EFFECT OF SHRIMP HEAD MEAL ON THE GROWTH OF SILVER BARB (*Puntius gonionotus*)

A Project Thesis

By

Mohd. Shafiqur Rahman

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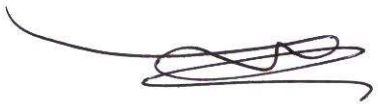

Dr. Khandaker Anisul Huq

Professor and Head

Fisheries and Marine Resource Technology Discipline

Khulna University, Khulna

Declaration

Effect of Shrimp Head Meal on the Growth of Silver Barb (*Puntius gonionotus*).

The results presented in the Project thesis with the above mentioned title are entirely of the candidate's own investigations and no part of the results has been accepted for any degree nor is it being concurrently submitted for any degree.

Mohd. Shafiqur Rahman

(Signature of the candidate)

Mohd. Shafiqur Rahman

Examination Roll No: MS 030608

Session: 2002-03

(Signature of the Supervisor)

Dr. Md. Nazmul Ahsan

Professor

Fisheries and Marine Resource Technology Discipline

Khulna University, Khulna, Bangladesh

February, 2008

DEDICATED

TO

MY BELOVED PARENTS

Acknowledgement

It is my pleasure for being able to complete this project thesis; so first of all, all my appreciation and indebtedness go for the creator of the universe, the Almighty Allah.

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Mohd. Shafiqur Rahman

Khulna University

February, 2008

Abstract

An experiment was conducted to observe the effect of shrimp head meal as a dietary protein supplement on the growth of Thai silver barb (*Puntius gonionotus*) for 107 days in 10 mini earthen ponds. Five experimental diets were formulated. They were designated as T_1 (control), T_2, T_3, T_4 and T_5. Each feed contain 25% protein. In T_1 (control) fishmeal was used as protein supplement. In T_2, T_3, T_4 and T_5; 100, 75, 50, and 25% fishmeal was replaced with shrimp head meal protein respectively. Fry was collected from hatchery having 1.10g weight and stocked at a density of 60 per decimal. Water quality was monitored regularly and found within the acceptable limit. Feed was given twice a day at a rate of 10 % of the total body weight. Ratio was adjusted by sampling. The highest specific growth rate (SGR%/day) was observed in T_5 (3.21±0.08) and lowest in T_4 (3.135±.01), although no significant difference ($p>0.05$) among the treatments. Besides, the highest weight gains (32.99g) and FCR value (4.43±.24) was observed in T_5 also. The maximum survivality (85.29±2.83) and protein efficiency ratio (1.18±0.19) was observed in T_1 that showed significant difference ($p<0.05$) with other treatments. The highest protein content of the fish sample was observed in the treatment T_1 (17.11±0.18) was followed by T_5 (16.80±0.56). Considering the yield and protein content of the treatments, T_5 was good for its culture. From the result of weight gain, SGR and FCR T_5 (75% Fishmeal+ 25% Shrimp head meal) is recommended for Thai silver barb culture.

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List of Abbreviations and non-English Words

ANOVA	Analysis of Variance
DO	Dissolved Oxygen
DOF	Department of Fisheries
FAO	Food and Agriculture Organization
FCR	Feed Conversion Ratio
FM	Fish Meal
HCI	Hydrochloric Acid
PER	Protein Efficiency Ratio
MOC	Mustard Oil Cake
MP	Muriate of Potash
SD	Standard Deviation
SGR	Specific Growth Rate
SM	Shrimp Meal
TSP	Triple Super Phosphate

CHAPTER ONE

INTRODUCTION

Chapter-1

Introduction

1.1. Background of the study:

Bangladesh is blessed with a lot of natural resources both terrestrial and aquatic resources in the form of rivers, canals, depressions, reservoir, ox-bow lakes, ponds, tanks, seasonally flooded areas and others. Fish and fisheries resources cover a huge portion of the aquatic resources. Bangladesh is a country well suited for fish production. The fisheries resources of Bangladesh represents about one percent of the total world fish catch (FAO, 1997). According to an estimation, the inland fisheries resources covers an area of 4.47 million hectares in which perennial and seasonal water body constitute an area of about 2.83 million ha and 1.53 million ha respectively and also from this area 90% comprises capture fisheries and 10% closed water culture fisheries (DoF, 2003), surrounded by India on the West, North and Northeast, by Barma on the East and by the Bay-of-Bengal on the South. The bulk of fish production (more than 75 % of the total fish production) comes from inland fisheries (DoF, 2003).

Most of the farmers in Bangladesh have many of utilized or unutilized water bodies like ditches, canals, mini ponds etc. which retains water for 5-6 months. Therefore, farmers can effectively utilize these water bodies for fish culture. There are several species were transplanted from different countries to Bangladesh which have great culture potential. The silver barb, *P. gonionotus* is one of them has created a significant profitability in Bangladesh. It was introduced into Bangladesh from Thailand in 1977 and has become very popular because of its rapid growth and suitability for seasonal water bodies. It is a

herbivore, feeding mainly on aquatic plants, grasses and algae (Phaohorm 1970 and Srisuwantach 1981). Hussain *et al.* (1989) studied the production potential of this species in earthen ponds, while Kohinoor *et al.* (1993) reported on optimizing its production by using a mixture of rice bran (60%) and mustard oilcake (40%) as supplementary feed. It is excellent as food and the cheapest source of protein to the common people of Bangladesh. It has been introduced in many Asian countries including Bangladesh not only for its palatability and market ability but also for high yield potential (Hussain *et al.*, 1987).Silver barb, *P. gonionotus* is one of the best suited species for bringing the unutilized or underutilized water bodies under intensive culture . This fish was also found to feed well on supplemental feed (Hussain *et al.*, 1987). The fish can grow fast at high stocking densities (Sipitekhiat and Leenannod 1984).

The production potentiality of *P. gonionotus* for culture in seasonal ponds, ditches and canals has already been proven in Bangladesh (Sarker *et al.*, 2002). The farmers of our countries are practicing this *P. gonionotus* with almost all types of species. But there was no actual research on feeding practice needed for *P. gonionotus* culture. Feed selection is a key factor to get highest yield from any sorts of culture. It is an important phenomenon to know silver barb mono and mixed culture with carpio that fed on different natural resources may play a significant management technique to efficiently utilize the production potential of the ditches.

Generally fishmeal is used as the protein source in feed formulation. But it is a prime concern yet because of its scarcity and expensive value. Moreover, the massive aquaculture intensification program of the government is expected to result in a total annual fish and shrimp production of about 3.0 million tons during the course of Sixth Five Year plan that would require approximately 1.5 million tons of nutritionally rich and

balanced fish feed (Mazid, 2002). This would necessarily mean a huge gap between the demand and supply of fish feed under the backdrop of only 22,000 metric tons annual fish feed production capacity of the four existing feed manufacturers (Mazid, 2002).

Therefore, shrimp processing waste is an excellent source of cheap and abundant substitute of fishmeal. Usually shrimp processing is done to get de-headed and peeled exportable items leaving behind shrimp head, tail, hull and exoskeleton. Although any systematic studies have not been done so far on the total amounts of wastes produced and their utilization, it is estimated that 1, 14,000 metric tons of shrimp head is produced annually from the shrimp processing industries located in the coastal region (Khan and Hussain, 1996). The processed waste of shellfish also contains chitin and its derivative chitosan. The shells of these animals are richest source of chitin. Chitin is the most abundant natural polymer after cellulose. The natural occurrence of chitin is widespread. The exoskeleton of shellfishes is the only practical and considerable source of chitin also. This exoskeleton contains 18-24% chitin on a dry basis (Ockerrman, 1992). Some recent studies indicate that faster growth rate of Bagda shrimp could be found by the use of supplemented feed with required amount of chitin. Studies on protein digestibility of Bagda shrimp by using chitin as supplement in the diet confirm that the use of chitin at 5% to the diet could enhance protein digestibility and mineral uptake, hence growth, significantly than using normal commercial shrimp feed (Shiau and Yu, 1998; Kamal 1999; Shiau and Liu, 1994). To increase the fish production intensive and semi-intensive culture practice is necessary. The availability of low cost feed is one of the vital factors that may contribute to success of intensive and semi-intensive fish culture that is suitable for small-scale farmers in a developing country like Bangladesh. The success of intensive and semi-intensive fish culture is primarily depends on the use of well-balanced artificial feed. So it is essential to formulate a low cost aquafeed from locally available indigenous

feed ingredients to meet this requirement. Traditionally, fishmeal has been the major protein source in commercial fish feeds elsewhere in the world. Shrimp meal can be used as a cheaper protein source of ingredients which are locally available that can replace the fishmeal without changing the protein level. For this replacement the cost of feeds will become lower also. Under this consideration, present investigations were designed to fulfill the following objectives.

