diets for 60 days

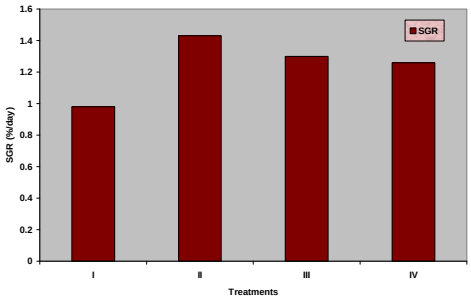

Fig.6: Specific Growth Rate (SGR) of *A. testudineus* fry at the end of experimental period in responce to different diets

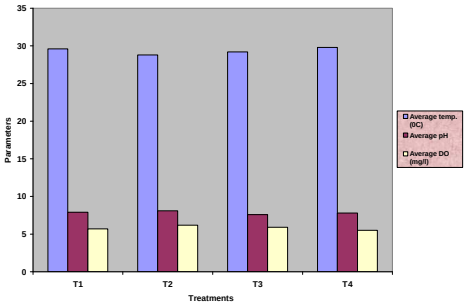

Fig. 7: Water quality parameters during the experimental period

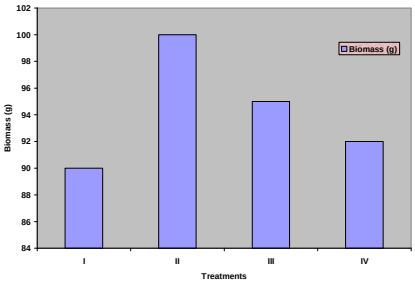

Fig. 8: Biomass (g) *A. testudineus* fry at the time of stocking

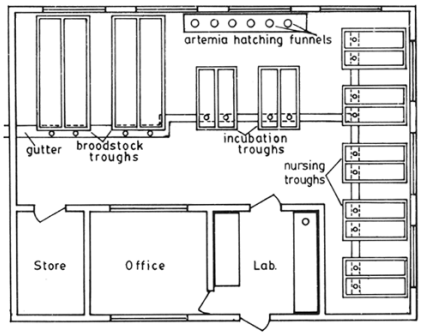

Fig. 10: Lay out of the hatchery

Fig. 11: *A. testudineus* fry

CHAPTER V

5. DISCUSSION

For successful culture of any fish suitable feed is one of the most important prerequisites. Availability of quality feed or ingredients of feed are the vital factors that affect commercial fish culture. In the present study Feed II was Sabinco Feed. Feed I, Feed III and IV were prepared from locally available ingredients. Among the feeds, Feed II contained highest protein percentage (on dry matter basis) followed by Feed III, IV and I. Better growth, survival and FCR were observed in fish fed with Feed II Feed III produced second highest growth and survival.

5.1 Growth

Growth and production in fish culture are generally dependent on the daily feed consumption, qualities of

feed and feeding frequency (Mookerjee and Mazumdar ,1946). According to Chakraborty *et al.* (1995) the growth of carp (*Cyprinus carpio)* increases with protein levels, and there was an approximately linear increase of growth with feeding level for any given diet. Authors also mentioned that increasing the feeding level resulted in a larger amount of ingested energy available for growth.

At the end of the present experiment on rearing fish in concrete tanks, fish growth was not significantly different between treatment III and IV, but was significant between treatment I and II. The best growth was observed in treatment II with fish fed dietary that contained 50.92% protein. Results showed that fish fed on Sabinco feed grew with the weight gain of 0.11 g/day and SGR of 1.43%/day that were significantly higher ($p<0.05$) compared to other treatments. The significant lower growth was observed in fish fed Feed-I that the growth rate and SGR were 0.04 g/day and 0.98%/day, respectively. There was no significant difference ($p<0.05$) between treatment III and IV. The mean weight and length of fish in treatment II were the highest because of high protein content in the test diets.

