

**GASTRIC EVACUATION RATE AND DAILY RATION OF  
*HYPOPHthalmichthys molitrix* (VAL.) FINGERLINGS**

*A Thesis*

*Submitted to the*

*West Bengal University of Animal and Fishery Sciences,  
in partial fulfilment of the requirements for the degree of*

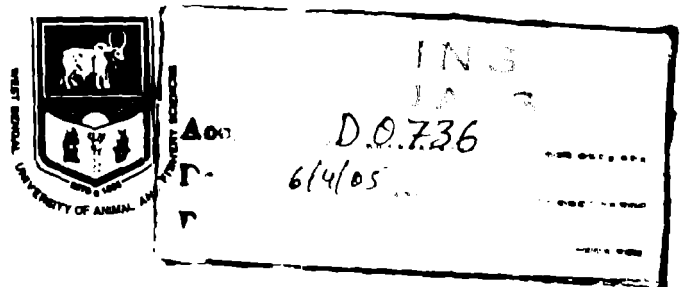
***Master of Fishery Science***

*in*

**FISHERY BIOLOGY**

**By**

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**# 2002 #**

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## Certificate

This is to certify that the work recorded in the thesis entitled **“GASTRIC EVACUATION RATE AND DAILY RATION OF HYPOPHTHALMICHTHYS MOLITRIX (VAL.) FINGERLINGS”** submitted by **Mr. Sabyasachi Bandyopadhyay** in partial fulfilment of requirement for the Degree of **Master of Fishery Science (Fishery Biology)** in the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, is the faithful and bonafide research work carried out under my supervision and guidance. The results of the investigation reported in this thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.

Date : 12.09.2002

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## APPROVAL OF EXAMINERS FOR THE AWARD OF THE DEGREE OF MASTER OF FISHERY SCIENCE (FISHERY BIOLOGY)

We, the undersigned, having satisfied with the performance of **Mr. Sabyasachi Bandyopadhyay**, in the viva-voce examination, conducted today, the 8<sup>th</sup> NOV 2002 recommend that the thesis be accepted for award of the degree.

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*Dated: 31<sup>st</sup> August, 2002*

*Place: Mohanpur, Nadia*

*Sabyasachi Bandyopadhyay*  
*(SABYASACHI BANDYOPADHYAY)*

# CONTENTS

<b>CHAPTER NO.</b>	<b>PARTICULARS</b>	<b>PAGE NO.</b>
<b>1</b>	<b><i>INTRODUCTION</i></b>	<b>1 - 3</b>
<b>2</b>	<b><i>REVIEW OF LITERATURE</i></b>	<b>4 - 19</b>
<b>3</b>	<b><i>MATERIALS AND METHODS</i></b>	<b>20 - 26</b>
<b>4</b>	<b><i>RESULTS</i></b>	<b>27 - 32</b>
<b>5</b>	<b><i>DISCUSSION</i></b>	<b>33 - 38</b>
<b>6</b>	<b><i>SUMMARY</i></b>	<b>39 - 41</b>
<b>7</b>	<b><i>BIBLIOGRAPHY</i></b>	<b>42 - 58</b>
<b>8</b>	<b><i>ANNEXURE</i></b>	<b>-</b>

## LIST OF TABLES

TABLE NO.	PARTICULARS
1	Composition of vitamin-mineral mixture in 250 g of "Supplevite-M"
2	Composition of feed (% ingredients) used in preparation of diets of different protein levels
3	Composition of feed (% ingredients) used in the preparation of diets where fish meal was partially replaced by soybean
4	Composition of feed (% ingredients) used in the preparation of diets where fish meal was partially replaced by silkworm pupae
5	Composition of feed (% ingredients) used in the preparation of conventional diets
6	Proximate composition of feed ingredients
7	Proximate composition of experimental diets
8a	Percentage of food recovered at each sampling intervals from guts of <i>Hypophthalmichthys molitrix</i> fingerlings fed with plankton and conventional diet
8b	Instantaneous rate of evacuation (b), co-efficient of determination ( $r^2$ ) and gut evacuation time (GET) of <i>Hypophthalmichthys molitrix</i> fingerlings fed with plankton and conventional diet

8c	Gut evacuation time (GET) of <i>Hypophthalmichthys molitrix</i> fingerlings fed with plankton and conventional diet: Estimation on the basis of linear regression equation
9a	Percentage of food recovered at each sampling intervals from guts of <i>Hypophthalmichthys molitrix</i> fingerlings fed with different protein levels
9b	Instantaneous rate of evacuation (b), co-efficient of determination ( $r^2$ ) and gut evacuation time (GET) of <i>Hypophthalmichthys molitrix</i> fingerlings fed with different protein levels
9c	Gut evacuation time (GET) of <i>Hypophthalmichthys molitrix</i> fingerlings fed with different protein levels: Estimation on the basis of linear regression equation
10a	Percentage of food recovered at each sampling intervals from guts of <i>Hypophthalmichthys molitrix</i> fingerlings fed with feeds where fish meal was partially replaced by soybean meal at different levels
10b	Instantaneous rate of evacuation (b), co-efficient of determination ( $r^2$ ) and gut evacuation time (GET) of <i>Hypophthalmichthys molitrix</i> fingerlings fed with feeds where fish meal was partially replaced by soybean meal at different levels
10c	Gut evacuation time (GET) of <i>Hypophthalmichthys molitrix</i> fingerlings fed with diets where fish meal was partially replaced by soybean meal at different levels: Estimation on the basis of linear regression equation

11a	Percentage of food recovered at each sampling intervals from guts of <i>Hypophthalmichthys molitrix</i> fingerlings fed with diets of where fish meal was partially replaced by silkworm pupae at different levels
11b	Instantaneous rate of evacuation (b), co-efficient of determination ( $r^2$ ) and gut evacuation time (GET) of <i>Hypophthalmichthys molitrix</i> fingerlings fed with diets where fish meal was partially replaced by silkworm pupae
11c	Gut evacuation time (GET) of <i>Hypophthalmichthys molitrix</i> fingerlings fed with diets where fish meal was partially replaced by silkworm pupae at different levels: Estimation on the basis of linear regression equation
12	Average length (mm) and weight (g) of <i>Hypophthalmichthys molitrix</i> fingerlings (n = 10) during sampling time with respect to percentage body weight feed consumed
13	Daily ration of <i>Hypophthalmichthys molitrix</i> fingerlings (n=10) corresponding to their body weight

## LIST OF FIGURES

<b>FIGURE NO.</b>	<b>PARTICULARS</b>
1	Linear regression between percentage of food recovered and sampling interval from guts of silver carp fingerlings fed with phytoplankton and conventional diet.
2	Linear regression between percentage of food recovered and sampling interval from guts of silver carp fingerlings fed with different protein levels.
3	Linear regression between percentage of food recovered and sampling interval from guts of silver carp fingerlings fed with diets where fish meal was partially replaced by soybean meal at different levels.
4	Linear regression between percentage of food recovered and sampling interval from guts of silver carp fingerlings fed with diets where fish meal was partially replaced by silkworm pupae at different levels.
5	Average weight (g) increment of silver carp fingerlings (n=10) during sampling period with respect to percentage body weight feed consumed.

# Chapter – 1

## Introduction

# 1. INTRODUCTION

When the world's population moves into an arena that puts forward a challenge to the scientists to look for an alternative source of food, other than terrestrial source, to abate the widening gap between supply and demand of protein. In this direction, aquatic resources play a significant role to overcome the problem of shortage of protein. Despite fluctuations in supply and demand, caused by the changing state of fisheries resources, the economic climate and environmental conditions, fisheries and aquaculture remain very important as a source of food, employment and revenue in many countries and communities. The proteins derived from fish, crustaceans and molluscs account for between 13.8 and 16.5 percent of the total animal protein intake of the human population ([www.fao.org](http://www.fao.org)). The contribution of inland and marine capture fisheries to per capita food supply has been stabilized (at 10 to 11 kg per capita in the period 1984 to 1998). Recent increases in per capita availability have, therefore, been obtained from aquaculture production, from both traditional rural aquaculture and intensive commercial aquaculture of high-value species ([www.fishbase.org](http://www.fishbase.org)). One of the constraints in intensification of aquaculture is the cost of input, i.e. mainly the feed. Unfortunately, the supplementary feed is indispensable to achieve higher production in semi-intensive and intensive culture systems. Hence, development of nutritionally well-balanced and cost-effective diets for farmed fishes is a challenge before farmer. In order to develop suitable cost-effective feed for cultivable fishes, there is a need to have a clear understanding of the nutritional requirements and feeding schedule to be adopted in the culture systems. Knowledge on gastric evacuation rate (digestion rate) and daily ration on cultured species of fish is essential to formulate such feeding regimes in order to minimize the wastage of feed and cost of feed. Many studies have been addressed to reduce the cost of feed by replacing the high cost source of protein in the diet of fish, i.e.

fish meal with various plant and animal origin. The most widely used plant sources include soybean meal, corn meal, wheat, rapeseed etc., and the animal sources include silkworm pupae, chicken viscera, earth worm meal etc. (Ray and Das, 1993; Shyama and Keshavanath, 1996; Naik, 1998; Nandeeshha *et al.*, 1988; Nandeeshha *et al.*, 1999; Cailliez, 2001).

Gastric evacuation rate is defined as the rate of passage of food from gut/stomach to intestine. The time taken to evacuate (gut evacuation time) may vary from a few hours to several days depending on various biotic and abiotic factors. Knowledge on gastric evacuation rate is very much required in order to find out daily ration (amount of food consumed by a fish in 24-h period) and food consumption (Jobling, 1981; Brodeur and Pearcy, 1987). Further, information on gastric evacuation rate also provide a basis for a number of related problems such as determination of modes of energy pathway within the food web (Tyler, 1970), maximal stomach capacity (Brett and Higgs, 1970; Elliott, 1975), different feeding frequency (Sarokon, 1975) and feed conversion efficiency (Pandian, 1967). Several studies have been carried out to understand the influence of various factors on gastric evacuation rate (Windell, 1967; Persson, 1982; De-Silva and Owoyemi, 1983; Singh-Renton and Bromely, 1996; VanDer-Lingen, 1998; Annappaswamy *et al.*, 2000; Saha, 2002).

Freshwater aquaculture production of the world is dominated by finfish, particularly silver carp, grass carp and other carps. Farming of Indian major carp along with exotic carps is expanding at a rapid rate in India. Silver carp *Hypophthalmichthys molitrix* (Val.) is the most widely cultured species in the world and the aquaculture production of which recorded the highest quantity of 3,308 thousand tonnes during 1998 (FAO, 1998). It has got significant position in the world market due to its faster growth rate compared to other carp species. Since consumer acceptance is low in India, Siddaiah *et al.* (1999) recommended

the suitability of silver carp in producing acceptable surimi and its further incorporation in value added products.

Despite the fact that the number of studies have been carried out in respect to food and feeding habits, effect of partial replacement of fish meal on growth, etc. yet to be addressed are the detailed studies on gastric evacuation rate and daily ration of silver carp.

The present study was, therefore, proposed with the following objectives:

1. To understand the influence of natural food (phytoplankton) and conventional diet (rice bran : groundnut oilcake :: 50: 50) on gastric evacuation rate.
2. To determine the effect of different protein levels on gastric evacuation rate.
3. To study the effect of partial replacement of fish meal by plant protein source (soybean meal) and animal protein source (silkworm pupae) on gastric evacuation rate and
4. To estimate the maintenance and maximum daily ration.



# DEPARTMENT OF FISHERY BIOLOGY

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From

T. S. Nagesh  
Lecturer and Incharge

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Date 11.11.2002

To

The Controller of Examination  
WBUAFS,  
Belgachia. Kot-37.

Sir,

sub: Submission of M.F.S. Viva-voce report of 4 PG students  
of department.

I am here with submitting the viva-voce report and evaluated  
thesis of the following M.F.S. students of Department of  
Fishery Biology and Resources Management for necessary  
action.

1. Mr. Dilip Kumar
2. Mr. Sabyasachi Bandyopadhyay
3. Mr. Gdam Ziauddin
4. Miss. Anjana.

Thanking You

Yours faithfully

- Encl: ① Viva-voce report of 4 students in duplicate  
② detailed report of external examiner  
③ Thesis (1st copy) of four students

T. S. Nagesh

# Chapter — 2

## Review Of Literature

## **2. REVIEW OF LITERATURE**

### **2.1. Gastric evacuation rate/time in fishes**

Gastric evacuation rate (GER) refers to rate of passage of food from gut/stomach to intestine. The time taken for complete elimination of food from the gut is regarded as gut/gastric evacuation time (GET). The GET explains the total time required by a fish for complete digestion of the ingested food. Information on GER is imperative to determine daily feed consumption level as well as suitability of a feed. Estimate of daily food consumption by a fish in the wild often derived from gut content analysis and laboratory measurements of gut evacuation (Eggers, 1977; Elliott and Persson, 1978; dos Santos and Jobling, 1995). The process of feeding and the digestion rate of food in fishes are influenced by copious factors of biotic and abiotic origin, which include physical, chemical and biological factors. The abiotic factors include habitat, water temperature, salinity, pH, dissolved oxygen, meal size, food concentration, chemical composition of food, starvation, feeding frequency and stress; while the biotic factors include density of fish, fish species, size and age of fish, behaviour of fish and fish physiology (Windell, 1967; Persson, 1982; Brodeur and Pearcy, 1987; Elliott, 1991). However, Bromely (1994) and Andersen (1999) reported that major variables involved in gastric evacuation rate are predator size, prey size and type, meal size and temperature. Thus, GET and GER differ not only from species to species, but also from one individual to other of the same species. The important factors, which influence the GER/GET are reviewed hereunder.

#### **2.1.1. Gastric evacuation and temperature**

Each species of fish has preferred water temperature range at which feeding, digestion and metabolism are optimal. Thus, it appears that temperature is one of the significant factors, which regulate many of the physiological activities

in fish including digestion rate. Generally, the gut evacuation rate tends to increase with the raise in temperature upto that optimal limit and reaching a maximum rate near the upper temperature tolerance limit for the species (Molnar and Tolg, 1962; Smith, 1967; Molnar *et al.*, 1967; Shrable *et al.*, 1969; Brett and Higgs, 1970; Tyler, 1970). But, above the maximum tolerance limit, the gut evacuation rate plunges sharply (Tyler, 1970) as the fish loses its appetite, ceases feeding and becomes extremely lethargic.