1.2. Objectives

The present study was undertaken with the following objectives:

- To utilize the shrimp processing discards by preparing low cost feed for Silver barb (*Puntius gonionotus*) which is economically viable.

- To prepare feed from locally available indigenous feed ingredients for the development of fish feed with varying levels of shrimp processing wastes.

- To observe growth performance of these feeds on the growth of Silver barb (*P. gonionotus*) in monoculture system.

CHAPTER TWO

LITERATURE REVIEW

Chapter-2

Literature Review

Hussain *et al.* (1989) reported that use of rice bran in the production of Thai sharpunti, *Punius gonionotus* under semi-intensive culture system is more effective than fertilizer. The ponds were stocked with fingerlings at a density of 16000/ha. Each treatment had three replicates. The food used was rice bran at a rate of 5% of the standing biomass and fed daily in treatment T_1. The T_2 treatment ponds were fertilized with cattle dung, urea and TSP at a rate of 750 kg, 8 kg and 16 kg/ha/week respectively. Average fish production from T_1 treatment was 1953 kg/ha/5 months and from T_2 treatment, was 690 kg/ha/5 months. Analysis of variance of total yield showed highly significant differences ($P>0.01$) between two treatments.

Noor *et al.* (2000) observed that suitability of duckweed as dietary fishmeal substitute for silver barb had a less effect than fishmeal from a 60-day long growth trial conducted. Five iso-nitrogenous diets were formulated to contain 35% protein and each treatment had three replicates with 15 fish in each aquarium with a mean initial weight of 1.5±0.2 g. Duckweed was used in the experiment to replace 10, 20, 30 and 35% of the dietary fish meal in diet 2, 3, 4 and 5 respectively. Fish meal was used as the sole source of protein in control diet (Diet 1). In terms of growth, food conversion and protein utilization, the control diet and diet containing 17.07% duckweed showed the best ($P>0.05$) performance followed by diets containing 34.14%, 51.21% and 59.24% duckweed. Fish fed diets containing higher levels of duckweed had higher moisture and lower lipid content

compared to the control diet. It was noted that 10% of the dietary fish meal protein could be replaced by duckweed in the diet of silver barb.

Hossain *et al.* (1994) revealed that up to 50% of the fish meal protein in *P. gonionotus* diet could be replaced by mustard and sesame protein without reducing growth from a 75-day laboratory trial conducted. He showed that fish fed on a diet containing 25% mustard oilcake protein produced the best results as compared to the control diet. Fish fed on diets containing 50% mustard oilcake and sesame meal protein produced comparable growth to that of the control. Apparent protein digestibilities (APDs) for all the diets were fairly high ranging from 80.12 to 84.46% with the control diet producing the lowest. In general, APD values decreased with increasing oilseed meals in the diets. The carcass moisture was highest and the protein and lipid lowest in fish fed on the control diet.

Islam *et al.* (1998) indicated that duckweed can be used as an effective supplementary feed for the culture of *P. gonionotus.* An experiment was conducted to show the effect of duckweed as a dietary supplement on Thai silver barb (*P. gonionotus*) for a period of 180 days. The highest production of 2281.5 kg/ha was obtained from T_1 where only duckweed was supplied as a dietary supplement and lowest 1907.5 kg/ha from T_2 where rice bran was used. A combination of duckweed and rice bran (50:50) yielded 2107.5 kg/ha production. There was no significant difference ($P>0.05$) in final body weight, AFCR, net production and total production of silver barb among the treatments. Apparent feed conversion ratio varied from 4.5 to 5.2 among the treatments.

Hossain (2002) concluded that boiled soybean might be used as a fishmeal substitute to formulate simple feed at farm levels. A 4-month long experiment was conducted in cemented cisterns to evaluate raw and boiled soybean as fishmeal substitute in

CHAPTER THREE

MATERIALS AND METHODS

Chapter-3

Materials and Methods

3.1. Study Area

The experiment was conducted in 10 earthen mini ponds at Khulna University from 01 August to 15 November for a period of three months and a half.

The ponds were equal in size and rectangular in shape. The ponds depth varies from 1-1.5 meter. The size of each pond was 1.50 decimal. These ponds were well-organized to maintain the water level. Dike was constructed properly to protect floodwater.

3.2. Pond preparation:

Preparation of ponds is the important factor to increase the survival rate and for the better production.

3.2.1. Liming and Fertilization:

The ponds were sun dried for several days before stocking. At the rate of 1kg/ decimal lime was applied before one week of stocking. After five days of liming fertilizer viz. Urea, TSP and Cowdung were used. Doses are given in the table (1). Then the ponds were filled with water and the experimental fish was stocked. The ponds were selected randomly prior the experiment starts.

Table 1: Doses of different organic and inorganic fertilizers used during the culture period

Fertilizer	Dose (gm/dm/wk)	Dose (kg/ha/yr)
Urea	100	1284.4
TSP	50	542.2
MP	25	321.1
Cow dung	200	2568.8

3.3. Treatments and replication:

Five experimental feed were formulated replacing fish meal with shrimp head meal at different levels (0%, 25%, 50%, 75% & 100%). The treatments using feeds were designated as T_1, T_2, T_3, T_4 and T_5. Each treatment consisted of two replications.

3.4. Water quality parameters:

Temperature, pH, alkalinity and dissolve oxygen (DO) were measured. Temperature and pH were measured in every morning and evening. Alkalinity and dissolved oxygen were measured after seven and fifteen day's interval respectively.

3.5. Feed formulation, manufacturing and cost:

Selection of ingredients, proximate analysis of ingredients, amount of feed stuff, diet formulation and manufacturing, proximate composition of diet and diet cost.

3.6. Stocking:

60 per decimal.

3.7. Feeding method:

Feed was given twice a day at a rate of 10% of the total body weight.

3.8. Growth monitoring:

Sampling was done every 15 days interval to observe the growth by netting. In every sampling 10 pieces of fry were collected.

3.9. Harvesting:

Complete harvesting was done dewatering the pond water.

3.10. Data analysis & presentation:

SGR, PER, FCR & ANOVA was done by Microsoft word, Excel and SPPSS.

3.11. Feed Formulation, Manufacture and Cost

3.11.1. Selection of feed ingredients:

Considering both protein supplement and price locally available feed ingredients were selected for feed formulation.

Table-2: Selection of diet ingredients and their costs.

Diet ingredients	Cost (Tk / Kg)
Fish meal	40.00
Shrimp head meal	11.50
Wheat flour	10.50
Wheat bran by product	9.25
Rice polishing	6.00
Oyster shell	2.50
Shark oil	50.0
*Pegabind binder (Bentoli, Inc. USA)	190.00

3.11.2. Collection of feed ingredient:

To formulate of experimental diets, various feed ingredients such as fish meal, shrimp head meal, wheat flour, wheat bran by product, rice polishing, oyster shell, shark oil, pegabind binder were collected from Khulna local market. The fishmeal and pegabind binder were Australian and USA origin respectively. The other ingredients were indigenous origin. The ingredients were analyzed for their protein levels are shown in table-3.

3.11.3. Different feed ingredients and their protein level (dry weight basis):

Table-3: Different feed ingredients and their protein level (dry weight basis)

Diet ingredients	**Protein %**
Fish meal	60.40
Shrimp head meal	29.12
Wheat flour	15.75
Wheat flour by product	19.00
Rice polishing	14.81

3.12. Method of analyzing proximate composition

The level of crude protein, crude lipid, and ash in samples were determined according to AOAC (1980).