In the present experiment maximum average length and weight of 66 mm and 7.3 g respectively of *Anabas testudineus* fry were obtained from the treatment fed with Sabinco feed (containing 50.92% protein). The experimented result is in agreement with the report of Sangrattanakhul, C. (1989) in which he estimated required dietary protein level for climbing perch ranged from 35-45%. Doolgindachabaporn (1994) also recommended that the feed containing 38.6% protein as the best feed formula in term of growth and survival for *Anabas* fry. Mookerjee, H.K. and S.R. Mazumdar, 1946, tested the performance of different diets containing 30, 34.7, 39.5, 44.1 and 48.9% protein in dry weight basis and reported that 39.5% protein is optimum in diet for commercial rearing of *Anabas testudineus*. In this experiment, the highest growth and survival were found with the Sabinco Feed containing 50.92% protein. In a trial conducted by Ray and Patra (1989) he indicated that climbing perch can achieve a rate of growth from 0.5-0.9 g/day when culture in earthen pond. The daily weight gain was observed in fish fed Feed-II that was .105. The experimented result is in agreement with the report of Sangrattanakhul (1989) reported that the ADG of *A. testudincus* fish ranging from (0.10-0.12). The above finding has more or less similarities with us.

5.2 Survival rate

After 60 days of culture period, fish in treatment I showed the lowest survival rate that was 74% and the highest survival rate was 85%. However, there was no significant different ($P>0.05$) among the survival rate of fish in treatment I, II, III and IV where survival rates were 74%, 85%, 81%, and 79% respectively. Mookerjee and Mazumdar (1946) in a study with climbing perch with prepared feeds (containing 35-45% protein) observed survival rate ranging from75%-89%. This observation is within our observed value of survival rate of *A. testudineus*. High survival rate of climbing perch was also reported by Rao (1971). These indicated that climbing perch is an excellent candidate for aquaculture perhaps more so than other culture species. Rearing fish using artificial food gave higher survival rate. The present results were higher at treatment II and III, but lower at treatment I. In general, treatment II gave the best results among experimental treatments.

5.3 Specific Growth Rate

It is evident from the results of SGR values of *A. testudineus* fish fed on Sabinco Feed and formulated feed represented in table 3 that with the increase of age the values of SGR decrease. From this point of view the formulated feed II gives best result in comparison with the other three feed.

This finding resembles the Medawars (1945) fifth law "the specific growth 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 and his epigram. “Organism age fastest, when they are young" is expressed by Medawars (1945) fifth law. The SGR% value of koi fish in our experiment also shows the same trend mentioned in Medawars *(*1945*)* fifth law.

5.4 Feed conversion ratio (FCR)

FCR were higher in the diets with the lowest protein content. In this experiment fish fed Feed I had the highest FCR that is 4.63. Fish fed Sabinco feed performed the lowest (2.88), and no significant differences were found in treatment III and treatment IV that was 3.50 and 3.92 respectively at ($p<0.05$) level. Doolgindachabaporn, (1994) found that the FCR value of *A. testudineus* ranges from 1.8-3.0. Chakraborty (1999) found that the FCR value of *Labeo rohita* 1.30-1.99. The above findings are similarities with us.

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

respectively. There were some explanations, firstly in other trials that have been conducted on small size of fish (2g) and ended when fish reach to the size of 10-12g, with bigger size of climbing perch may be resulted in higher FCR. Secondly, most of fish in present experiment matured from the 3rd month that caused slow growth. Maturation of climbing perch is a critical in commercial farming.

In this study, feed conversion ratio was higher at higher feeding level due to feed losses increased with feeding level. Moreover, increase of feed conversion ratio at feeding rates might result from the in-completed digestion of feed Rao (1971).

5.5 Water quality parameters in the experiment

During nursing period, most water parameters in cement tank were in suitable ranges for fish growth. Dissolve oxygen ranged from 5.5 to 6.2 mg/l whereas temperature ranged from 28 to 29.8˚C. The water temperature and dissolved oxygen in all experimental tanks are showed in table 4. Although there were little fluctuation in the parameters of water temperature and dissolved oxygen concentration from morning to afternoon in three treatments of experiment, but the ranges of these values were still suitable for the growth of climbing perch fry (Khan *et al.*, 1996).