Darnell and Meierott (1962) stated that mackerel *Scomber japonicus* took 21-24 h at 20°C for complete (100%) evacuation, whereas *Lepomis macrochirus* took only 5 h for 50% gut evacuation when the temperature was 21-22°C (Windell, 1967). The possibility of temperature and metabolic compensation for seasonal temperatures has also been studied (Smith, 1967; Molnar *et al.*, 1967; Brett and Higgs, 1970). Baur (1970) and Beamish (1972) reported that black bullhead *Ictalurus melas* and large mouthbass *Micropterus salmoides* required 3 h and 6.6 h for passing 50% of food from stomach at 21-24°C and 25.8°C, respectively. An increase in gastric evacuation rate with the increased water temperature has been observed in the case of juvenile sock-eye salmon *Oncorhynchus nerka* (Doble and Eggers, 1978), brown trout *Salmo trutta* (Jensen and Berg, 1993) and cod and whiting fishes (Singh-Renton and Bromely, 1996).

Swenson and Smith (1973) found that *Stizostedion vitreum vitreum* required 7 h for 50% of food emptying from stomach at 15°C, whereas, it took only 4 h when the temperature was increased to 20°C. Similarly rainbow trout *Salmo gairdneri* took longer duration (24 h) at reduced temperatures of 11°C (Sarokon, 1975). Windell *et al.* (1976) established the relationship between gastric evacuation time and temperature in rainbow trout *Salmo gairdneri* fed with oligochaetes. They noticed that rainbow trout *Salmo gairdneri* needed 20.0 h, 11.3 h, 7.1 h and 4.5 h for 50% stomach evacuation at 5°C, 10°C, 15°C and 20°C, respectively.

Brodeur (1984) recorded gastric evacuation time as 72 h in *Sebastes melanops* at 11°C. Garcia and Adelman (1985) studied the rate of food evacuation in common carp *Cyprinus carpio* and reported gastric evacuation rate values ranging from 6.0% of gut contents per hour at 9°C to 32.7% per hour at 26.5°C and concluded that temperature accounted for 72-91% of the variation in gut evacuation rate. In 1991, Jiang and Claussen studied the seasonal changes of gastric evacuation in *Notophthalmus viridescens* and reported that stomachs were completely evacuated in 48 h at 25°C, whereas it took more than 15 days at 5°C indicating that the fish would be able to digest only few meals throughout the winter even though plenty of food was available. A functional relationship to describe the influence of fish weight, temperature, prey size and ration size on gastric evacuation in cod fed four natural prey types has been derived by dos Santos and Jobling (1991).

It is interesting to note that the instantaneous gastric evacuation rate of young pinctado *Pseudoplatystoma corruscans* was negatively correlated with temperature (Marques *et al.*, 1992). Similarly, there was no significant relationship between gastric evacuation rate and temperature in sardine *Sardinops sagax* (VanDer-Lingen, 1998).

The gastro-intestinal evacuation of inert particles (markers) started 48 h after a meal was digested at 0.5°C in the case of polar cod *Bareogadus saida* and the average time needed for total gastric evacuation was 400 h (Saether *et al.*, 1999).

### **2.1.2. Gastric evacuation rate and protein source/level**

Proteins are essential ingredients in the diets of fishes as source of amino acids. Dietary requirements of protein vary from species to species as well as different age groups of the same species. Feed containing high level of proteins of plant origin or protein derived from animals other than fish may require longer

time to digest. Such diets may also require supplementation of essential amino acids to meet the requirements of a particular fish species. Thus, the level and source of protein not only decide the food quality but also play important role in determining the digestion rate in fish.

Storebakken *et al.* (1999) estimated gastrointestinal evacuation time in Atlantic salmon (*Salmo salar*) using inert markers with different sources of protein and found that soybean based meal took more time for 50% evacuation than fish meal and bacterial meal. Annappaswamy *et al.* (2000) found that casein based diets evacuated faster (7.17 h) than soybean meal (10.69 h) and fish meal (9.43 h) based diets in rohu *Labeo rohita* fingerlings. Similar studies were conducted by Naik *et al.*, (2000a), whereas Saha (2002) reported that casein based diet evacuated at faster rate (7.19 h and 7.29 h) compared to soybean (10.89 h and 10.75 h) and fish meal (9.14 h and 9.64) based diets in catla *Catla catla* and bata *Labeo bata*, respectively. Though Naik *et al.* (2000a) could notice significant difference between casein and fish meal based diets but there was no significant difference between soybean meal and fish meal based diets. However, Saha (2002) could not find any significant difference among the different protein sources.

Several studies have been conducted to replace the source of protein (fish meal) with several plant and animal origin to reduce the cost of feed. The most commonly used plant sources include soybean meal, aquatic weeds, corn gluten meal, wheat, rapeseed, lupin, etc. The animal origin sources comprise of silkworm pupae, chicken viscera, etc. Though the effect of replacement of these sources on growth has been studied (Ray and Das, 1993; Shyama and Keshavanath, 1996; Kikuchi, 1999; Regost *et al.*, 1999; Nandeeshha *et al.*, 1999; Naik *et al.*, 2000c), but the reports on the effect of replacement on gastric evacuation rate are very scanty. The level of replacement has been suggested to be varying between 10% and 70% depending on the source of protein.

Apart from protein source, the level of protein in the diet of fish also play an important role in deciding the rate of gastric evacuation. Annappaswamy *et al.* (2000) reported that food passage time ranged from 9.43 h to 10.37 h when rohu *Labeo rohita* fingerlings were fed with different protein levels (30 to 40%). They could also notice significant difference in gastric evacuation rate due to 30 % and 35% protein levels and 35 % and 40% protein levels. In the case of catla *Catla catla* and bata *Labeo bata* fingerlings, the evacuation rate was faster when 30% protein (9.14 and 9.64 h) was fed compared to 35% protein (10.15 h and 10.01) and 40% protein (10.55 and 10.50) levels, respectively (Naik *et al.*, 2000a; Saha, 2002).

### **2.1.3. Gastric evacuation rate and lipid concentration**

Dietary lipids are the source of essential fatty acids in feed. They provide a rich source of energy and dietary phospholipids, which are the structural components of biomembranes. Foods rich in lipids are generally considered to be digested slowly (Medved, 1985; Basimi and Grove, 1985a; Grove *et al.*, 1985). The meals of several natural food items fed to blue gill sunfish *Lepomis macrochirus* were evacuated more rapidly than meals of worm larvae containing 35% lipid (Windell, 1967). Hunt and Stubbs (1975) reported that the quality of fatty acid is responsible for varying the digestion in fishes. On the other hand, De Silva and Owoyemi (1983) observed that density of diet or lipid content had no effect on evacuation time in *Serotherodon mossambicus*. Windell *et al.* (1972) also made similar observations in the case of rainbow trout *Salmo gairdneri*. Lipid concentration of more than 15% of the dry weight probably have an inhibitory effect on gastric motility (Basimi and Grove, 1985b). They also found the evacuation rate in plaice *Pleuronectes platessa* was slower when fed krill rich in lipid. Santulli *et al.* (1993) found that gastric evacuation rate in sea bass (*Dicentrarchus labrax*) increased with a reduction in lipid absorption at higher temperature. The gastric evacuation rate in rohu *Labeo rohita* fingerlings

(Annappaswamy *et al.*, 2000), catla *Catla catla* and common carp *Cyprinus carpio* (Naik *et al.*, 2000a and b) fingerlings decreased with the increase in lipid level. Similarly, Saha (2002) observed that gastric evacuation rate was faster in bata *Labeo bata* fingerlings fed on diet incorporated with 15% (16.10 h) than 10% (13.55 h) and 5% (11.86) cod liver oil.

#### **2.1.4. Gastric evacuation and natural food**

Gastric evacuation rate also varies with type of food. Seaburg and Moyle (1964), reported that black crappie *Pomoxis nigromaculatus* took 7 h for evacuation of 50% natural food. Sarokon (1975) found that rainbow trout *Salmo gairdneri* took 24 h to pass natural food (caddis fly larvae) whereas, the fish took 10 h when fed with pelleted feed (Windell *et al.*, 1972). Similarly Elliott (1972) reported that natural food evacuated much faster (2.4 h) than the artificial diets. Gastric evacuation time of *Perca flavescens* decreased when fed with zooplankton as compared to dry diets (Mills *et al.*, 1984). Sumagaysay (1993) recorded a lower gastric evacuation rate in milkfish *Chanos chanos* fed natural food (1.57 h) than those fed on supplemental diet (1.79 h). Andrade *et al.* (1996) found variations in gastric evacuation rate of gill head sea bream (*Sparus aurata*) when fed with natural feed (12 h) and pelleted feed (2 h). Annappaswamy (1997) has also observed that gastric evacuation time was lower when rohu *Labeo rohita* fingerlings were fed with natural food (6.85 h) than fish meal based pelleted diets (9.19 h).

The gastric evacuation time (GET) in the fingerlings of rohu *Labeo rohita* was found to be 6.85 h and 9.9 h for natural food and pelleted feed, respectively. (Annappaswamy *et al.*, 2000). Whereas, it was 6.87 h and 8.65 h when fed with natural food and pelleted feed in the case of fingerlings of catla *Catla catla* (Shanbhogue *et al.*, 2000).

### 2.1.5. Gastric evacuation rate and meal size

Selection of appropriate meal size and feeding schedule are critical elements in effective feed management. The ration size is one of the major factors that influences digestion rate and food conversion in fishes. Hence the study of effect of meal size on rate of gastric emptying has been received considerable attention. Barrington (1957) reported that fish digest small meals (prey) more hastily than large meals (prey). However, Daan (1973) and Bagge (1977) noticed that the rate of gastric evacuation was constant for different meal sizes in *Gadus morhua*. Similarly, in *Perca fluviatilis* fed on fish larvae, gut evacuation rate was not influenced by the meal size (Persson, 1981). Jobling (1980) stated that the gastric evacuation rate depends on energy density of the meals. Talbot (1984) found that large meals were evacuated in the similar way as that of smaller meals. Bromely (1988; 1991) concluded that gastric evacuation rate in whiting and cod was independent of meal size. Elliott (1991) opined that use of wet or dry weight could lead to different conclusions about the effects of meal size on evacuation rates. He-Enqiang and Wetsbaugh (1993) and Hop and Tonn (1998) noticed that predator size did not significantly influence rates of evacuation of *Salmo trutta*, and Arctic cod *Boreogadus saida*, respectively. Similarly, Andersen (1999) found that prey size had no influence on the gastric evacuation rate in whiting *Merlangius merlangus*. He stated that variation in gastric evacuation rate could be explained by difference in energy density among prey fish species and individuals. Further, he stated that conclusions can not be drawn on the effect of prey size on gastric evacuation rate without the information on energy density.

Studies conducted by Paeaekkoenen *et al.* (1999) revealed that the gastric evacuation rates were 0.8, 1.3 and 1.6 g / day when fed meal sizes of 1%, 5% and 10% of body weight in *Lota lota*. Knutesen and Salvanes (1999) observed direct relationship with gastric evacuation rate and meal size. Observations made by Sveier *et al.* (1999) revealed that gastric evacuation time in Atlantic salmon

(*Salmo salar*) fed with course ground fish meal was slower than micro ground fish meal. The gastric evacuation rate in African catfish *Clarias gariepinus* was faster when fed with smaller food particles of 1 mm and 1.5 mm than that of larger food particle of 2 mm and 3 mm diameter (Hossain *et al.*, 2000). Boyce *et al.* (2000) noticed an increase in absolute gastric evacuation rate of Antarctic spiny plunder fish *Harpagifer antarcticus* Wybelin, with increasing ration size. However, there was no effect of prey size on the gastric evacuation rate of pike perch *Stizostedion pucioperca* (Koed, 2001).

### **2.1.6. Gastric evacuation rate and feeding frequency**

Feeding frequency is yet another factor that influences the gastric evacuation rate in fishes. Many workers reported that increased feeding frequency generally accelerates the digestion rate in most of the fishes (Dawes, 1930; Grove *et al.*, 1978; Persson, 1982; Rosch, 1987). The rate of food passage in plaice *Pleuronectes platessa* is related to the amount of food recently consumed (Karzinkin, 1935). However, as long as the total amount of food consumed remains constant, gastric evacuation rate is not affected by the number of daily feedings. Jobling (1983) studied the effect of feeding frequency on food intake and growth of Arctic charr *Salmo alpinus* and stated that these effects could not be attributed to direct competition for food since fish were fed to satiation at each feeding period. Rosch (1987) noticed higher stomach evacuation rates when fish fed three meals per day rather than one meal per day in *Coregonus lavaretus*. Paul *et al.* (1990) have shown that multiple meals of whole shrimp produced higher evacuation rates than single meal. The gastric evacuation rates increased in rohu *Labeo rohita*, catla *Catla catla* and common carp *Cyprinus carpio* fingerlings as the feeding frequency increased from one meal to three meals per day (Annappaswamy *et al.*, 2000; Naik *et al.*, 2000a and b).

### **2.1.7. Gastric evacuation rate and starvation**

The study of effect of starvation on gastric evacuation rate, digestibility, absorption efficiency and growth is being paid considerable attention. It was observed by Windell (1966) that fasting periods of 7, 14 and 25 days substantially decreased the rate of gastric evacuation of bluegill sunfish from 22% to 51%, when compared to control (two days fasting); while fasting periods of less than six days did not affect the rate of evacuation in brown trout fed *Gammarus gammarus*. It was reported that prolonged starvation prior to feeding delays the rate of digestion probably due to absence of gastric juice when the food enters the gut (Elliott, 1972; Fange and Grove, 1979). Sarokon (1975) investigated the short-term effect to three and six days starvation period on gastric evacuation rate of rainbow trout. He found that trout famished for three days and six days had significantly lower gastric evacuation rate than those famished only for 18 h. Talbot (1984) reported that post-prandial starvation decreased evacuation rate in juveniles of Atlantic salmon *Salmo salar*. Further he stated that this effect was inversely related to the quality of food consumed by a fish.

Interestingly, Russell and Wootton (1993) could not notice any significant effect of starvation on gastric evacuation rate in the adults of *Phoxinus phoxinus* (Cyprinidae) starved for 4 -16 days. Similar observations have been made by Annappaswamy *et al.*, (2000) in the case of rohu *Labeo rohita* and Naik *et al.*, (2000a) in catla *Catla catla* and Naik *et al.*, (2000b) in the case of common carp *Cyprinus carpio*. Saha (2002) reported that the gastric evacuation in bata *Labeo bata* fingerlings fed 30% protein diet after five days of starvation was comparatively faster (12.12 h) than those starved for 10 days (14.21 h) and 15 days (15.64 h).