3.12.1. Crude Protein

Sample was digested using concentrated H_2SO_4 and resolvent. After that distillation was done. Finally by titration crude protein was determined. (Annexure –A).

3.12.2. Crude Lipid

Crude lipid was determined by Soxhlet method where acetone was used as fat extractor. Recycling of acetone was done through Soxhlet fat extractor. After six hours mixture of oil and acetone was collected and evaporated to collect the lipid. (Annexure –B)

3.12.3. Ash

Ash determination was done by Muffle Furnace ignition. After ignition sample was kept in a desiccator and weight was gained by an electric balance. (Annexure–C)

3.12.4. Crude Fiber

Crude fiber content in sample was determined according to the procedure cited by Lim and Yeow (1965). (Annexure–D)

3.12.5. Moisture

The moisture content (%) of sample was determined by using an electric Moisture determination apparatus (model no. MA 30 – 000V3, SARTORIUS AG Gottingen, Germany).

3.13. Nitrogen Free Extract (Carbohydrate)

Carbohydrate content was determined by deducting the total of crude protein, crude lipid, ash, crude fiber and moisture from one hundred (Das *et al.,* 1995).

3.14. Diet formulation

In the study, 5 types of diets with one control and 4 types of test diets were formulated by using 'Pearson sequare' method (De silva and Anderson, 1995) (Table-4).

Table-4: Amount of diet stuff in different treatments.

Treatment	Amount of diet stuff (%)							
	FM	SM	Wheat flour	Wheat bran by product	Rice polish	Oyst shell	Shark oil	Pegabind binder
T_1 (100% FM)	18.87	0	24.34	32.45	24.34	3.0	3.0	0.6
T_2 (100% SM)	0	66.65	10.01	13.34	10.01	3.0	3.0	0.6
T_3 (75% SM & 25% FM)	10.20	30.61	17.76	23.68	17.76	3.0	3.0	0.6
T_4 (50%FM & 50% SM)	14.71	14.71	21.18	28.24	21.18	3.0	3.0	0.6
T_5 (75% FM & 25% SM)	17.24	5.75	23.10	30.80	23.10	3.0	3.0	0.6

3.15. Nutritional value of test diets:

For the determination of feed quality the nutrient composition of feed, the level of crude protein, crude lipid, ash, crude fiber, carbohydrate in feed (Table-5) were analyzed.

Table-5: Nutrient composition in different test diets on dry-weight basis.

Treatment	**Nutrient composition** (Dry-weight basis)					
	Protein %	Lipid %	Ash %	Crude fiber %	NFE[1]	Dry matter
T_1	25.48	7.70	10.51	6.95	49.36	85.02
T_2	25.90	4.12	21.28	3.99	44.71	90.03
T_3	24.24	4.52	20.31	4.56	45.37	88.52
T_4	24.86	5.31	15.45	5.09	49.29	89.84
T_5	25.94	6.49	13.96	6.12	47.49	89.48

Nitrogen Free Extract (Carbohydrate) content was determined by 100 - (crude protein+ crude lipid + ash + crude fiber + moisture)

[1] According to Das *et al.*, 1995.

3.16. Feed manufacture

The feed were manufactured in the following process represented schematically. (Fig: 1)

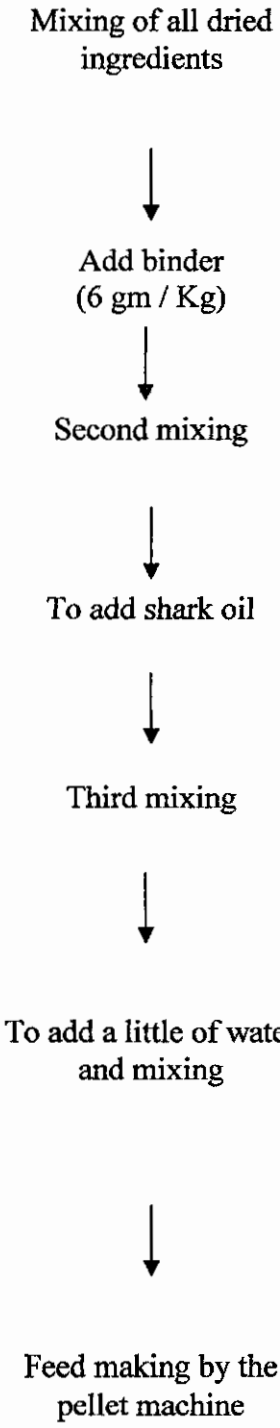

Fig.1: Flow chart of feed preparation process

3.17. Feed cost

Feed cost included only the cost of raw materials. The cost of each test diet is given below (Table-6):

Table-6: Costs of different test diets.

Treatments	Cost (Tk/Kg)
T_1	17.30
T_2	16.10
T_3	16.80
T_4	17.10
T_5	17.20

3.18. Collection of fry:

The fry of *Puntius gonionotus* were collected from chachra, Jessore. The sizes of fry were of 25 days old. The prices of fry were 120 taka/kg. These fry were brought to Khulna University campus in polythine bags with oxygen. The fry were stout and naturally moving at the time of release into the pond.

3.19. Stocking

Fry of Silver barb are locally called 'Thai sharputi' of 1.10 g were hand counted and stocked at a rate of 60 piece per decimal. Prior to release fry into the pond, fry were acclimatized. In acclimatization, pond water was added to the container of fry and then fry were kept in the mixed water for several minutes.

3.20. Grow - out Management

3.20.1. Feeding method

Experimental feed was given twice a day at 9.0 hrs and 17.0 hrs regularly. During the first month fish fed at the rate of 10% body weight of the experimental species. In the second month fish were given formulated feed at the rate of 8% body weight and finally last one month and a half it was given 6% of the body weight. The amount of feed was adjusted fortnightly on the basis of sampling.

3.21. Water Quality Monitoring

3.21.1. Temperature

Method of temperature determination has been given in Annexure–E.

3.21.2. Dissolved Oxygen

Water oxygen was measured fortnightly by Winkler method (Azide Modification of iodometric method, APHA, 1992) in the time between 10 A.M. and 11 A.M. (Annex-F)

3.21.3. Alkalinity

The alkalinity of water measurements were measured once a week with the use of procedure cited by APHA, 1992. (Annexure-G)

3.21.4. pH

The water pH was measured once a week by using RI 02895 (Woon Socket, HANNA) pH meter.

3.22. Growth Monitoring

3.22.1. Sampling

During sampling of the study, 10% individuals from each pond were sampled fortnightly and every individual fish sample was weighed (wet-weight) to nearest unit and then

3.22.5. Determination of the quality of diet

The qualitative value of feed was evaluated by the following parameters (De Silva and Anderson, 1995).

$$\text{FCR (feed conversion ratio)} = \frac{\text{Mass of food consumed (Dry)}}{\text{Increase in mass of animal produced (wet)}}$$

$$\text{PER (protein efficiency ratio)} = \frac{\text{Increase in the mass of animal produced (wet Wt)}}{\text{Mass of protein in fed (dry Wt)}}$$

3.23. Harvest

At the end of the experiment all the fish were totally harvested by netting. After capturing individual weights of the fish were recorded.

3.24. Data Analysis and Presentation

Statiscal analysis

One way analysis of variance (ANOVA) was used to determine the effect of water quality and different feed on the growth of fish. Data analysis was taken by SPSS software according to Duncan's New Multiple Range Test (Duncan, 1955) to identify the 5% level of significance of variance among the different treatment means.

Data presentation:

Data presentation was done tabular and graphical and diagrammatical.

CHAPTER FOUR

RESULTS

Chapter-4

Results

4.1. Water quality parameters:

Different water quality parameters such as temperature, dissolve oxygen, pH and alkalinity during culture period were measured regularly as a routine work. Mean values of these parameters are presented in Annexure H.