CONCLUSION

Koi fish (*A. testudineus)* is traditionally a popular type of fish in this region. This fish is highly popular for its high nourishing quality and prolonged freshness. It is found in most of the fresh water bodies and is abundantly available from June to September. The investigation reported here had been carried out with the main objective to find the fish growth (*A. testudineus)* fed on formulated fish feeds reared in eight different tanks for a period of 60 days. Objective of the experiment also included to find the feed utilization by the experimental fish (*A. testudineus).* Optimum level of protein for the fish growths was also determined. Sabinco and three formulated fish feeds were used in the feeding and rearing trial of the fish (*A. testudineus).* Different bio parameters, such as condition factor, survival rate, feed conversion ratio (FCR), specific growth rate (SGR) etc. were used to see the growth performance and feed utilization during the study period. Data were analyzed statistically using ANOVA & DMRT (Duncan's New Multiple Range Test). Probabilities of 0.05 were considered statistically significant.

Specific growth rate (SGR %) of the koi fish (*A. testudineus)* during the present study period were also determined. SGR were found ranging from 0.98-1.43.

Feed conversion ratio (FCR) of the koi fish were computed at different time intervals with a view to determining the ratio between feed and converted flesh. Result showed FCR value ranged from 2.88-4.63 (at the end of 60days study period).

Effects of water temperature on feed intake, gain in weight, SGR, FCR, of the koi fish *(A. testudinetis)* had been observed. The observation revealed that feed intake is temperature dependant, if the water temperature remains between (27-30°C).

Water temperature has been found to have strong and negative correlation with SGR%. Maximum SGR% was reported in the early developmental stages.

FCR of the experimental fish was found to have negative correlation with the water temperature (27-30°C).

Weight gain had been found to be temperature dependent. Gain in weight increased with the increase of water temperature (27-30°C).

The following conclusions may be drawn on the basis of the observations made during the rearing and feeding experiment of the koi fish (*A. testudineus)* in eight different tanks, fed on four different types of prepared fish feed in the laboratory.

A. Proximate composition of the fish Feeds were determined and have been found to have 20%, 50.92%, and 30% 24.7 % protein content.

B. The koi fish is comparable to other fresh water fishes as a source of protein.

C. The articial feed are found to be effective for the better growth and culture of the experimental koi fish (*A. testudineus).*

D. The fish feed containing 50.92% protein is the best feed for better growth.

E. Condition factor, SGR% of the koi fish at the Lab. condition showed results similar to the results of other worked scientists both at home and abroad.

F. FCR and feed efficiency of the rearing koi fish during the study period showed results in favour of the use of prepared fish feed.

G. Water temperature is found to have strong and negative correlation with SGR%.

H. FCR is found to have negative correlation with the water temperature (27-30°C).

I. Weight gain of the koi fish is temperature dependent. Gain in weight is observed to increase with the increase of water temperature (27-30°C).

J. Water quality parameters (such as temperature, DO, pH) are observed and have the values within the acceptable range for the growth of the koi fish (*A testudineus).*

K. DMRT is done. On the basis of DMRT it is observed that the prepared fish Feed II is the most effective feed among the four categories of fish fee.

In all treatment, the growth of fish was slow; most of fish did not reach the market size after 3 months of culture; and the FCRs' were high. These may be caused by maturation of climbing perch in the early spawning season.

Based on results of this experiment, it can be concluded that the culture of climbing perch in tanks using Sabinco and Feed III feed can be applied in order to improve the income of farmers in our country. The feed containing 50.92% protein resulted in the best growth, survival, FCR and yield of climbing perch, which can be recommended for the culture of climbing perch.

CHAPTER VI

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