### **2.1.8. Gastric evacuation rate and body size**

Among numerous gastric evacuation studies, a few experiments have also been designed to determine the relationship between evacuation time and body

size. Noble (1973) stated that evacuation rate is slower in larger fish. Larval stages of large mouthbass *Micropterus salmonides* fed with zooplankton evacuated more rapidly than adults (Laurence, 1971). In the case of dab *Limanda limanda*, absolute gastric evacuation rate increased as the fish size increases (Jobling, 1987). The rate of gastric evacuation decreased with fish size in the case of juvenile sock eye salmon *Oncorhynchus nerka* (Doble and Eggers, 1978). Jobling (1981) reanalyzed the data for the influence of predator size in a number of fish species. He found that, predator size co-efficient (C) ranging from 0.234 to 0.619. Opuszynski and Shireman (1991) reported that the food passage time of big head carp *Aristichthys nobilis* weighing 13-28.50 g was 7.1 to 12.8 h. Food passage rate was higher for larger fish (323 mm/h) than smaller fish (67 mm/h). Temming and Andersen (1994) examined the effect of body size in cod and found 'C' values in the range of 0.018-0.487 depending on food type. Dong and Deshang (1994) reported that the removal rate of silver carp *Hypophthalmichthys molitrix* for phytoplankton was higher than that of bighead carp *Aristichthys nobilis*. Whereas, zooplankton evacuated faster in bighead carp than that of silver carp. dos Santos and Jobling (1995) reported that relative gastric evacuation rate decreases as body mass decreases in case of cod *Gadus morhua*.

In contrast to above studies, Olsen and Boggs (1986) opined that there is no effect of body mass on gastric evacuation or gut evacuation time in yellow fin tuna *Thunnus albacares*. Similar observations have also been reported by Elliott (1991) in brown trout *Salmo trutta* and Hayward and Weiland (1998) in rainbow trout *Oncorhynchus mykiss* (Walbaum).

## **2.2. Maintenance ration and daily ration**

Knowing the optimal ration for farmed fish is vital for both economic and ecological reasons. Minimizing feed wastage not only decreases the nutrient loss but also the water degradation. Maintenance ration is the ration, which is sufficient

enough to maintain the basic physiological activities with very minimum growth. Ricker (1946) defined daily ration as the size of daily meal expressed as percentage of the body weight. Estimation of optimal ration could be obtained based on theoretical consideration of growth and feed conversion (Smith, 1989) or on individual determination of feed intake. Estimation of gastric evacuation rates is requisite for in-situ determination of daily ration of fish (Elliott and Persson, 1978). Rajasilta (1980) used measures of rate of digestion to estimate the daily ration of sticklebacks in brackish water in the Baltic sea. Maximum daily ration (C<sub>MAX</sub>) estimates were also useful in such studies as reference values against which daily ration estimates can be compared (Hartman and Brandt, 1995; Weiland and Hayward, 1997).

The daily ration differs from species to species and individual to individual due to various exogenous and endogenous factors.

In most fishes, the relationships between growth rate and ration size is decelerating curve (Brett and Grove, 1979). The conversion efficiency is highest at an intermediate ration and decreases with further increase in the ration. Grass carp *Ctenopharyngodon idella* fed on pelleted feed showed a similar pattern of growth-ration relationship (Huisman and Valentijn, 1981). However, daily feeding rhythms are reported not to be uniform for many benthivorous species (Fugi *et al.*, 1996).

Doble and Eggers (1978) estimated the lower rations (maintenance ration) 0.62% body weight per day for juvenile sock eye salmon *Oncorhynchus nerka*. They also observed a general decrease in the daily ration with increase in body weight. Godin (1981) reported that pink salmon fry of mean length ranging from 42.1 – 44.0 mm consumed 6.6% and 13.1% body weight daily. Troschel and Rosch (1991) reported that in juvenile *Coregonus lavaretus*, the maximum daily ration was about 27.0% of fish body weight, which corresponds to 75% of body dry weight and no correlation was found between daily ration and specific growth

rate. Kim and Kang (1992) recorded the daily ration (as percentage of body weight) values of 9.35%, 6.65%, 5.76%, 4.72% and 3.31% in the order of ages 0-4 years for *Agrammus agrammus*. Urtans (1992) reported that the daily ration ranged from 4.9-9.7% body weight for young fish and from 2.4-5.9% for adult ones of eelpout *Zoarces viviparous*. The maximum daily ration of large bloaters *Coregonus hoyi* (over 100 g) to be ranged between 5 and 12% per day (Binkowski, 1994). Hoelker and Temming (1996) reported that the daily ration of ruffe (*Gymnocephalus cernuus*) to be 1.3% to 2.3% of body weight for adult ruffe and 2.3% to 4.3% for juvenile. The daily ration of yellowfin tuna increased from 2.1% to 5.5 % body weight with increasing size up to 70 cm fork length, beyond which it decreased (Maldeniya, 1996). Hop and Tonn (1998) reported that the daily rations for Arctic cod *Boreogadus saida* in Resolute Bay N. W. T. to be 0.51% body weight for fish of size 45 g and 1.13% body weight for fish of size 51 g. The daily rations of common carp *Cyprinus carpio* and calta *Catla catla* fingerlings were estimated by Annappaswamy *et al.* (2000) and Shanbhogue *et al.* (2000). The values varied between 9.9 and 14% of body weight, and 5.9 and 13.7%, respectively depending on the size of fish, while the daily rations of rohu *Labeo rohita* fingerlings varied from 9.20 to 11.12% (Annappaswamy *et al.*, 2001). Saha (2002) reported that daily ration of bata *Labeo bata* fingerlings (4-5 g) was varying between 9.87% and 11.48% of body weight.

Daily ration varies with temperature of the water in which fish resides. Generally, increase in temperature increases the feeding activity, hence, the daily ration. Fish will consume maximum food at optimum temperature rather decreased or increased temperature than this level. Rajasilta (1980) reported that stickleback consumed 24.2 mg, 48.3 mg and 132 mg daily at 10°C, 14°C and 18°C, respectively. Garcia and Adelman (1985) observed that the daily food consumption of common carp *Cyprinus carpio* varied from a low of 3.87 mg/g of fish per day at 14°C (0.39% body weight) to 40.75 mg/g per day (4.08% body weight) at 26.5°C. Mac (1985) reported that selected temperature and ration size

were linearly correlated in lake charr *Salvelinus fontinalis*. Based on a caloric value of 2.0 kcal/g of food, yellowfin tuna would require 0.35-0.39% body weight/day at 3°C and 0.63% body weight/day at 5-6°C (Smith *et al.*, 1991). The daily ration of bighead carp *Aristichthys nobilis* was 5.7-2.0% of body weight at water temperatures ranging from 21-28.5°C (Opuszynski and Shireman, 1991). Sturgeon fingerlings (20.0 g) consumed 6.5% of their body weight at a water temperature of 18-20°C fed for 130 min when food concentration was 2 g /m<sup>2</sup> (Gershanovich and Taufik, 1992). The studies made by Russell *et al.* (1996) revealed that rations on the growth of juvenile sea bass *Dicentrarchus labrax* fed squid mantle at 16, 10, 14 and 18°C ranged between 0.7 and 23% body weight/day with the increase in temperature. Optimum ration was found to be 7.4% body weight/day at 18°C. Hayward and Weiland (1998) estimated maximum daily ration (C<sub>MAX</sub>) in rainbow trout *Oncorhynchus mykiss* at different temperatures. The mean C<sub>MAX</sub> values were substantially increased with the increase in temperature, i.e., from 10.21% (dry g food/dry g fish x 100) at 7.8°C to 15.41% at 12.8°C.

Daily ration is also influenced by different seasons. Manzer (1976) estimated that stickleback in Great Central Lake had a daily consumption of 6.6% of their body weight in July and 7.8% in October. The estimated daily ration of coho salmon in Chignik during early June ranged from 2.1 to 4.4% body weight per day (Eggers, 1979). Brodeur and Pearcy (1987) found that daily ration varied from 2.4% to 3.7% body weight per day. It's daily ration declined sharply in late June. Amundsen and Klemetsen (1988) noticed that the Arctic charr *Salvelinus alpinus* consumed maximum food in July after which it decreased markedly towards autumn. The daily meal of juvenile Coho salmon *Oncorhynchus kisutch* was found to be varied from 0.22 to 0.43 g (2.1 to 4.4%) body weight (Ruggerone, 1989). Seki and Somerton (1994) recorded the daily ration for armor head *Pseudopentaceros wheeleri* as 1.17 g (0.28% body weight) and 12.87 g (2.86% body weight) in summer seasons during 1987 and 1988. The daily ration was

generally high during the post-spawning season with the highest (47.5%) in May than the other months in the case of brown sole *Pleuronectes hersensteini* (Tominaga *et al.*, 1994). Pederson (2000) reported that pelagic and demersal North Sea whiting *Merlangius merlangus* fed at night and day had daily ration ranged from 4.38 to 7.84%, respectively.

## **2.3. Models related to food consumption**

### **2.3.1. Models related to gastric evacuation rate**

Mammalian physiologists have attempted to describe gastric emptying using a wide range of mathematical expressions (Stubbs, 1977) all of which imply that the emptying of food from the stomach is a continuous process. However, there is considerable disagreement about which mathematical expression gives the best description of gastric evacuation curves in fish species (Jobling, 1981; Lambert, 1985; Medved, 1985). Simultaneously, attempts have also been made to find a theoretical basis to support the use of mathematical expressions (Hopkins, 1966; Stubbs, 1977; Jobling, 1986). But no single mathematical expression has been found to be best fit consistently to empirical data and suggested all are inadequate for describing gastric evacuation under different conditions.

Jones (1974) developed a model in which evacuation is independent of meal size and evacuation rate was described as a 'power function' of stomach content. Later, Windell *et al.* (1976) used another model to describe gastric evacuation rate in rainbow trout and this model was referred to as 'the log time model', which is given by

$$\begin{array}{ll} \% \text{ meal remaining} = a + b \log_{10} & \text{where, } a \text{ and } b \text{ are linear regression} \\ \text{(hours after feeding)} & \text{constants} \end{array}$$

Linear, exponential and square root models (Jobling, 1981 and 1986) are widely used to describe the evacuation of stomach content with time.

The models proposed are as follows,

The linear model :  $W_t = W_0 - rt$

The exponential model :  $W_t = W_0 e^{-ret}$

The square root model :  $\sqrt{W_t} = \sqrt{W_0} - r_{0.5}t$

Where, 'W<sub>0</sub>' is initial food weight, 'W<sub>t</sub>' is food weight, remaining at time 't' and 'r', 're' and r<sub>0.5</sub> are the rates of evacuation.

Jobling (1981 and 1986) suggested that the best evacuation model depends on type of food consumed by fish. Persson (1986) reported that food evacuation curves of most fish should be exponential. In contrast, Robert and Mark (1998) concluded from their experiment with rainbow trout *Salmo gairdneri* fed chironomid larvae at different temperatures (7.8°C, 10.0°C and 12.8°C), that log time model is most suitable as compared to other models. Further, Persson (1986) and Simenstad and Cailliet (1986) had noted that the exponential and square root models are quantitatively similar. However, Bromely (1988) found that a linear model was appropriate for describing gastric evacuation of whiting. From a review and re-analysis of data, Jobling (1987) opined that a square root model is the most appropriate model for describing gastric evacuation rate. This was supported by Andersen (1998) who demonstrated that evacuation in whiting *Merlangus merlangus* could be described independently of meal size using a simple power function model with an exponent of 0.5.

The power function model could be used to know the effects of predator and meal size on the evacuation rate (Jobling, 1981; Bromely, 1994; Temming and Andersen, 1994). They have used the following modified model

$$ds/dt = -g e^{bT} W^c M^d S^a$$

Where, 'T' is temperature, 'W' is predator weight, 'M' is initial meal size and 'S' is the weight of total stomach content. The parameters to be estimated are g, b, c, d and a. The predator length (L) could also be used instead of predator weight (W), simply by substituting L for W in the above equation.

Similarly, The model developed by Jones (1974) was modified and applied by dos Santos and Jobling (1992) and Temming and Andersen (1994) for *Gadus morhua* to estimate gastric evacuation rate. Here the gastric evacuation was expressed as  $ds/dt = - pS^a$  ;

Where 'S' is the weight of stomach content and 'a' and 'p' are parameters to be estimated and 't' is time.

### **2.3.2. Models related to daily ration**

Bajcov (1935) developed a formula for measurement of the daily ration (linear-evacuation model) when the fish is feeding continuously and rate of food passage is continuous and uniform for different kinds and amount of food. The model is expressed as follows,

$$C = S (24/n)$$

Where 'C' is food consumption, 'S' is the mean weight of food consumed over a 24 h period and 'n' is hours required for complete gastric evacuation.

The linear evacuation model was challenged by Elliott (1972) working on gastric digestion in brown trout *Salmo trutta*. The evacuation rate in the trout follows the exponential function, which is given by

$$dw/dt = - r W^b$$

Where, 'W' is the weight of stomach content, 't' is the time, 'r' is the evacuation rate and 'b' is exponential rate.

Teichmann (1992) described MAXIMS - a computer programme for estimating the food consumption of fish, which allows for the estimation of daily ration and population food consumption of fish based on diet cycle of stomach content weights.

# Chapter — 3

## Materials and Methods

## **3. MATERIALS AND METHODS**

### **3.1. Collection and acclimatization of experimental fishes**

Fingerlings of silver carp *Hypophthalmichthys molitrix* (60 - 71 mm; 2.3 - 3.0 g) were collected from Bat Tala seed market, Naihati, North 24 Parganas. Approximately, 500 fishes were brought and maintained in equal numbers in 5 fiberglass reinforced plastic (FRP) rectangular tanks (300 l capacity; 125 x 60 x 40 cm<sup>3</sup>) with aeration. Fishes were acclimatized to the laboratory conditions for 32 days in the wet laboratory of Department of Fishery Biology and Resources Management. During this period fishes were fed with pelleted feed (30% protein) twice a day (morning 7 a.m. and evening 5 p.m.) to satiation. The residual feed and faecal matter were siphoned out daily and partial water exchange was done thrice a week.