4.1.1. Temperature

A wide range of fluctuation in temperature among the ponds in the morning and that of the evening was observed during the experimental period (24.75 ~ 32^0 C and 26 ~ 34^0 C). However it was observed no significant difference (P>0.05) on mean temperature of the ponds were observed in the morning and that of the afternoon (28.3 ~ 28.4^0 C and 29.3 ~ 29.4^0 C respectively, Fig.2, Annexure-I)

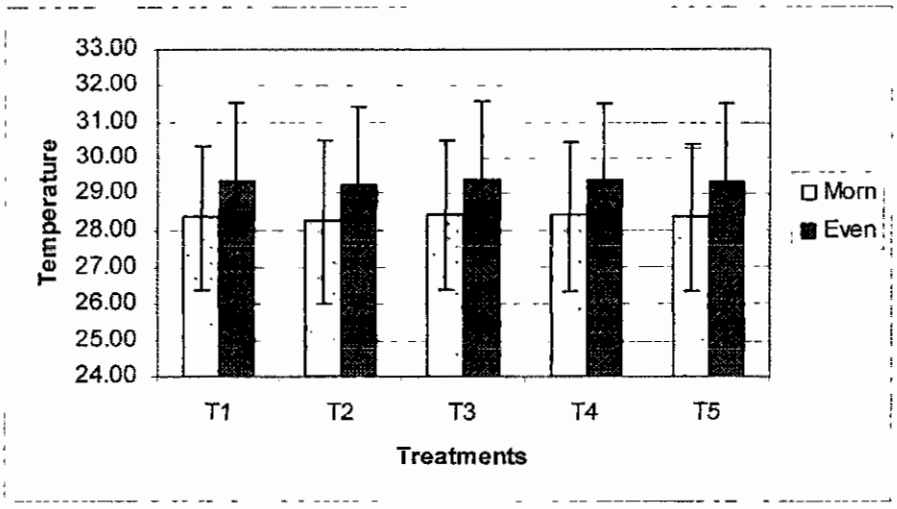

Fig.2: Temperature differences among treatments in morning and evening.

4.1.2. pH

In the morning the lowest (7.91±0.16) and highest (8.02±0.15) pH was found for treatment T_4 and T_5 respectively (Fig.3). On the other hand the lowest pH (8.00±0.32) and highest (8.16±0.15) was found for treatment T_4, and T_5 respectively in the evening. There was no significant difference ($P>0.05$) among the treatments in the morning and in the evening (Annexure J).

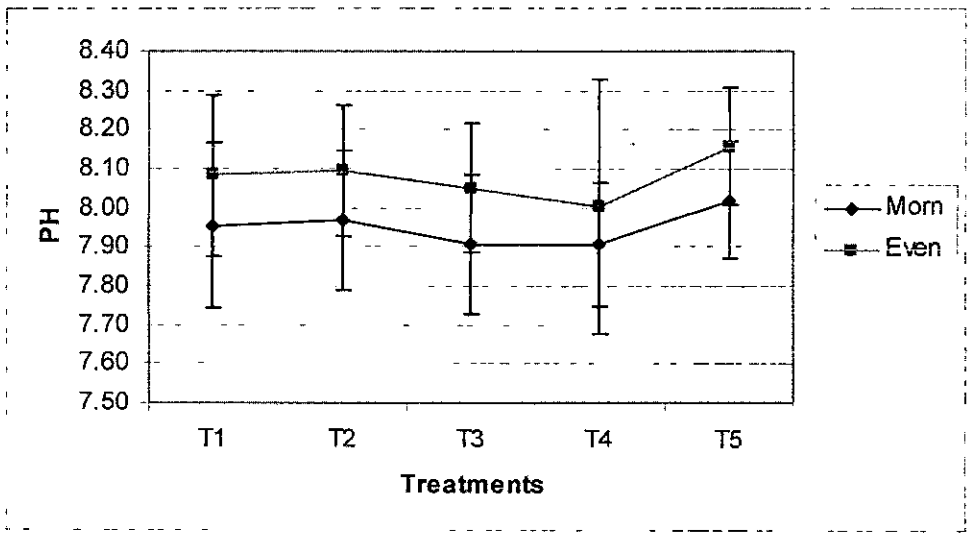

Fig.3: The pH differences among treatments in morning and evening.

4.1.3. Alkalinity

A significant difference ($P<0.05$) in the mean alkalinity levels among the ponds was observed during the culture period (Annexure K). The highest (208.04±6.19 mg $CaCO_3$/l) and lowest (180.29±1.83 mg $CaCO_3$/l) alkalinity was found for treatment T_5 and T_1, respectively (Fig.4).

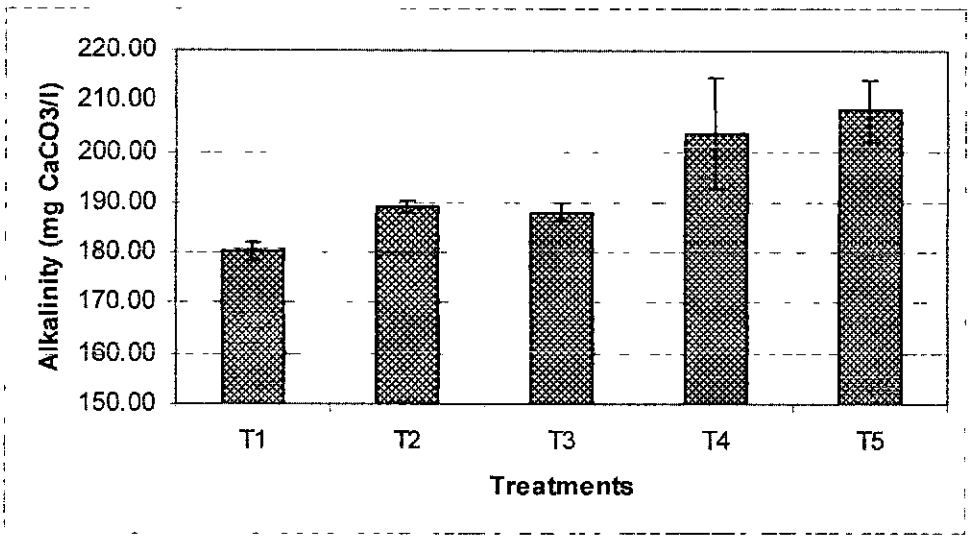

Fig.4: Alkalinity differences among treatments during the experimental period.

4.1.4. Dissolve oxygen (DO)

The highest (7.01±0.19 mg/l) and lowest (6.87±0.46 mg/l) DO was found for treatment T_3 and T_1 (Fig.5).The mean values of dissolved oxygen were 6.87±0.46, 7.00±0.30, 7.01±0.19, 6.93 ±0.41 and 6.98±0.50. There was no significant difference (P>0.05) among the treatments of T_1, T_2, T_3, T_4 and T_5. (Annexure L)

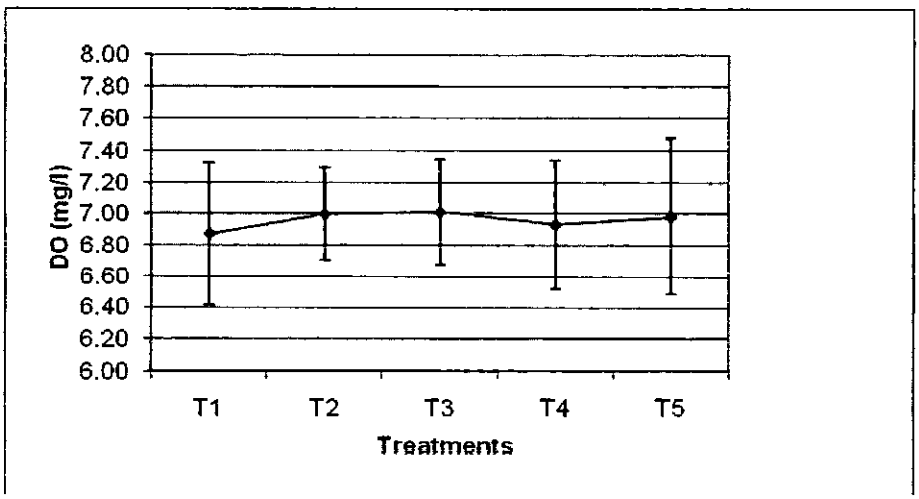

Fig.5: Dissolve Oxygen differences among different treatments.