### **3.2. Feed**

#### **3.2.1. Feed ingredients**

The ingredients used in the preparation of different formulated pelleted feeds for various gastric evacuation, maintenance ration and daily ration experiments were fish meal, groundnut oilcake, wheat flour, rice bran, soybean meal, silkworm pupae and vitamin-mineral mixture. All the ingredients except soybean meal, vitamin-mineral mixture and silkworm pupae, were procured from the local market. The soybean meal was purchased from 'Loba Chemie Pvt. Ltd.' company. Dried silkworm pupae powder was procured from Malda district. The vitamin-mineral mixture used in the preparation of feed was 'Supplevite-M' (M/S Sarabhai Chemicals, Baroda). The composition of the vitamin-mineral mixture is given in Table 1.

**Table 1. Composition of vitamin-mineral mixture in  
250 g of “Supplevite-M”**

<b>Item</b>	<b>Unit</b>
Vitamin A	500,000 IU
Vitamin D <sub>3</sub>	100,000 IU
Vitamin B <sub>2</sub>	0.20 g
Vitamin E	75 units
Vitamin K	0.10 g
Calcium pantothenate	0.25 g
Nicotinamide	1.00 g
Vitamin B <sub>12</sub>	0.60 mg
Choline chloride	15.00 g
Calcium	75.00 g
Magnesium	2.75 g
Iodine	0.10 g
Iron	0.75 g
Zinc	1.50 g
Copper	0.20 g
Cobalt	0.04 g

### **3.2.2. Proximate composition of feed ingredients**

All the ingredients used in feed preparation were subjected to proximate composition analysis such as moisture, crude protein, crude fat, ash, crude fibre and nitrogen free extract (NEF) in the laboratory before formulation of experimental diets. Moisture content was determined by drying the sample at elevated temperature (105°C) for 30 min and then drying at 65°C till constant weight was obtained following the method of Boyd (1969). Percent moisture was derived from the difference in weight of sample before and after complete drying. Total nitrogen content was estimated using Micro-Kjeldahl's method (AOAC, 1984) and crude protein was calculated by multiplying nitrogen content of sample with the factor 6.25. The crude fat content was estimated by ether (petroleum ether) extraction method (Boiling point 40-60°C for 6-8 h) in the Soxhlet extraction apparatus (Bligh and Dyer, 1959). Ash content was estimated by drying the sample at 550°C ± 20°C for 6 h in muffle furnace (AOAC, 1984). Crude fibre was determined by following the modified method of De Silva (1985). The nitrogen free extract (NFE) or carbohydrate was found out by difference method (Hastings, 1976) by using the following formula,

Nitrogen free extract (NEF) (%) = 100 – (% crude protein + % crude fat + % crude fibre + % ash + % moisture).

### **3.2.3. Pelleted feeds**

#### **3.2.3.1. Preparation of formulated pelleted feeds**

The composition of pelleted feeds used in the preparation of various experimental diets is presented in Tables 2, 3, 4 and 5. The respective ingredients required for the pelleted feed were mixed thoroughly as per the square method (Hardy, 1980). Dough was prepared by adding sufficient potable water (1:0.8) to get the required consistency in the dough. The dough, thus obtained was cooked in a pressure cooker for 30 min at 105°C and cooled at room temperature. After

**Table 2. Composition of feed (% ingredients) used in preparation of diets of different protein levels**

Diets	P <sub>1</sub>	P <sub>2</sub>	P <sub>3</sub>
Ingredient	(30% Protein)	(35% Protein)	(40% Protein)
Fish meal	40	45	45
Ground nut oil cake	10	20	30
Rice bran	30	25	15
Wheat flour	19	9	9
Vitamin– mineral mixture	1	1	1

**Table 3. Composition of feed (% ingredients) used in the preparation of diets where fish meal was partially replaced by soybean meal**

Diets	SB <sub>1</sub>	SB <sub>2</sub>	SB <sub>3</sub>
Ingredient	(10%)	(20%)	(40%)
Fish meal	36	32	24
Ground nut oil cake	10	10	15
Rice bran	25	25	20
Wheat flour	24	24	24
Soybean meal	4	8	16
Vitamin– mineral mixture	1	1	1

**Table 4. Composition of feed (% ingredients) used in the preparation of diets where fish meal was partially replaced by silkworm pupae.**

<b>Diets</b>	<b>SW<sub>1</sub></b>	<b>SW<sub>2</sub></b>	<b>SW<sub>3</sub></b>
<b>Ingredient</b>	<b>(10%)</b>	<b>(20%)</b>	<b>(40%)</b>
Fish meal	36	32	24
Ground nut oil cake	10	10	10
Rice bran	25	20	20
Wheat flour	24	29	29
Silkworm pupae	4	8	16
Vitamin– mineral mixture	1	1	1

**Table 5. Composition of feed (% ingredients) used in the preparation of conventional diet.**

<b>Ingredient</b>	<b>Level (%)</b>
Ground nut oil cake	50
Rice bran	50

cooling, vitamin-mineral mixture was added, mixed thoroughly and pelletized in a pelletizer (5 mm dia). The pellets were collected in aluminium tray and dried in an hot air oven at 60°C for 24 h till the moisture content was reduced to less than 10%. The dried pellets were then broken into small pieces, crumbled by using mixer grinder and sieved to get uniform sized particles. The prepared feeds were packed in airtight polythene bags, labeled and stored in a cool dry place.

### **3.2.3.2. Proximate composition of pelleted feeds**

Proximate composition of various pelleted feeds used in the different set of experiments were analysed following the methods adopted for analysis of the feed ingredients (Section 3.2.2.).

### **3.3. Gastric evacuation experiment**

Prior to starting of experiment, fishes were segregated into three sets and transferred to glass aquaria (60 cm x 30 cm x 30 cm, @ 15 nos/aquarium) with constant aeration. Each set of fishes was fed with respective experimental diets, twice a day to satiation, for 15 days to make them habituated with the experimental diets. The excess feed and faecal matter were removed daily and loss of water was replenished. The fishes ( $75 \pm 5$  mm) were then starved for 24 h before the start of gastric evacuation experiment. All the experiments were conducted in triplicate at  $28 \pm 1.5^\circ\text{C}$ . Randomly, three fishes from each set (one from each aquaria) were selected and their total length and weight were recorded. Then fishes were sacrificed and initial (empty) gut (anterior bulged portion of gut) weight (I) was recorded to the nearest mg after removing excess water by absorbent paper.

Fishes were fed *ad libitum* with respective diets to satiation for 20 min and excess feed removed by siphoning. Immediately, a random sample of three fishes (one from each aquarium) was removed and their total length (mm) and

weight (g) were recorded. The fishes were then sacrificed to examine the amount of feed in the digestive tract and weighed (F) to the nearest mg after removing excess water by absorbent paper. The difference in the initial (I) and final (F) weight was taken as the weight of food that a fish has consumed during 20 min and taken as 100% for further calculations. This procedure was continued at an interval of every one hour until the guts of sampled fishes were empty.

### **3.3.1. Influence of natural food and conventional diet on gut evacuation time**

For this experiment, 2 sets of fishes ( $75 \pm 5$  mm) were arranged. First set of fishes kept in three aquaria (12 fishes in each aquaria) and fed with conventional diet (rice bran : groundnut oilcake :: 50:50) twice a day to satiation. Gastric evacuation experiment was conducted as explained in Section 3.3.

Second set of fishes was kept in FRP tanks and fed with natural food (phytoplankton) cultured in wet laboratory of Department of Fishery Biology and Resources Management by using groundnut oilcake and lime (inoculum from 'Shanti' jheel). After 7 days dense plankton growth was observed. Among them the dominating species were *Spirogyra*, *Oscillatoria*, *Coscinodiscus*, *Volvox*, *Chlorella*, *Microcystis* and *Anabaena*. Above 24 h prior to the gastric evacuation experiment fishes were transferred to aquaria containing potable water (plankton free) for fasting. On the day of experiment after recording the initial (empty) gut weight fishes were transferred to the FRP tank containing plankton and allowed to feed for 20 min. Again they were transferred to aquaria till the end of the experiment.

Sampling and gastric evacuation experiment conducted were similar as described in Section 3.3.

### **3.3.2. Influence of protein level on gut evacuation time**

Fish meal based diets containing 30% (diet P<sub>1</sub>), 35% (diet P<sub>2</sub>) and 40% (diet P<sub>3</sub>) protein were prepared for this experiment using square method (Hardy, 1980). Fishes (75±5 mm) were divided into three groups of 45 nos. each (15 in each aquarium) and gastric evacuation experiment was carried out as described in Section 3.3.

### **3.3.3. Influence of fish meal replacement by plant protein source on gut evacuation time**

For this set of experiment, three diets containing 30% protein were prepared by replacing fish meal by plant protein source (soybean meal) using square method (Hardy, 1980). Three diets were designated as diet SB<sub>1</sub> (10% replacement), diet SB<sub>2</sub> (20% replacement) and diet SB<sub>3</sub> (40% replacement). Three groups (45 numbers each) of fishes (75 ± 5 mm) were acclimatized to three diets separately. The methodology of gastric evacuation experiment was similar as mentioned in earlier Section 3.3.

### **3.3.4. Influence of fish meal replacement by animal protein source on gut evacuation time**

For this set of experiment, three diets containing 30% protein were prepared by replacing fish meal by animal protein source (silkworm pupae) using square method (Hardy, 1980). Three diets were designated as diet SW<sub>1</sub> (10% replacement), diet SW<sub>2</sub> (20% replacement) and diet SW<sub>3</sub> (40% replacement). Three groups (45 numbers each) of fishes (75 ± 5 mm) were acclimatized to three diets separately. The methodology of gastric evacuation experiment was similar as mentioned in earlier Section 3.3.

### **3.4. Estimation of daily ration**

#### **3.4.1. Estimation of maintenance ration**

Maintenance ration is the minimum amount of food needed by a fish for basic physiological activities. For estimation of maintenance ration, five groups of fishes were stocked (@ 10 fishes) in 5 aquaria (60cmx30cmx30cm). These groups of fishes were fed 1.0%, 2.0%, 3.0%, 5.0% and 10.0% of body weight per day, respectively with pelleted feed separately for a period of 6 weeks. Sampling (n = 5) was done at the end of every week to record the growth of an individual group in terms of length (mm) and weight (g) increment of fishes. The quantity of feed given was readjusted after each sampling. The ration at which the group of fish showed no or little response to growth in terms of length and weight increment was considered as the minimum daily ration or maintenance ration.

#### **3.4.2 Estimation of maximum food consumption/daily ration**

Daily ration is the total feed consumed by a fish in 24 h period. For the estimation of daily ration, the fishes were fed with a known quantity of feed (5% body weight of fish) at 4 h interval over 24 h period. After 30 min of each feeding, the excess feed was removed and its dry weight determined. The total amount of food consumed over a 24 h period (Daily ration) was estimated using Bajkov's (1935) formula, i.e.,  $(24/h)A$ ,

Where,  $h$  = food passage time and  $A$  = food consumed (% BW)

This model is based on the assumption that changes in fish diurnal feeding activity do not occur.

### **3.5. Statistical analyses**

The mean weight of food in the gut obtained during different sampling periods in different gut evacuation experiments are subjected to one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1968).

The evacuation data obtained in different set of experiments were fitted to linear model. The co-efficient of determination ( $r^2$ ) was determined linearly and exponentially.

The linear model used is as follows

The linear model, 
$$W_t = W_o - rt$$

Where  $W_o$  is the initial food weight,  $W_t$  is the food weight remaining at time  $t$  and  $r$  is the rate of evacuation.

The increment in average weight (g) of fishes fed with different percentage body weight during the study period (42 days) was subjected two-way analysis of variance (ANOVA) (Snedecor and Cochran, 1968).

# Chapter — 4

## Results

## **4. RESULTS**

### **4.1 Proximate composition of feed ingredients**

The results of proximate composition of different ingredients used to prepare various formulated feeds of various experiments are given in Table 6. The moisture content was highest in rice bran (12.26%) followed by wheat flour (10.23%), soybean meal (9.75%), fish meal (9.20%), silkworm pupae (8.20%) and lowest in groundnut oilcake (8.18%). The dry matter content of the ingredients was highest in groundnut oilcake (91.82%) and lowest in rice bran (87.74%). The crude protein content estimated in different ingredients varied between a maximum of 53.20% in fish meal and a minimum of 12.40% in rice bran. The protein level of other ingredients was found to be 42.50%, 41.81%, 13.20% and 48.30% in soybean meal, groundnut oilcake, wheat flour and silkworm pupae, respectively. The crude fat content was highest in silkworm pupae (11.20%) followed by groundnut oilcake (9.33%), fish meal (6.66%), wheat flour (5.15%) and soybean meal (3.36%). The crude fibre content ranged from 2.16% (fish meal) to 22.65% (rice bran). The intermediate values were found to be 3.20% (wheat flour), 6.25% (silkworm pupae) 8.20% (soybean meal) and 8.41% (groundnut oilcake). The ash content was highest in fish meal (20.27%) and lowest in soybean meal (2.13%); while the values recorded in rice bran, silkworm pupae, wheat flour and groundnut oilcake were 18.23%, 16.65%, 6.36% and 4.36%, respectively. The carbohydrate (nitrogen free extract) content of ingredients varied between 9.40% (silkworm pupae) and 61.86% (wheat flour).

### **4.2. Proximate composition of formulated feeds**

The results of proximate composition of formulated pelleted feeds used in different sets of experiments throughout the study period are presented in Table 7.

**Table 6. Proximate composition of feed ingredients**

<b>Ingredients</b>	<b>Proximate composition (Mean <math>\pm</math> Standard deviation) (%)</b>						
	<b>Moisture</b>	<b>Dry matter</b>	<b>Crude protein</b>	<b>Crude fat</b>	<b>Crude fibre</b>	<b>Ash</b>	<b>Nitrogen free extract</b>
Fish meal	9.20 $\pm$ 0.31	90.80 $\pm$ 0.69	53.20 $\pm$ 0.52	6.66 $\pm$ 0.15	2.16 $\pm$ 0.15	20.27 $\pm$ 0.64	8.51
Soybean meal	9.75 $\pm$ 0.21	90.25 $\pm$ 0.60	42.50 $\pm$ 0.60	3.36 $\pm$ 0.11	8.20 $\pm$ 0.16	2.13 $\pm$ 0.05	34.06
Groundnut oilcake	8.18 $\pm$ 0.24	91.82 $\pm$ 0.76	41.81 $\pm$ 0.46	9.33 $\pm$ 0.20	8.41 $\pm$ 0.21	4.36 $\pm$ 0.11	27.91
Rice bran	12.26 $\pm$ 0.25	87.74 $\pm$ 0.75	12.40 $\pm$ 0.20	9.50 $\pm$ 0.20	22.65 $\pm$ 0.42	18.23 $\pm$ 0.11	24.96
Wheat flour	10.23 $\pm$ 0.11	89.77 $\pm$ 0.89	13.20 $\pm$ 0.32	5.15 $\pm$ 0.05	3.20 $\pm$ 0.10	6.36 $\pm$ 0.15	61.86
Silkworm pupae	8.20 $\pm$ 0.26	91.80 $\pm$ 0.74	48.30 $\pm$ 0.17	11.20 $\pm$ 0.17	6.25 $\pm$ 0.13	16.65 $\pm$ 0.36	9.4

**Table 7. Proximate composition of experimental diets**

Diets	Proximate composition (Mean $\pm$ Standard deviation) (%)						
	Moisture	Dry matter	Crude protein	Crude fat	Crude fibre	Ash	Nitrogen free extract
P <sub>1</sub>	9.40 $\pm$ 0.20	90.60 $\pm$ 0.80	30.25 $\pm$ 0.15	7.20 $\pm$ 0.10	15.23 $\pm$ 0.11	16.20 $\pm$ 0.17	21.72
P <sub>2</sub>	8.30 $\pm$ 0.26	91.70 $\pm$ 0.74	34.96 $\pm$ 0.15	7.70 $\pm$ 0.14	14.23 $\pm$ 0.15	16.28 $\pm$ 0.10	18.53
P <sub>3</sub>	9.35 $\pm$ 0.23	90.65 $\pm$ 0.77	40.90 $\pm$ 0.10	7.83 $\pm$ 0.05	12.96 $\pm$ 0.15	17.43 $\pm$ 0.11	11.53
SB <sub>1</sub>	8.84 $\pm$ 0.25	91.16 $\pm$ 0.75	30.77 $\pm$ 0.16	7.33 $\pm$ 0.05	15.57 $\pm$ 0.16	16.01 $\pm$ 0.18	21.48
SB <sub>2</sub>	8.72 $\pm$ 0.34	91.28 $\pm$ 0.66	30.97 $\pm$ 0.20	6.96 $\pm$ 0.15	16.22 $\pm$ 0.12	15.68 $\pm$ 0.10	21.45
SB <sub>3</sub>	8.87 $\pm$ 0.20	91.13 $\pm$ 0.80	30.14 $\pm$ 0.31	6.63 $\pm$ 0.22	16.37 $\pm$ 0.21	14.53 $\pm$ 0.32	23.46
C	8.70 $\pm$ 0.36	91.30 $\pm$ 0.64	24.96 $\pm$ 0.15	7.13 $\pm$ 0.15	15.03 $\pm$ 0.20	14.60 $\pm$ 0.43	29.58
SW <sub>1</sub>	8.64 $\pm$ 0.41	91.36 $\pm$ 0.59	30.07 $\pm$ 0.25	7.70 $\pm$ 0.09	15.21 $\pm$ 0.12	16.35 $\pm$ 0.07	22.03
SW <sub>2</sub>	9.07 $\pm$ 0.25	90.93 $\pm$ 0.75	30.76 $\pm$ 0.15	7.81 $\pm$ 0.10	14.78 $\pm$ 0.17	16.23 $\pm$ 0.07	21.35
SW <sub>3</sub>	9.20 $\pm$ 0.36	90.80 $\pm$ 0.64	30.43 $\pm$ 0.25	8.77 $\pm$ 0.11	15.08 $\pm$ 0.12	15.98 $\pm$ 0.13	20.54

The maximum moisture content was recorded in diet P<sub>1</sub> (9.40%) and minimum in diet P<sub>2</sub> (8.30%). The intermediate values were recorded in other formulated feeds. The crude protein content was adjusted to 30%, 35% and 40% level in diets P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub>, respectively in the second set of evacuation experiment. For all other diets (except diet C) the crude protein was adjusted to 30%. The crude protein content was minimum in conventional diet (diet C), i.e., 24.96%. The crude fat was highest in diet SW<sub>3</sub> (8.77%) and lowest in diet SB<sub>3</sub> (6.63%). The crude fibre content of the formulated feeds varied between 12.96% (diet P<sub>3</sub>) and 16.37% (diet SB<sub>3</sub>). The ash content of formulated diets ranged from 14.53% (diet SB<sub>3</sub>) to 17.43% (diet P<sub>3</sub>). The maximum carbohydrate (nitrogen free extract) content was observed in diet C (29.58%) and minimum was noticed in diet P<sub>3</sub> (11.53%). Intermediate values were recorded in other experimental diets.

### **4.3. Gastric evacuation experiments**

#### **4.3.1. Influence of natural food and conventional diet on gastric evacuation time**

The percentage of food recovered from guts of silver carp *Hypophthalmichthys molitrix* fingerlings fed with natural food (phytoplankton) and conventional diet (rice bran : groundnut oilcake :: 50:50) are presented in Table 8a. There was a gradual decline in percentage of food recovery from guts with time. The instantaneous rate of evacuation (Table 8b) was 0.2829 for natural food and 0.2217 for conventional diet. The time taken for 25%, 50%, 75% and 100% gut evacuation was estimated on the basis of linear regression equation, which is presented in Table 8c. The 100% gut evacuation time was observed to be 5.82 h and 7.24 h for natural food and conventional diet, respectively.

The  $r^2$  values ranged from 0.95 to 0.97 in linear model and 0.92 to 0.99 in the exponential model.

**Table 8a. Percentage of food recovered at each sampling intervals from guts of *Hypophthalmichthys molitrix* fingerlings fed with plankton and conventional diet**

Sampling time (min) X	Percentage of food recovered	
	Plankton (Y <sub>1</sub> )	Conventional diet (Y <sub>2</sub> )
20	100.00	100.00
60	75.540	77.512
120	64.378	67.234
180	42.918	52.250
240	35.622	42.500
300	14.163	34.250

**Table 8b. Instantaneous rate of evacuation (b), co-efficient of determination ( $r^2$ ) and gut evacuation time (GET) of *Hypophthalmichthys molitrix* fingerlings fed with plankton and conventional diet**

Diets	Average length (mm)	Average weight (g)	Water temperature (°C)	Instantaneous rate of evacuation (b)	Co-efficient of determination $r^2$		100%GET (h)
					L	E	
Plankton	75 ± 5	3.0±0.5	28 ± 1.0	- 0.2829	0.97	0.92	5.82
Conventional diet	75 ± 5	3.0±0.5	28 ± 1.0	- 0.2217	0.95	0.99	7.24

**L = Linear; E = Exponential.**

**Table 8c. Gut evacuation time (GET) of *Hypophthalmichthys molitrix* fingerlings fed with plankton and conventional diet: Estimation on the basis of linear regression equation**

Diets	a	b	Time (h) to percent evacuation			
			25	50	75	100
Plankton	98.8180	- 0.2829	1.40	2.87	4.35	5.82
Conventional diet	96.2790	- 0.2217	1.59	3.48	5.36	7.24

The linear regression equation of natural food and conventional diet on gastric evacuation time is given below (Fig 1).

$$\begin{aligned} \text{Natural food} & : Y = 98.8180 + (-0.2829) X \\ \text{Conventional diet} & : Y = 96.2790 + (-0.2217) X \end{aligned}$$

#### 4.3.2. Influence of protein level on gastric evacuation time

The percentage of food recovered from guts of silver carp *H. molitrix* fingerlings fed with diets of different protein levels are shown in Table 9a. The instantaneous rate of evacuation (Table 9b) was 0.1871, 0.1562 and 0.1410 for diet P<sub>1</sub> (30% protein), diet P<sub>2</sub> (35% protein) and diet P<sub>3</sub> (40% protein), respectively. The co-efficient of determination ( $r^2$ ) values for diets P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub> were 0.98 in all the diets in linear model and 0.93, 0.97 and 0.94 in exponential model, respectively (Table 9b).

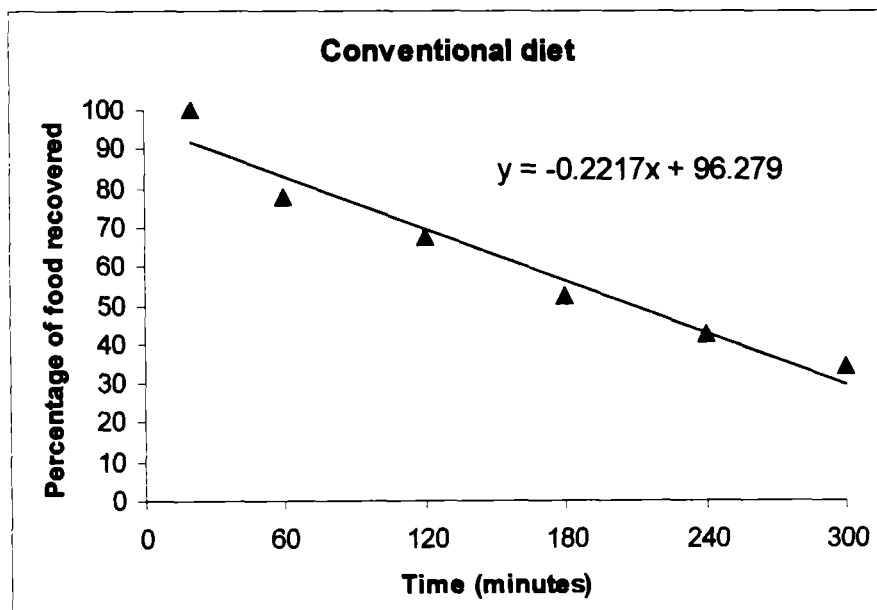
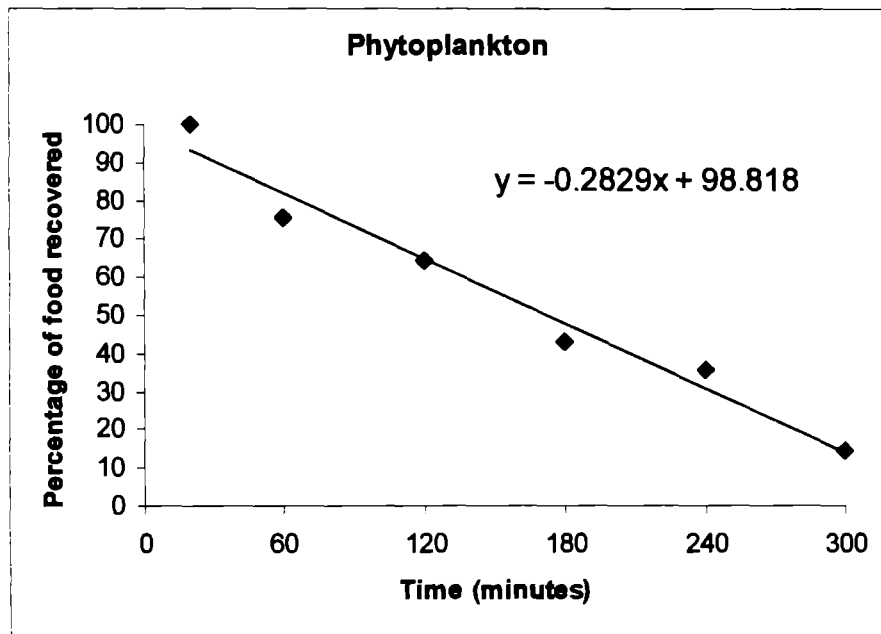
The linear regression equations computed for the three diets are given below (Fig 2).

$$\begin{aligned} \text{Diet P}_1 \text{ (30\% protein diet)} & : Y = 94.3786 + (-0.1871) X \\ \text{Duet P}_2 \text{ (35\% protein diet)} & : Y = 96.2910 + (-0.1562) X \\ \text{Diet P}_3 \text{ (40\% protein diet)} & : Y = 97.8360 + (-0.1410) X \end{aligned}$$

The estimated time for 25%, 50%, 75% and 100% gut evacuation determined using the above linear regression equation is given in Table 9c. The 100% gut evacuation time ranged from 8.41 h (diet P<sub>1</sub>) to 11.56 h (diet P<sub>3</sub>); while it was 10.27 h for diet P<sub>2</sub> (Table 9c).