4.2. Growth performance

Sampling was done fortnightly to check the weight of the species fed on different experimental diets. First three samplings showed almost the same weight of the fish in all the treatments. But the highest and lowest sampling weight was observed in the treatments T_5 and T_3 respectively. (Fig.6)

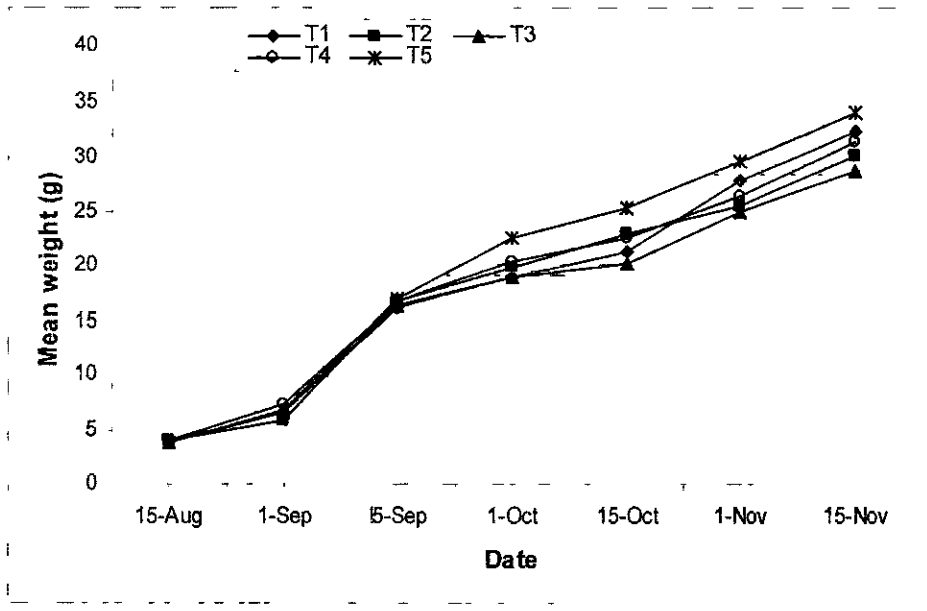

Fig.6: Fortnightly growth performance in five different treatments.

4.3. Production

In the culture of three and a half months, the mean weight gains were found 31.26g, 29.15g, 27.63g, 30.34g and 32.99g from the treatment of T_1, T_2, T_3, T_4 and T_5 respectively. The highest mean final weight gain (32.99g) was found in T_5 while the lowest (27.63g) was found in T_3. The highest production (1.84kg/dm/107 days) per pond was found after 107 days culture in T_1 while the lowest production (1.41kg/dm/107 days) was found in T_3. There was no significant difference ($P>0.05$) in the treatments of T_1, T_4 and T_5. But T_2 and T_3 differ significantly ($P<0.05$) each other. The production from per hectare pond might be 1550.33, 1377.60, 1183.81, 1470.29 and 1466.08kg/ha/year from T_1, T_2 T_3, T_4 and T_5 respectively (Table 7).

Table 7: Yield parameters of experimental species in five experimental ponds

Parameters	**Treatments**				
	T_1	T_2	T_3	T_4	T_5
No. of stocked	102	102	102	102	102
No. of harvested	87	83	75	85	78
Survivality (%)	$85.29^{c}\pm2.83$	$81.37^{bc}\pm2.83$	$75.53^{a}\pm1.41$	$83.33^{c}\pm2.83$	$76.47^{ab}\pm1.41$
Mean initial weight(g)	1.10	1.10	1.10	1.10	1.10
Mean final weight (g)	$32.36^{b}\pm0.57$	$30.25^{ab}\pm3.26$	$28.73^{a}\pm2.79$	$31.44^{b}\pm0.33$	$34.09^{b}\pm2.85$
Net weight gain(g)	31.26	29.15	27.63	30.34	32.99
FCR	$3.21^{a}\pm0.52$	$3.70^{abc}\pm0.11$	$3.99^{bc}\pm0.20$	$3.67^{ab}\pm0.06$	$4.43^{c}\pm0.24$
PER	$1.18^{b}\pm0.19$	$0.99^{ab}\pm0.03$	$0.94^{ab}\pm0.05$	$1.05^{ab}\pm0.02$	$0.83^{a}\pm0.06$
SGR (%/day)	$3.155^{a}\pm0.02$	$3.195^{a}\pm.05$	$3.15^{a}\pm.06$	$3.135^{a}\pm.01$	$3.21^{a}\pm0.08$
Yield (Kg/dm/107 d)	1.84^{b}	1.64^{ab}	1.41^{a}	1.75^{b}	1.74^{b}
Yield (Kg/ha/Year)	1550.33^{b}	1377.60^{ab}	1183.81^{a}	1470.29^{b}	1466.08^{b}

Data are mean values of two replicates. Values are mean ± SD. Values within the same row having the same superscripts shows no significant difference (P>0.05).

4.4. Survival rate

The lowest survival rate was observed in T_3 that showed a significant difference ($P<0.05$) with T_1 and T_4 (Table 7, Annexure–P). The survival rates were 85.29±2.83%, 81.37±2.83%, 75.523±1.41%, 83.33±2.83% and 76.47±1.41% in the treatments T_1, T_2, T_3, T_4 and T_5 respectively (Fig.7). There was no significant difference ($P>0.05$) among the treatments of T_1 and T_4. (Annexure–P)

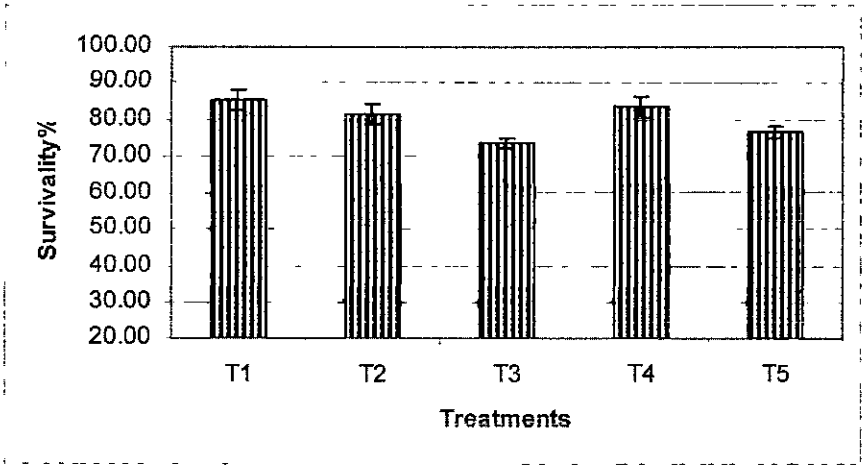

Fig.7: Survivality in five treatments.

4.5. SGR

In the experiment the specific growth rate ranged between 3.21±0.08/day to 3.135±.01/day (Fig.8). The highest specific growth rate was observed in T_5 and the lowest in T_4. There was no significant difference ($P>0.05$) among the treatments of T_1, T_2, T_3, T_4 and T_5. (Annexure–O)

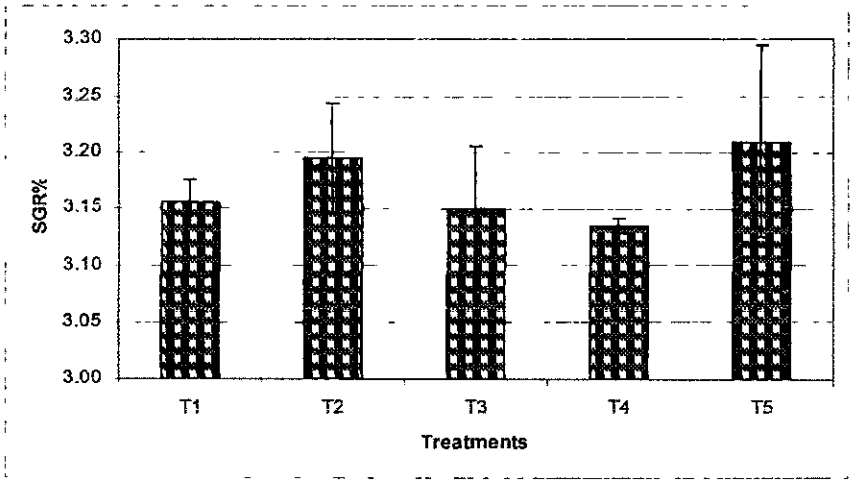

Fig.8: Specific growth rates in five treatments.

4.6. FCR

Apparent food conversion (FCR) of the treatments varied from 3.21±0.52 to 4.43±0.24 (Fig.9, Annexure-M). The highest FCR was observed in T_5 followed by T_3 (4.43± 0.24 and 3.99±0.20 respectively). The lowest FCR was observed in T_1. There was a significant difference ($P<0.05$) among the treatments. (Annexure-M)

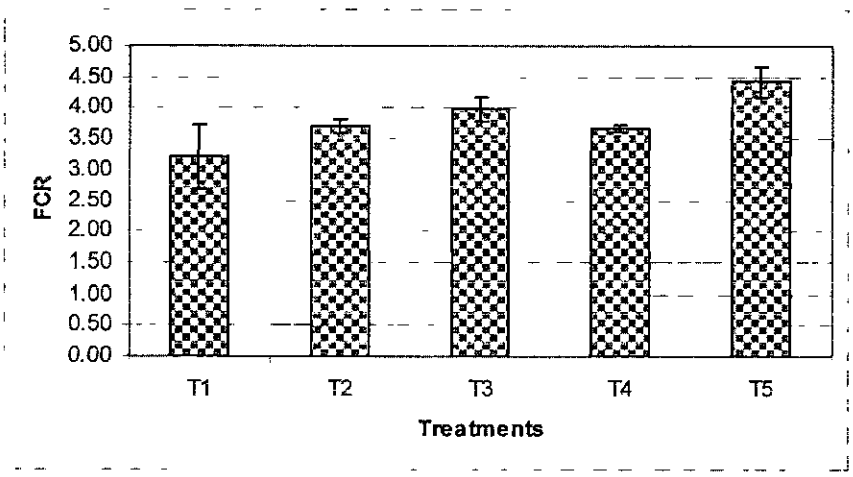

Fig.9: FCR differences among the treatments after harvesting.

4.7. PER

The apparent protein efficiency ratios (PER) of the treatments observed to vary from 0.83±0.06 to 1.18±0.19. The highest PER was observed in T_1 and the lowest was in T_5 showed a significant difference ($P<0.05$). But there was no significant difference ($P>0.05$) among the treatments of T_2, $T_3$and T_4. (Fig.10, Annexure- N)

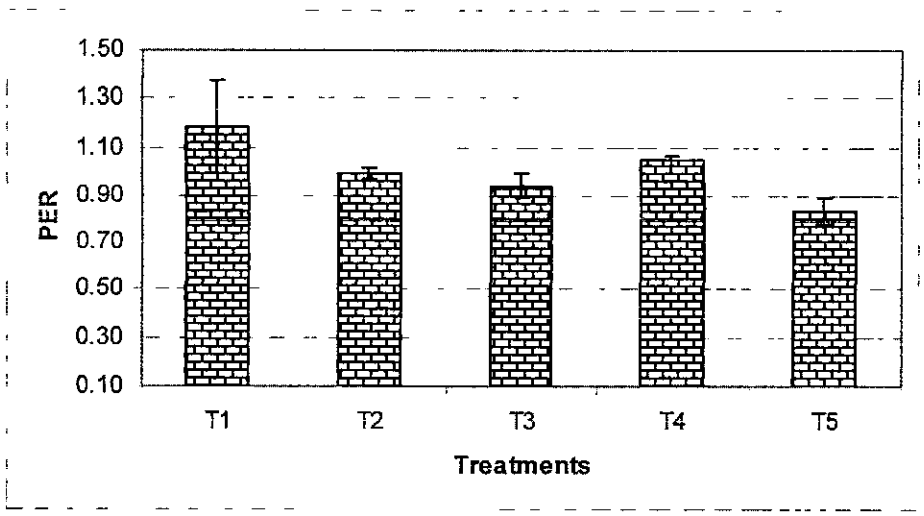

Fig.10: PER differences among the treatments.

4.8. Proximate Composition of harvested fish

4.8.1. Protein

At the end of the experiment protein percentage in different treatments were measured. The values were 17.11±0.18, 15.34±0.45, 15.40±0.63, 16.52±0.49 and 16.80±0.56 in T_1, T_2, T_3, T_4 and T_5 treatments respectively (Fig.11). The highest protein percentage was observed in T_1 (100% FM) and lowest in T_2 (100% SM). Besides in the treatment T_5 the protein percentage were also good where (75% FM+ 25% SM) was applied.

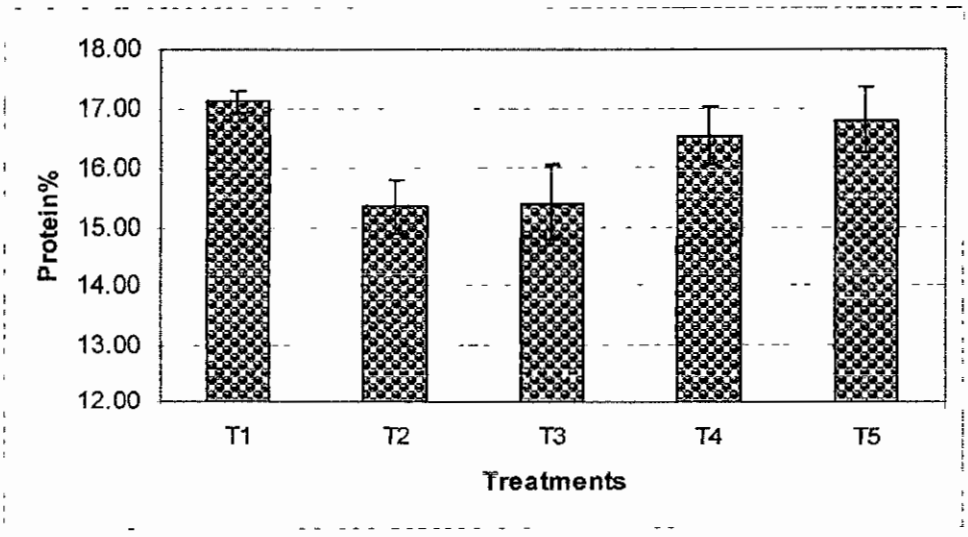

Fig.11: Protein percentage of harvested fish in different treatments.

4.8.2. Lipid

The lipid percentage of the harvested fish sample were 2.38±0.36, 3.20±0.33, 3.45±0.31, 2.97±0.26 and 3.04±0.40 in the treatments T_1, T_2, T_3, T_4 and T_5 respectively. The highest and lowest lipid contents were observed in T_3 (75% SM+25% FM) and T_1 (100% FM) treatments. The lipid content of T_4 and T_5 were almost the same.

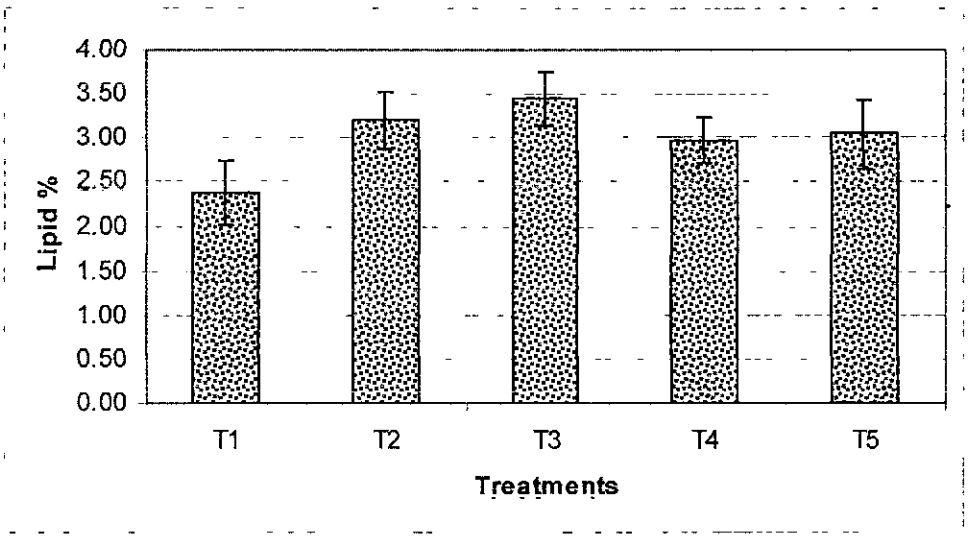

Fig.12: Lipid percentage of harvested fish in different treatments.