#### 4.3.3. Influence of partial replacement of fish meal by soybean meal on gastric evacuation time

The percentage of food recovered from the guts of silver carp *H. molitrix* fingerlings fed with diets where fish meal was partially replaced by soybean meal



**Figure 1. Linear regression between percentage of food recovered and sampling interval from guts of silver carp fingerlings fed with phytoplankton and conventional diet.**

**Table 9a. Percentage of food recovered at each sampling intervals from guts of *Hypophthalmichthys molitrix* fingerlings fed with different protein levels**

Sampling time (min) X	Percentage of food recovered		
	Diet P <sub>1</sub> (30% Protein) Y <sub>1</sub>	Diet P <sub>2</sub> (35% Protein) Y <sub>2</sub>	Diet P <sub>3</sub> (40% Protein) Y <sub>3</sub>
	20	100.00	100.00
60	79.13	82.32	85.25
120	69.21	75.25	78.62
180	55.50	67.81	71.91
240	49.50	59.25	64.64
300	38.45	47.50	58.25
360	26.12	39.33	45.50
420	15.25	31.50	38.12
480	8.25	23.12	30.81

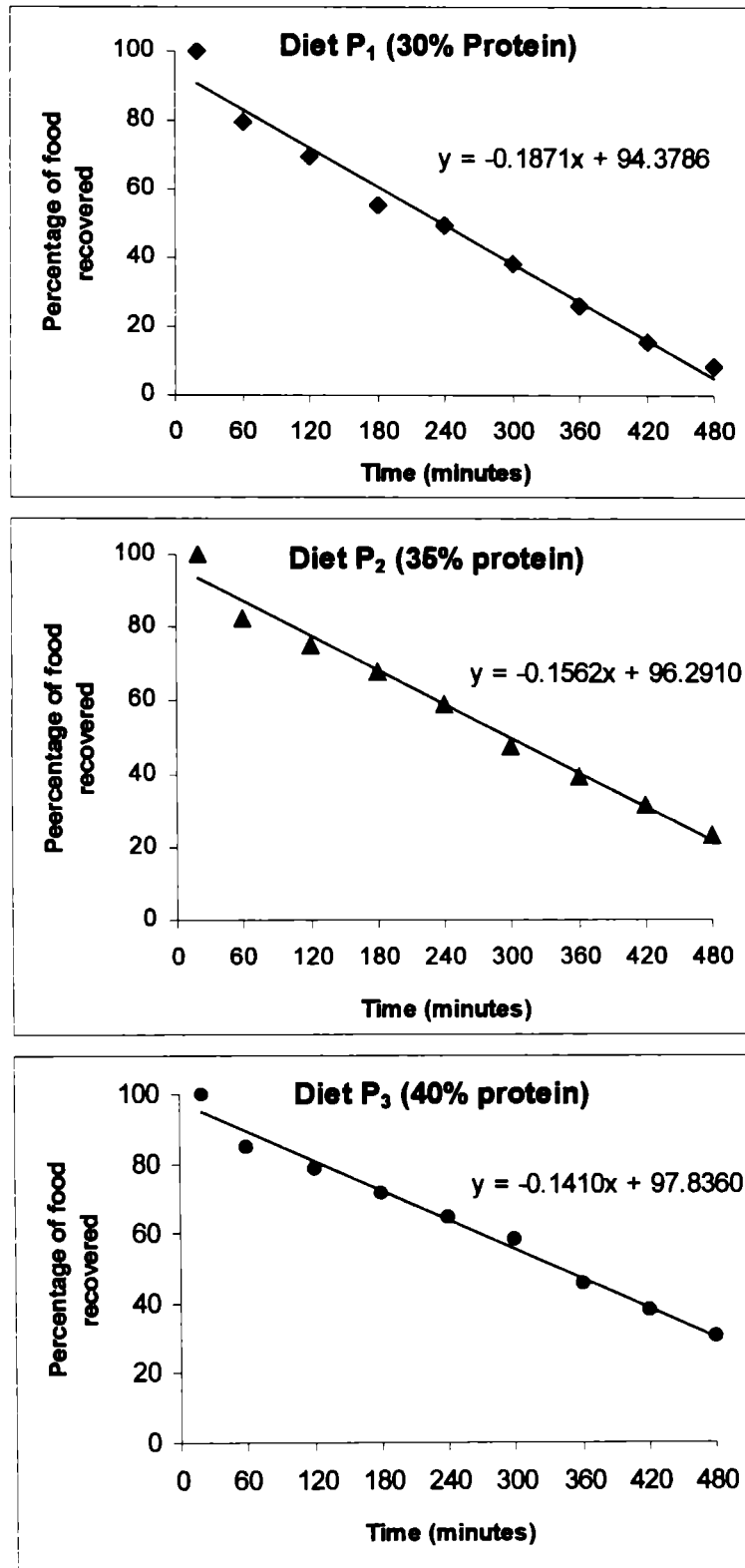
**Table 9b. Instantaneous rate of evacuation (b), co-efficient of determination ( $r^2$ ) and gut evacuation time (GET) of *Hypophthalmichthys molitrix* fingerlings fed with different protein levels**

Protein levels	Average length (mm)	Average weight (g)	Water temperature (°C)	Instantaneous rate of evacuation (b)	Co-efficient of determination $r^2$		100% GET (h)
					L	E	
30% Protein	75 ± 5	3.0 ± 0.5	28 ± 1.5	- 0.1871	0.98	0.93	8.41
35% Protein	75 ± 5	3.0 ± 0.5	28 ± 1.5	- 0.1562	0.98	0.97	10.27
40% Protein	75 ± 5	3.0 ± 0.5	28 ± 1.5	- 0.1410	0.98	0.94	11.56

L = Linear; E = Exponential

**Table 9c. Gut evacuation time (GET) of *Hypophthalmichthys molitrix* fingerlings fed with different protein levels : Estimation on the basis of linear regression equation**

Protein levels	a	b	Time (h) to percent evacuation			
			25	50	75	100
30% Protein	94.3786	- 0.1871	1.73	3.95	6.18	8.41
35% Protein	96.2910	- 0.1562	2.27	4.94	7.61	10.27
40% Protein	97.8760	- 0.1410	2.69	5.65	8.61	11.56



**Figure 2. Linear regression between percentage of food recovered and sampling interval from guts of silver carp fingerlings fed with different protein levels.**

at different levels are presented in Table 10a. The instantaneous rate of evacuation (b) was found to decrease with the increase in percentage incorporation of soybean meal in the diet. The values of 'b' were found to be 0.1631, 0.1504 and 0.1243 for diets SB<sub>1</sub> (10% replacement), SB<sub>2</sub> (20% replacement) and SB<sub>3</sub> (40% replacement), respectively (Table 10b).

The r<sup>2</sup> values ranged between 0.98 to 0.99 and 0.94 to 0.96 in the linear and the exponential models, respectively.

The linear regression fits computed for the three diets are given below (Fig 3).

$$\text{Diet SB}_1 \text{ (10\% replacement)} \quad : \quad Y = 93.1940 + (-0.1631) X$$

$$\text{Diet SB}_2 \text{ (20\% replacement)} \quad : \quad Y = 96.6510 + (-0.1504) X$$

$$\text{Diet SB}_3 \text{ (40\% replacement)} \quad : \quad Y = 96.8220 + (-0.1243) X.$$

Complete evacuation (100%) of gut was faster in fishes fed with diet SB<sub>1</sub> (9.52 h) compared to diet SB<sub>2</sub> (10.71 h) and diet SB<sub>3</sub> (12.98 h) diets (Table 10c).

#### **4.3.4. Influence of partial replacement of fish meal by silkworm pupae on gastric evacuation time**

Table 11a represents the percentage of food recovered from guts of silver carp *H. molitrix* fingerlings after different intervals of time when fish meal was partially replaced (10%, 20% and 40%) by silkworm pupae in the diets. The instantaneous rate of evacuation (Table 11b) was found to decrease with the increase in level of incorporation silkworm pupae in the diets. The values of 'b' were 0.1758, 0.1695 and 0.1524 for diets SW<sub>1</sub> (10% replacement), SW<sub>2</sub> (20% replacement) and SW<sub>3</sub> (40% replacement), respectively. The r<sup>2</sup> values varied from 0.97 to 0.98 and 0.95 to 0.97, respectively in linear and exponential models.

**Table 10a. Percentage of food recovered at each sampling intervals from guts of *Hypophthalmichthys molitrix* fingerlings fed with feeds where fish meal partially was replaced by soybean meal at different levels**

Sampling time (min) X	Percentage of food recovered		
	Diet SB <sub>1</sub> (10%) Y <sub>1</sub>	Diet SB <sub>2</sub> (20%) Y <sub>2</sub>	Diet SB <sub>3</sub> (40%) Y <sub>3</sub>
20	100.00	100.00	100.00
60	80.71	83.25	86.25
120	71.43	75.75	79.12
180	57.14	66.62	70.75
240	52.29	62.50	66.62
300	44.50	54.12	62.50
360	34.75	41.75	54.12
420	23.29	33.25	45.75
480	14.43	24.25	35.50
540	9.71	15.75	29.25

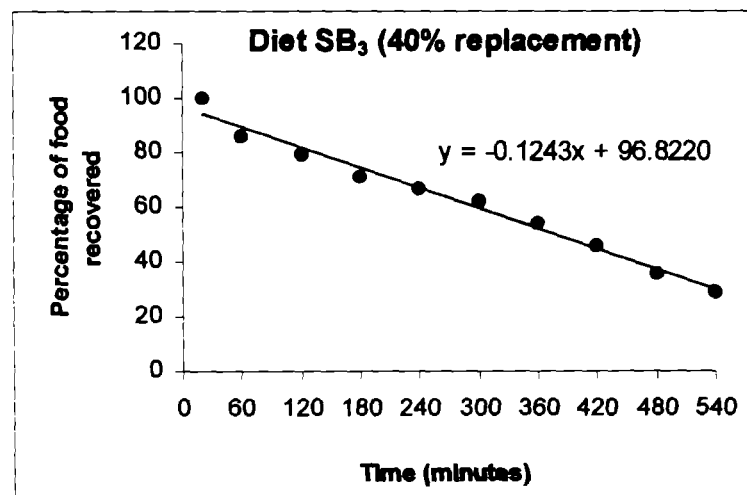
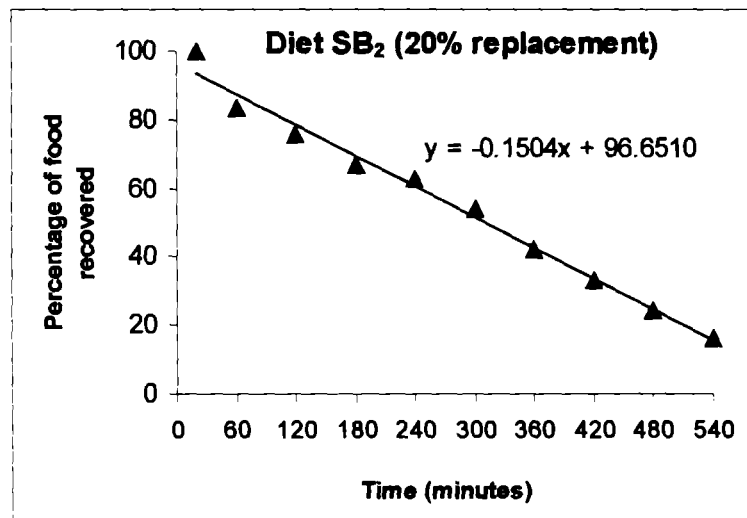
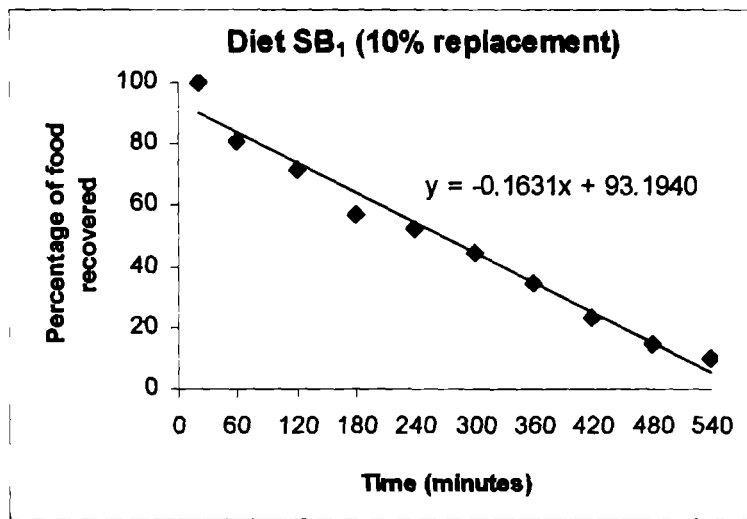
**Table 10b. Instantaneous rate of evacuation (b), co-efficient of determination ( $r^2$ ) and gut evacuation time (GET) of *Hypophthalmichthys molitrix* fingerlings fed with feeds where fish meal was partially replaced by soybean meal at different levels**

Replacement level	Average length (mm)	Average weight (g)	Water temperature (°C)	Instantaneous rate of evacuation (b)	Co-efficient of determination ( $r^2$ )		100% GET (h)
					L	E	
10%	75 ± 5	3.0 ± 0.5	28 ± 1.5	- 0.1631	0.98	0.94	9.52
20%	75 ± 5	3.0 ± 0.5	28 ± 1.5	- 0.1504	0.99	0.94	10.71
40%	75 ± 5	3.0 ± 0.5	28 ± 1.5	- 0.1243	0.98	0.96	12.98

**L = Linear; E = Exponential**

**Table 10c. Gut evacuation time (GET) of *Hypophthalmichthys molitrix* fingerlings fed with diets where fish meal partially was replaced by soybean meal at different levels: Estimation on the basis of linear regression equation**

Replacement level	a	b	Time (h) to percent evacuation			
			25	50	75	100
10%	93.1940	- 0.1631	1.86	4.41	6.97	9.52
20%	96.6510	- 0.1504	2.39	5.17	7.94	10.71
40%	96.8220	- 0.1243	2.92	6.28	9.63	12.98



**Figure 3. Linear regression between percentage of food recovered and sampling interval from guts of silver carp fingerlings fed with diets where fish meal was partially replaced by soybean meal at different levels.**

**Table 11a. Percentage of food recovered at each sampling intervals from guts of *Hypophthalmichthys molitrix* fingerlings fed with diets where fish meal was partially replaced by silkworm pupae at different levels**

Sampling time (min) X	Percentage of food recovered		
	Diet SW <sub>1</sub> (10%) Y <sub>1</sub>	Diet SW <sub>2</sub> (20%) Y <sub>2</sub>	Diet SW <sub>3</sub> (40%) Y <sub>3</sub>
20	100.00	100.00	100.00
60	80.03	82.62	83.12
120	69.97	75.12	76.87
180	56.25	61.82	65.25
240	51.12	56.25	62.50
300	43.96	45.04	53.12
360	30.71	32.15	41.75
420	19.03	25.28	32.43
480	12.91	18.75	23.93

The linear regression equations computed for the three diets are given below (Fig 4).

$$\text{Diet SW}_1 \text{ (10\% replacement)} : Y = 94.1520 + (-0.1758) X$$

$$\text{Diet SW}_2 \text{ (20\% replacement)} : Y = 96.2180 + (-0.1695) X$$

$$\text{Diet SW}_3 \text{ (40\% replacement)} : Y = 96.8150 + (-0.1524) X$$

The estimated time for complete (100%) gut evacuation was 8.93 h, 9.46 h and 10.59 h for diets SW<sub>1</sub>, SW<sub>2</sub> and SW<sub>3</sub>, respectively (Table 11c).

#### **4.4. Estimation of maintenance and daily ration**

##### **4.4.1. Estimation of maintenance ration**

The average length (mm) and weight (g) of silver carp *H. molitrix* fingerlings during different sampling periods with respect to percentage body weight of feeding are presented in Table 12. The fishes fed 1% of body weight have shown least growth (weight increment 0.04 g) at the end of the experimental period, and hence, considered as maintenance ration. The weight increment was found to be 1.13 g, 1.45 g, 1.91 g, 2.05 g in other groups of fishes fed 2%, 3%, 5% and 10% of body weight, respectively at the end of experimental period (6 weeks). The increment in length was found to be 1.50 mm, 10.20 mm, 13.40 mm, 13.92 mm and 15.40 mm in the groups of fishes fed 1%, 2%, 3%, 5% and 10% of body weight, respectively (Fig 5).

##### **4.4.2. Estimation of daily ration**

The estimated daily ration of silver carp *H. molitrix* fingerlings corresponding to their body weight is represented in Table 13. Fishes weighing 3.0 g to 4.5 g were used to find out the influence of size on food consumption. The amount of food consumption was increased with the increase in the size of fish.

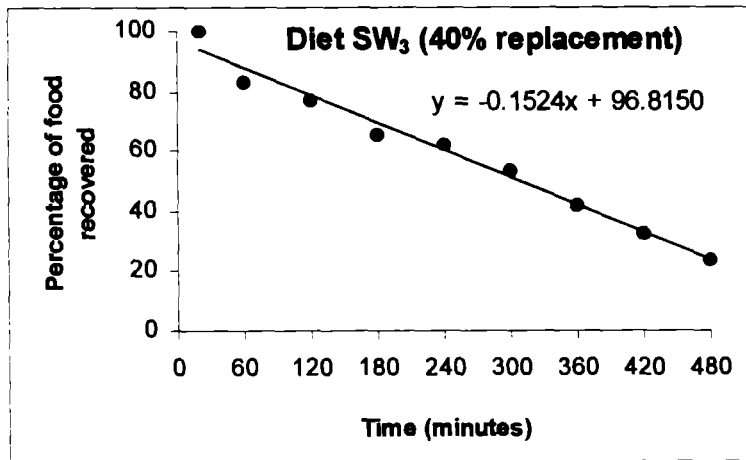
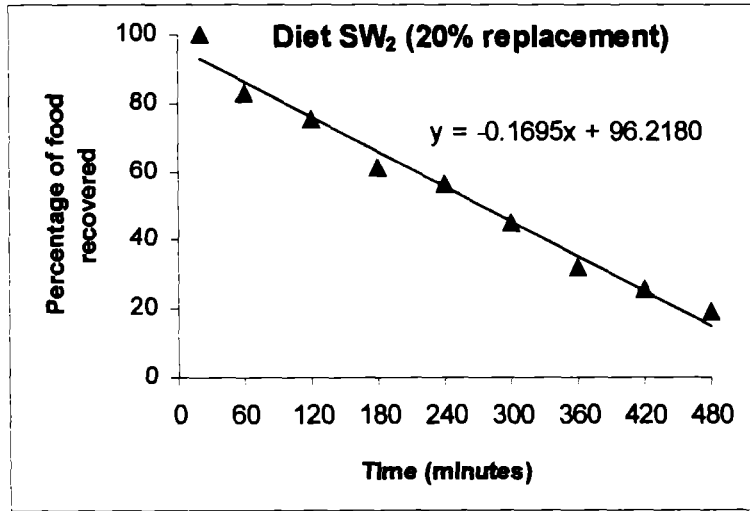
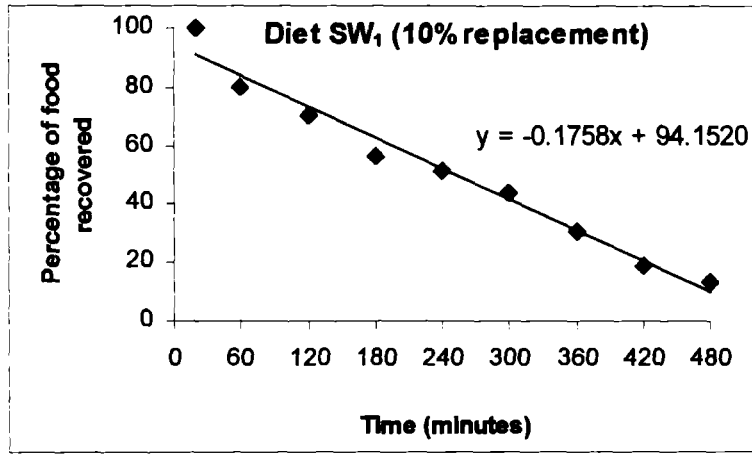
**Table 11b. Instantaneous rate of evacuation (b), co-efficient of determination ( $r^2$ ) and gut evacuation time (GET) of *Hypophthalmichthys molitrix* fingerlings fed with diets where fish meal was partially replaced by silkworm pupae**

Replacement level	Average length (mm)	Average weight (g)	Water temperature (°C)	Instantaneous rate of evacuation (b)	Co-efficient of determination ( $r^2$ )		100% GET (h)
					L	E	
10%	75 ± 5	3.0 ± 0.5	28 ± 1.5	- 0.1758	0.97	0.95	8.93
20%	75 ± 5	3.0 ± 0.5	28 ± 1.5	- 0.1695	0.98	0.97	9.46
40%	75 ± 5	3.0 ± 0.5	28 ± 1.5	- 0.1524	0.98	0.96	10.59

L = Linear; E = Exponential

**Table 11c. Gut evacuation time (GET) of *Hypophthalmichthys molitrix* fingerlings fed with diets where fish meal was partially replaced by silkworm pupae at different levels: Estimation on the basis of linear regression equation**

Replacement level	a	B	Time (h) to percent evacuation			
			25	50	75	100
10%	94.1520	- 0.1758	1.81	4.19	6.55	8.93
20%	96.2180	-0.1695	2.10	4.55	7.01	9.46
40%	96.8150	- 0.1524	2.39	5.12	7.85	10.59

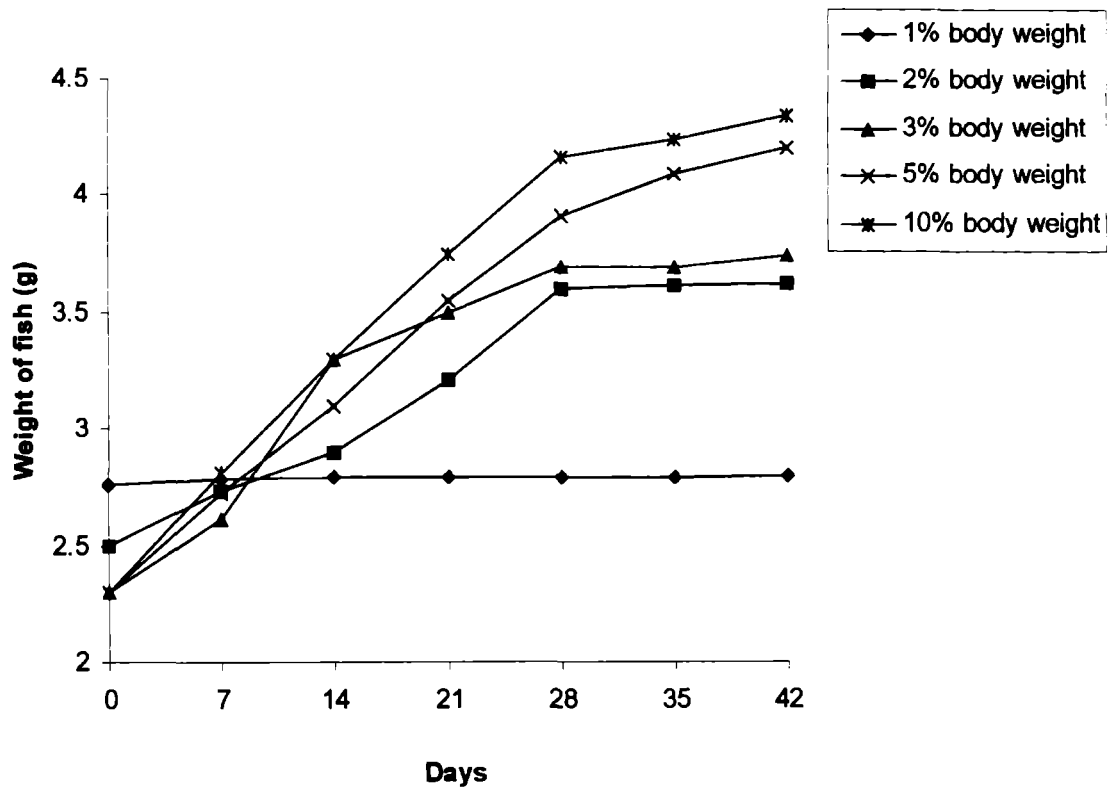


**Figure 4. Linear regression between percentage of food recovered and sampling interval from guts of silver carp fingerlings fed with diets where fish meal was partially replaced by silkworm pupae at different levels.**

**Table 12. Average length (mm) and weight (g) of *Hypophthalmichthys molitrix* fingerlings (n = 10) during sampling time with respect to percentage body weight feed consumed**

Feed given	1%		2%		3%		5%		10%	
	Average length (mm)	Average weight (g)	Average length (mm)	Average weight (g)	Average length (mm)	Average weight (g)	Average length (mm)	Average weight (g)	Average length (mm)	Average weight (g)
Initial weight	67.80	2.76	62.00	2.50	61.00	2.30	61.00	2.30	61.10	2.30
7	67.80	2.78	64.20	2.73	65.00	2.61	65.22	2.72	65.51	2.81
14	68.80	2.79	68.00	2.90	68.30	3.30	70.03	3.10	71.20	3.30
21	68.80	2.79	70.10	3.21	71.30	3.50	72.11	3.55	73.20	3.75
28	69.20	2.79	71.50	3.60	73.20	3.70	73.50	3.92	75.31	4.17
35	69.30	2.79	72.10	3.62	74.30	3.70	74.71	4.10	76.20	4.25
42	69.30	2.80	72.20	3.63	74.40	3.75	74.92	4.21	76.50	4.35
Increment in length/weight	1.50	0.04 <sup>a</sup>	10.20	1.13 <sup>b</sup>	13.40	1.45 <sup>b</sup>	13.92	1.91 <sup>c</sup>	15.40	2.05 <sup>c</sup>

Cd = 0.344. Values with same superscript do not differ significantly (P>0.05)



**Fig 5. Average weight (g) increment of silver carp fingerlings (n = 10) during sampling period with respect to percentage body weight feed consumed**

It varied between 0.088 g and 0.177 g. The percentage body weight of food consumed was ranged from 2.635 to 4.023. For the estimation of daily ration, feed consumed (as percentage body weight), i.e., 'A' was calculated (Table 13). Food passage time (h) was considered as 8.41 h (gut evacuation time of 30% fish meal based diet), as 30% fish meal based diet was used in this experiment. The estimated daily ration ranged between 7.52% and 11.48% body weight with an average value of 9.64% at room temperature ( $28\pm 2.0^{\circ}\text{C}$ ).

#### **4.4. Statistical analyses**

The analysis of variance (ANOVA) revealed insignificant differences ( $P>0.05$ ) in the gut evacuation time between plankton and conventional diet. Similarly there was no significant difference ( $P>0.05$ ) among the levels of protein, levels of incorporation of soybean meal and silkworm pupae (Annexure - I). There was significant difference ( $P<0.05$ ) in the weight increment was observed among the percentage of body weight fed to silver carp fingerlings (Annexure - II). Further, critical difference test revealed that there was a significant difference ( $P<0.05$ ) in weight increment among fishes fed 1% body weight and all other levels. However, there was no significant difference between 2% and 5%, and 5% and 10% body weight levels.

**Table 13. Daily ration of *Hypophthalmichthys molitrix* fingerlings (n=10) corresponding to their body weight**

<b>Fish weight (g)</b>	<b>Feed consumed (g)</b>	<b>Feed weight (% Body weight)</b>	<b>Daily ration (%)</b>
3.34	0.088	2.635	7.52
3.81	0.118	3.097	8.84
4.15	0.152	3.663	10.45
4.20	0.159	3.786	10.80
3.90	0.132	3.385	9.66
4.25	0.164	3.859	11.01
4.40	0.177	4.023	11.48
4.10	0.151	3.683	10.51
3.60	0.105	2.917	8.32
3.52	0.097	2.756	7.86

# Chapter — 5

## Discussion

## **5. DISCUSSION**

### **5.1. Gastric evacuation experiments**

In the present study, all the gastric evacuation data were explained by linear regression model. Though there was no significant difference between co-efficient of determination ( $r^2$ ) values of the linear and exponential models, the linear model was used for estimating the regression functions (a and b) for the sake of simplicity. Further, the linear regression fitted better than other models like exponential, square root model etc. (Jobling, 1980) and the gut evacuation appear to proceed in a linear function of ingestion under continuous feeding conditions.

All the results with respect to gut weight and food weight were expressed on wet weight basis as the amount of food ingested and that remained in the gut was very small.

#### **5.1.1. Influence of natural food and conventional diet on gastric evacuation time**

The role of plankton as natural food for fishes is of prime importance in fish culture. Silver carp is a planktophagous. The principal food of advanced fry and fingerlings is phytoplankton (Ghosh *et al.*, 1973) and it secondarily feeds on zooplankton. The ratio of phytoplankton and zooplankton is being 248:1 (Chondar, 1999). In the present investigation, it was observed that the gastric evacuation time (GET) of silver carp fingerlings was less when fed with phytoplankton (5.82 h) than conventional diet (rice bran : groundnut oil cake :: 50 : 50) (7.24 h). This might be due to the fact that natural food exposes the centre of the food to the action of digestive enzymes and potentially increases the digestion rate. Further, the higher content of water (92%) as well as lower contents of protein (2.5%) and lipid (0.3%) would have contributed for the faster evacuation

rate (Jobling, 1994). The GET of silver carp fingerlings was comparatively higher when fed with conventional diet probably due to higher protein (24.96%) and fat (7.13%) content. However, the time taken for complete evacuation was lesser than those of pelleted feeds. Similar observations were made by Annappaswamy *et al.* (2001). They found that the fingerlings of common carp *Cyprinus carpio* had taken 6.83 h and 8.68 h for complete gut evacuation when fed with natural food and conventional feed, respectively.