4.8.3. Moisture

The moisture content of the fish sample harvested ranged between 79.03± 0.31 to 77.82± 0.62. The highest and lowest moisture content were observed in the treatment T_2 and T_5 where 100% SM and 75% FM+25% SM were applied (Fig.13).

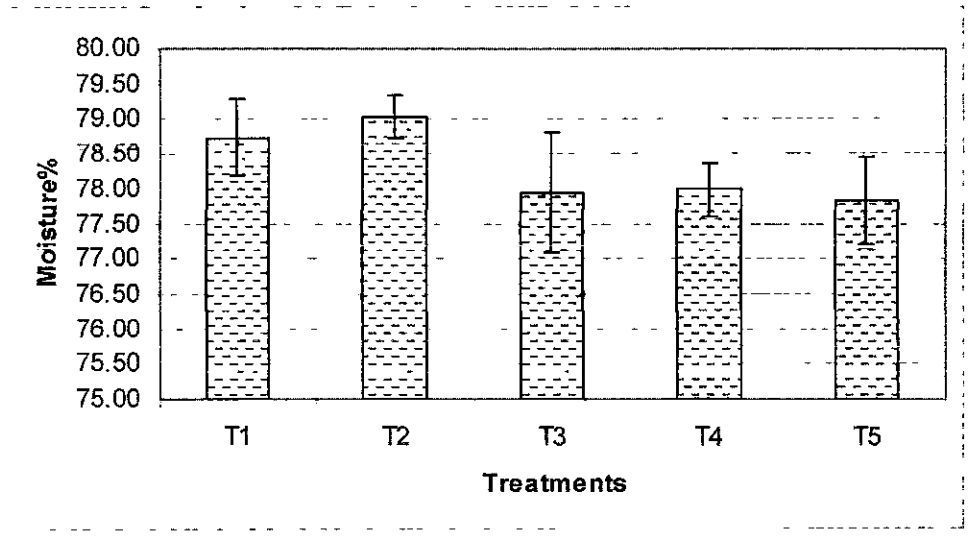

Fig.13: Moisture percentage of harvested fish in different treatments.

CHAPTER FIVE

DISCUSSION

Chapter-5

Discussion

Temperature is the essential factor for maximum growth attain for fish specially Silver Barb. The temperature was measured weekly during the study period at morning and evening. The minimum (28.25±2.22°C) and maximum (28.44±2.06°C) temperature was found for treatment T_2 and T_3, respectively in the morning season (Fig. 2). The minimum temperature (29.25±2.17°C) and maximum (29.42±2.15°C) was found for treatment T_2 and T_3, respectively in the evening season. During the evening season the temperature was higher for all treatments from the morning season. During the experimental period the value of temperature were varied among 34^{0}C and 24.75^0 C which were suitable for this study (Annexure-H). There was no significant difference ($P>0.05$) in the treatment of T_1, T_2, T_3, T_4 and T_5 in both morning and evening. Dewan *et al.* (1991) reported a temperature range from 30.2 to 34.0 ^{0}C (June-August) while Wahab *et al.* (1996) recorded a temperature range 28.5 to 31.3^{0}C (August-November) in their experiment with carps. Ali *et al.* (1982) recorded the temperature fluctuations between 31.62^{0}C and 30.71^{0}C. Therefore the mean temperatures of the culture pond were satisfactory.

pH is one of the most important factors in pond fish culture system. The variations in the values of pH were marginal and were almost similar throughout the study period. Michael (1968) reported that the best pH for fishes is 7.5 to 8.5 as observed in the experimental period. On the other hand Alam *et al.* (1996) reported that pH values ranged from 6.65 to 7.80 suitable for fish culture. He stated that the higher value of pH was found in early August which might be due to the increased rate of photosynthetic activities of phytoplankton with concomitant decrease of carbon dioxide. The decreased value of carbon dioxide and precipitation of $CaCO_3$ might increase the values of pH. Jia-Mo *et al.*

(1988) reported that the pH value ranged from 7.5 to 8.5 was suitable for *M. rosenbergi* in earthen ponds. Kohinoor *et al.* (1998) who obtained the pH range of 7.18 to 7.24 in the research ponds of Bangladesh Agricultural University Campus. Swingle (1967) considered a pH of 6.5 to 9.0 as satisfactory for fish culture. Ali *et al.* (1982) observed a pH range of 7.5 to 9.5 in a freshwater pond at the BAU campus. So the pH values were suitable in different treatments.

The total alkalinity was measured weekly during study period. The mean dissolved content was observed 180.29±1.83, 189.12±1.12, 188.13±1.71, 203.58±10.84 and 208.04±6.19 in the treatment T_1, T_2, T_3, T_4 and T_5 respectively. . There was no significant difference ($P>0.05$) among the treatments of T_4 and T_5. But treatments T_1, T_2 and T_3 were significantly different ($P<0.05$) from T_4 and T_5. Alikunhi (1957) reported that the highly productive water bodies should have more than 100mg/l alkalinity. Chisty and Rahman (1999) stated that total alkalinity of 100 mg $CaCO_3$/l to 175 mg $CaCO_3$/l indicates good water quality. From the above discussion it might be concluded that the values of alkalinity obtained in these experimental ponds were suitable for the culture.

Rahman (1997) stated that oxygen concentration is lower in summer season due to inverse relation of dissolved oxygen with temperature and higher in winter season. Islam *et al.* (1998) found higher dissolved oxygen content (7.92 mg/l) in August while the low DO content (4.37 mg/l). In general, an acceptable environment for aquatic life must contain not less than 5 ppm of oxygen. Andrew *et al.* (1972) reported that *Cyprinous carpio* can remain alive in water containing as little as 1-2 ppm dissolved oxygen. Ahmed (1993) also reported a similar trend of lower dissolved oxygen from fertilized and fed carp fingerling ponds in BAU campus. Various observations have been made on critical

as well as optimum level of DO in pond water. Ellis (1937) indicated that 3.0 ppm DO or less should be regarded as hazardous for fish and suitable DO level should be 5.00 ppm or more. Mckee and Wolf (1963) stated that DO for worm water fish habitat should not be less than 5 ppm at least 16 hour of any day. Bhuiyan (1970) reported that dissolved oxygen content from 5.0mg/l to 7 mg/l is required for high production. Boyed (1982) stated that dissolved oxygen content of 5 to 7 ppm is good for pond fish culture. In the treatments dissolved oxygen ranged from 7.01±0.19 mg/l to 6.87±0.46 mg/l (Fig.5). There was no significant difference ($P>0.05$) among the treatments. So it might be concluded that the values of dissolve oxygen obtained during the culture period were suitable.

The growth performance data of *Puntius gonionotus* fed diets containing different percentages of shrimp processing discards (shrimp meal) and fish meal are shown in the fig (6). The Weight gains of fish fed with these diets were not significantly different from each other. Mean final weight at the end of 107 days in the treatments of T_1, T_2, T_3, T_4 and T_5 were 32.26g, 30.25g, 28.73g, 31.44g and 34.09g, respectively. The highest growth was observed in T_5 (25% SM) while the lowest was found in T_3 (75% SM). The figure shows the fortnightly growth pattern of the experimental species in five different treatments. From the results it was found that growth in treatment T_5 was always in better position than in treatment T_3. In treatment T_1 feed 100% fish meal was provided where as T_2 and T_4 contained 100% SM and 50% SM respectively. Hossain *et al.*(1994) showed that fish fed on a diet containing 25% mustard oilcake protein produced the best results as compared to the control diet. Their study demonstrated that up to 50% of the fish meal protein in *P. gonionotus* diet could be replaced by mustard and sesame protein without reducing growth. Hossain and Jauncey (1989) observed poor growth performance of

Cyprinus carpio fed diets containing 25% MOC protein. Capper *et al.* (1982) also found that inclusion of untreated mustard meal (black mustard from Nepal) at 20% level in the diet of common carp resulted in reduced live weight gain. Noor *et al.* observed better performance in terms of growth fed with control diet and diet containing 17.07% duckweed that was the lowest of all other diets. Hossain (2002) showed that fish fed with diet containing boiled soybean meal resulted better growth than those fed raw soybean containing diet.