### **5.1.2. Influence of protein level on gastric evacuation time**

Protein makes up about 75% of the major organic materials in animal tissue on a dry weight basis. Level of crude protein in the diet plays a significant role in various physiological activities including the digestion rate. Each fish species has got its own optimum requirement for protein to achieve optimum digestion rate and growth. If the protein level in the diet is imbalanced, it delays the digestion rate and results in quick reduction or cessation of growth. In the present investigation, it was observed that a gradual decline in the percentage of food recovered from guts of silver carp *H. molitrix* fingerlings and the instantaneous rate of evacuation (0.1897, 0.1562 and 0.1410) with increase in protein levels in feed, i.e., Diets P<sub>1</sub> (30% protein), P<sub>2</sub> (35% protein) and P<sub>3</sub> (40% protein). The 100% gastric evacuation time ranged from 8.41 h (diet P<sub>1</sub>) to 11.56 h (diet P<sub>3</sub>), whereas it was 10.27 h in diet P<sub>2</sub>. Gastric evacuation rate is regulated by the dietary energy of the ingredients used (Andersen, 1999). Thus, reduction in digestion rate in the present study, may probably be due to chemical difference (proximate composition) of the diets (De Silva and Owoyemi, 1983), which have different levels of energy. Further, Lovel (1980) reported that too much energy in relation to percentage of protein in ration could impede the evacuation of food. The observations made in the present study are corroborated with that of Annappaswamy *et al.* (2000) in *Labeo rohita*, Naik *et al.* (2000a) in *Catla catla*, Naik *et al.* (2000b) in *Cyprinus carpio* and Saha (2002) in *L. bata*. All of them

noticed decrease in gastric evacuation time with the increasing level of protein. Annappaswamy *et al.* (2000) could notice a significant ( $P < 0.05$ ) difference in gastric evacuation rate of the species studied due to 30% and 40% protein levels in the feed. However, the rate of evacuation did not differ significantly ( $P > 0.05$ ) during the present study, among the different protein levels.

### **5.1.3. Influence of partial replacement of fish meal by soybean meal on gastric evacuation time**

The food quality in general and the protein source in particular is one of the key factors, which influence the gut evacuation in fishes. Soybean meal having high protein content (42.5%) and favourable amino acid profile, that closely meets the requirements of fish, is consistently available and reported to be palatable to most species of fishes (Lim and Akiyama, 1992). Hence, it is effectively used as substitute to fish meal to reduce the cost of feed. In the present study, soybean meal was partially incorporated (10%, 20%, and 40%) in diets of silver carp fingerlings as substitute of fish meal to understand its effect on gastric evacuation time. It was observed that time taken to complete (100%) gut evacuation was increased with the increased level of incorporation of soybean meal in diets. The 100% GET values of silver carp fingerlings when fed with diets SB<sub>1</sub>, SB<sub>2</sub> and SB<sub>3</sub> were 9.52 h, 10.71 and 12.98 h, respectively. The instantaneous rate of evacuation was found to be 0.1631, 0.1504 and 0.1243, for diets SB<sub>1</sub>, SB<sub>2</sub> and SB<sub>3</sub>, respectively. Type of the ingredients in the diet would influence gastric evacuation in fishes (Andersen, 1999). Further, the soybean meal is being plant origin has higher fibre content, the digestion of which might have taken longer time. According to Nengas *et al.* (1995), digestibility of soybean meal is lower than fish meal. The main limitations in the digestibility of soybean meal are related to its lower level of methionine and the presence of antinutritional factors (Wilson and Poe, 1985; Olli, *et al.*, 1994a). Antinutritional factors present in soybean meal are protease inhibitors, non-digestible carbohydrate, lectins, phytates and possibly

allergenic storage proteins (Liu, 1997). Fish meal based diet evacuated faster than soybean meal as the former is an animal protein containing the most essential amino acids. There was no significant difference ( $P>0.05$ ) in gut evacuation time among different levels of incorporation of soybean meal in the diets. The observations made for other fishes such as *Labeo rohita*, *Catla catla*, *Cyprinus carpio* and *L. bata* are in accordance with the present investigation (Annappaswamy *et al.*, 2000; Naik *et al.*, 2000a; Naik *et al.*, 2000b; Saha, 2002) when fish meal was completely replaced by soybean meal.

#### **5.1.4. Influence of partial replacement of fish meal by silkworm pupae on gastric evacuation time**

Earlier studies have indicated the suitability of silkworm pupae as a less expensive dietary ingredient for carps since it has high protein content (48.3%) (Nandeeshia *et al.*, 1990). But its level of incorporation in the diet is restricted to 60% due to its high fat content (11.2%) (Shyama and Keshavanath, 1996). In the present study, silkworm pupae had been partially incorporated (diet SW<sub>1</sub> – 10%, diet SW<sub>2</sub> – 20% and diet SW<sub>3</sub> – 40%) in diets of silver carp fingerlings as a substitute for fish meal. It was noticed that diet SW<sub>3</sub> took 10.59 h, whereas diets SW<sub>1</sub> and SW<sub>2</sub> took 8.93 h and 9.46 h, respectively for complete evacuation of gut of silver carp fingerlings. However, there was no significant difference ( $P>0.05$ ) among the different incorporation levels examined in the present study. The higher GET for diet SW<sub>3</sub> when compared to other diets may be due to higher fat content, which generally considered to be digested slowly (Basimi and Grove, 1985b; Medved, 1985). Raw silkworm pupae was reported to contain some attractants and appetite stimulants (Ina, 1976; Taushima and Ina, 1978), which might increase the digestibility, hence took less time for gut evacuation as compared to soybean meal. Hunt and Stubs (1975) stated that delay in digestion rate could be due to high amount of fat in the diets. The increased lipid content in the diet has decreased gut evacuation rate in plaice *Pleuronectes platessa*, rainbow trout *Salmo gairdneri* and

cod *Gadus morhua* (Windell and Norris, 1969; Jobling, 1980; dos Santos and Jobling, 1991). Higher level (15%) of incorporation of lipid (cod liver oil) took 16.10 h in *L. bata* fingerlings (Saha, 2002). Contradictory to the above studies, De Silva and Owoyemi (1983) could not notice any effect of lipid on the gut evacuation time in *Serotherodon mossambicus*.

## **5.2. Maintenance ration and daily ration**

### **5.2.1. Estimation of maintenance ration**

The minimum ration required to perform basic physiological activity of the fishes where no increment in growth or very little growth exists is considered as maintenance ration. It was observed in the present study that silver carp fingerlings fed 1% of body weight showed the minimum/least growth when reared under laboratory conditions for a period of 42 days and hence, it is considered as the maintenance ration. There was a significant difference ( $P < 0.05$ ) in weight increment among silver carp fingerlings fed 1% body weight and all other levels. However, there was no significant difference between 2% and 5%, and 5% and 10% body weight levels. Maintenance ration needed by a fish depends on various biotic and abiotic factors, viz., size of fish, species, water temperature etc. Usually, the temperate water fishes will have higher percentage of maintenance ration than those of tropical fishes. The maintenance ration of yellow fin sole *Limanda asperu* was found to be 0.26% (Smith *et al.*, 1991) whereas, it was ranged between 0.7 and 2.3 % body weight per day for seabass *Dicentrarchus labrax*. The maintenance ration of *L. rohita* and *L. bata* fingerlings was found to be 1% body weight (Annappaswamy, 1997; Saha, 2002).

### **5.2.2. Estimation of daily ration**

Estimation of daily ration is very much imperative in feed scheduling and feeding strategies in culture systems. In the present investigation, the estimates of

daily ration for silver carp *H. molitrix* fingerlings of weighing 3.34 – 4.40 g ranged from 7.52 to 11.48% body weight per day at a water temperature of  $28\pm 2^{\circ}\text{C}$ . These results are comparable to earlier studies in silver carp *H. molitrix* (5.72-11.10%) *L. rohita* (9.20-11.12%) and *L. bata* (9.87-11.48%) as being reported by Bialokoz and Krzywosz (1981), Annappaswamy (1997) and Saha (2002), respectively. Troschel and Rosch (1991) also reported that the maximum daily ration was higher for cyprinids than coregonids. The daily ration of bighead carp *Aristichthys nobilis* was found to be 2.0 to 5.7% of body weight per day (Opuszynski and Shireman, 1991). Elliott (1975) estimated daily ration of 11.3% of body weight for *Salmo trutta*. Brodeur and Percy (1987) noticed an increase in daily ration of Coho salmon with the increase in temperature. Persson (1982) found difference in the daily ration of *Rutilus rutilus* under field (4.6% of body weight) and laboratory (12.5% of body weight) conditions. The daily ration of sockeye salmon of more than 115 mm size was found to be as low as 0.62% of body weight per day (Doble and Eggers, 1978). The deviations in daily ration may indicate the insufficient quantity of dietary energy taken by fish from natural food alone (Sumagaysay, 1993).

The wide disparity in the results of the above studies reflects the difference in the method used to estimate daily food consumption. Other factors like temperature, season, habitat, density of fish, availability of feed, chemical composition of feed, size of fish, stress condition and method employed for the estimation would regulate gastric evacuation rate and, hence, the daily food consumption of fish. It is, therefore, important that the models should be employed with caution and only after thorough understanding of the causes of such incongruity.

# Chapter — 6

## Summary

## 6. SUMMARY

If a cultivable crop of fish is to be effectively managed for sustainable optimum production and hence profit, it is very much imperative to have a clear understanding of nutritional requirement of fish and its feeding schedule. In this regard, knowledge on gastric evacuation rate and daily ration of cultivable fish species is very much essential to formulate feeding regimes. Data on gastric evacuation rate also provide information for a number of related problems such as feeding activity, conversion efficiency etc. The present study was, therefore, carried out to evaluate the influence of natural food and conventional diet, protein level (30%, 35% and 40%), partial replacement of fish meal (10%, 20% and 40%) by soybean meal and silkworm pupae on gastric evacuation time in silver carp *Hypophthalmichthys molitrix* fingerlings. The maintenance and daily ration were also estimated on the basis of gastric evacuation data.

The salient findings of the investigation are summarized below,

1. The instantaneous rate of evacuation was higher in natural food (phytoplankton) than that of conventional diet (rice bran : groundnut oil cake :: 50:50). Phytoplankton took 5.82 h for complete (100%) evacuation, where as conventional feed took 7.24 h in fingerlings of silver carp. The gastric evacuation time for phytoplankton was also lowest when compared all the experimental feeds used.
2. Among different protein levels of the fish meal based diets, 30% protein diet evacuated faster (8.41 h) compared to 35% (10.27 h) and 40% (11.56 h) protein diets in the fingerlings of silver carp.

3. The gastric evacuation rate was higher in lower levels of incorporation of plant source (soybean meal). The 10% percent replacement took 9.52 h for complete evacuation whereas, 20% and 40% replacement levels evacuated after 10.71 h and 12.98 h, respectively.
4. The instantaneous rate of evacuation was found to decrease with increase in the level of replacement of silkworm pupae in the diet. Gut evacuation was faster in fingerlings of silver carp fed on diet replaced with 10% (8.93 h) followed by 20% (9.46 h) and 40% (10.59 h) of silkworm pupae, respectively.
5. The maintenance ration was found to be 1.0% of body weight for the fingerlings of silver carp.
6. The estimated daily ration of silver carp fingerlings weighing 3.34 g to 4.40 g were found to vary from 7.52% body weight to 11.48% body weight with the average value of 9.64% body weight at  $28\pm 2^{\circ}\text{C}$  temperature.
7. There was no significant difference ( $P>0.05$ ) between  $r^2$  values of the two models used, viz., linear model and exponential model.
8. The analysis of variance (ANOVA) revealed insignificant differences ( $P>0.05$ ) in the gut evacuation time between the natural food and conventional diet. There was no significant difference ( $P>0.05$ ) among the levels of protein, levels of partial replacement with soybean meal and silkworm pupae.
9. There was a significant difference ( $P<0.05$ ) in weight increment among silver carp fingerlings fed 1% body weight and all other levels. However, there was no significant difference between 2% and 5%, and 5% and 10% body weight levels.

It can be concluded from the results of the present study that silver carp *H. molitrix* exhibits a linear evacuation of food from the guts and hence, it can be considered as continuous feeder. The feeding schedule has to be devised accordingly for better feed conversion efficiency. The gastric evacuation rate of the silver carp fingerlings is influenced by type and composition of food including protein level and protein source. The higher protein and lipid contents not only increase the gastric evacuation time but also reduce the appetite and absorption efficiency, which in turn, may decline the growth rate. Hence, a well balanced diet with optimum requirement of these nutrients has to be developed and used in the culture systems, which not only increases the production but also reduces the cost of feed. The use of either simple protein or animal protein in the diet is preferred over the plant protein as the former takes lesser time for complete gut evacuation. The fingerlings of silver carp (3.34 to 4.40 g) can be fed with 9.64% of body weight in split dose i.e. one each in the morning and evening. The estimation of food consumption is reasonably accurate and can be used for application of various ecological problems.

# Chapter – 7

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# Chapter — 8

## Annexure

## Annexure - I

ANOVA (one way) for the effect of various diets on gastric evacuation rate in *Hypophthalmichthys molitrix* fingerlings

### 1. Natural food and conventional diet

Source of variation	Sum of squares	Degree of freedom	Mean sum of squares	F-value ( $\alpha = 0.05$ )	P-value	F <sub>crit</sub>
Between groups	137.7561	1	137.7561	0.180277	0.680125	4.964591
Within groups	7641.3520	10	764.1352			
Total	7779.1080	11				

### 2. Protein levels

Source of variation	Sum of squares	Degree of freedom	Mean sum of squares	F-value ( $\alpha = 0.05$ )	P-value	F <sub>crit</sub>
Between groups	989.71	2	494.8545	0.711559	0.500944	3.402832
Within groups	16690.83	24	695.4514			
Total	17680.54	26				

### 3. Partial replacement of fish meal by soybean meal

Source of variation	Sum of squares	Degree of freedom	Mean sum of squares	F-value ( $\alpha = 0.05$ )	P-value	F <sub>crit</sub>
Between groups	325.81	2	162.9034	0.215538	0.807651	3.402832
Within groups	18139.19	24	755.7996			
Total	18465.00	26				

### 4. Partial replacement of fish meal by silkworm pupae

Source of variation	Sum of squares	Degree of freedom	Mean sum of squares	F-value ( $\alpha = 0.05$ )	P-value	F <sub>crit</sub>
Between groups	1002.889	2	501.4446	0.716378	0.497571	3.354131
Within groups	18899.260	27	699.9724			
Total	19902.14	29				

**Annexure – II**

**ANOVA (two way) for the effect of percentage body weight feeding on weight increment in *Hypophthalmichthys molitrix* fingerlings**

<b>Source of variation</b>	<b>Sum of squares</b>	<b>Degree of freedom</b>	<b>Mean sum of squares</b>	<b>F-value (<math>\alpha = 0.05</math>)</b>	<b>P-value</b>	<b>F<sub>crit</sub></b>
Rows	7.863949	6	1.310658	13.05517	1.55E-06	2.508187
Columns	2.419389	4	0.604847	6.024745	0.00167	2.776289
Error	2.409451	24	0.100394			
Total	12.69279	34				