Kohinoor *et al.* 1993 showed an average production of 2384 kg/ha/6 months was obtained from the ponds receiving both supplemental feeds (rice bran and mustard oil cake) and fertilizers with a FCR value of 4.6. On the other hand, the semi-intensive culture of *P. gonionotus* produced 1953 kg/ha/5 months using rice bran with a FCR value of 6.1 (Hussain *et al.* 1989). Islam *et al.* concluded that duckweed can enhance fish production in the culture of silver barb due to its phytophagus feeding habit. Gupta (1991) reported an yield of 1.6 tons/ha/6 months when rajpunti was fed on rice bran. Hussain *et al.* 1989 observed about 689.8 kg/ha in 5 months of culture.

In the present experiment the highest two production per hectare pond was 1550.33 and 1466.08kg/ha/year in T_1 and T_5 (75% Fishmeal+25% Shrimp meal) respectively (Table 8). So for better growth performance shrimp meal can be used as a substitute of fishmeal.

Kohinoor *et al.* (1998) observed that the mean survival rate of the different species in the two treatments varied between 82-86% which was higher than the survival rate reported by Wahab *et al.* (1991) for Indian major carps in polyculture, where supplementary feed

was given. Lakshmanan *et al.* (1971) observed a carp survival rate of 80% in poly culture, where ponds were fertilized with both organic and inorganic fertilizers and fishes were fed with a mixture of rice bran and mustard oil cake. In another study, Kohinoor *et al.* (1993) obtained a survival rate of 86 to 94% in the monoculture of Thai sharpunti. Kohinoor *et al.* (1993) obtained survival rate of 86 to 94% in the monoculture of sharpunti. Wahab *et al.* (1995) also found survival of all fish including sharpunti was higher than 80% in polyculture of native carps. Haque *et al.* (1998) recorded 88.89 to 93.93% survival of rohu, catla and mirror carp in their experiment. Hussain *et al.* found the survival rate of *P. gonionotus* in three ponds ranged from 91 to 97% and the average survival rate was 945%.Islam et al. observed the highest survival rate of 82.50% from the ponds where only duckweed were supplied and the lowest 78.38% was recorded where rice bran was used as dietary supplement. Hossain found the survival rate of fish in different dietary groups was ranged between 83.3 to 100%.

Hossain (2002) observed significantly highest specific growth rate by using diet of 30% Mustered oilcake than diet of 20% fishmeal. Noor *et al.* (2000) observed the highest SGR value from the diet of 17.07% duckweed. Akter (1989) obtained better SGR of fishes fed with artificial feeds containing fishmeal than the feeds containing soybean meal.

From the experiment it was found that the FCR of the different treatments were 3.21±0.52, 3.70±0.11, 3.99±0.20, 3.67±0.06 and 4.43±0.24 in T_1, T_2, T_3, T_4 and T_5 treatments respectively (Fig.9). There was found a significant difference ($P<0.05$) among the treatments of T_1, T_2, T_3, T_4 and T_5. From the experiment it was revealed that FCR was highest when the feed was formulated with the mixture of Shrimp meal and fish meal. Islam *et al.* (1998) found the FCR value between 4.5 to 5.2 in an experiment of *Puntius*

gonionotus where duckweed was used as supplemental feed. Hossain *et al.* (1994) showed that *P. gonionotus* had a FCR value of 1.93 to 2.29 in the presence of oilseed meals as dietary protein source. Hussain *et al.* (2001) showed the FCR value of 5.8 to 6.4 in the production *P gonionotus* by using rice bran. Hossain (2002) reported the best FCR value 3.53 obtained in the treatment of *P. gonionotus* using 30% raw soybean meal as fishmeal substitute. Kohinoor *et al.* (1999) obtained a better FCR value of Thai sharpunti in a polyculture with carps using low-cost feed. Noor *et al.* (2000) found the FCR value ranged between 1.86 to 3.66 in their experiment of *P. gonionotus* where duckweed was used as dietary fishmeal substitute.

Highest PER value indicates maximum utilization of protein content. Noor *et al* (2000) found PER values varied between 0.79 and 1.59 which is more or less agreed the present findings. Among different factors protein quality has a significant effect on PER (Stiffens 1989).

Noor *et al.* (2000) observed 15.46% crude protein in fish sample fed by diet of duckweed as dietary fishmeal substitute for *Puntius gonionotus* where the initial fish had 14.06%. Hossain *et al.* (1994) found 17.07% crude protein in *P. gonionotus* fed on a diet containing 25% mustard oilcake as a substitute fishmeal where as the initial content was 14.02%. In this study T_5 showed a better replacement of protein in the fish sample where 25% shrimp meal was used.

Noor *et al.* (2000) observed 5.48% lipid in fish sample fed by diet of duckweed as dietary fishmeal substitute for *P. gonionotus* where the initial fish had 4.88%. Hossain *et al.*

(1994) observed 5.03% lipid in *P. gonionotus* fed on a diet containing 25% mustard oilcake as a substitute fishmeal where as the initial lipid content was 3.26%.

Noor *et al.* (2000) observed 77.12% moisture content in fish sample fed by diet of duckweed as dietary fishmeal substitute for *P. gonionotus* where the initial fish had 78.46%. Hossain *et al.* (1994) observed 74.26% lipid in *P. gonionotus* fed on a diet containing 25% mustard oilcake as a substitute fishmeal where as the initial lipid content was 79.17%.

The lipid and moisture content was also in an acceptable limit in this experiment. So in the case of lipid and moisture content the replacement of fishmeal by shrimp meal was satisfactory.

According to the feed formulation both the effects of protein supplementary ingredient and basal feed ingredient were observed. Though the effect of fish meal replacing by shrimp head meal was expected to investigate. The maximum variations among basal feed ingredient were found between T_1 (100% FM) and T_2 (100% SM) of about 14.33%, 19.11% and 14.11%. So further investigation is recommended for better result.

CHAPTER SIX

CONCLUSION

Chapter-6
Conclusion

A growth trial of *Puntius gonionotus* was conducted in 10 earthen mini-ponds at Khulna University Campus. Five experimental diets were applied to observe the effect of shrimp head meal as a substitute of fishmeal.

The water quality parameters viz temperature, pH, alkalinity and DO were almost same during the culture period. The variation in the morning and evening of the parameters was almost the same in all the ponds.

When fish is produced in intensive and semi-intensive culture system supplemental feed is very essential for better growth. In fish culture, feed cost accounts about 60-70% of the total cost, which is very expensive, especially for farmers of Bangladesh (Hossain, 2004). In this view the supplemental feed must be economic. To produce low cost supplemental feed locally available feed ingredients should be taken under consideration. In the present study, the diet T_1 (100% FM) containing higher level of fishmeal showed the best growth of fish in respect of survivality, yield, protein efficiency ratio (PER) and protein content. On the other hand, the lowest growth of fish was found in the diet T_3 (75% SM+ 25% FM) having higher level of shrimp head meal in respect of survivality, yield and protein efficiency ratio (PER). But in case of individual weight gain (32.99) and specific growth rate (3.21), treatment T_5 (75% FM+ 25% SM) showed the best results. The protein content was observed satisfactory in the treatment T_5 (16.80) also. Thus treatment T_5 containing 75% fish meal and 25% shrimp meal might be recommended as suitable for monoculture of *Puntius gonionotus* in culture ponds. Therefore it will be beneficial to utilize of these indigenous protein riched materials such as shrimp head meal as a substitute to fishmeal in fish feed. Further investigation is needed for monoculture of Thai silver barb.

CHAPTER SEVEN

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Chapter-7

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ANNEXURE

Annexure

Annexure- A

Crude Protein

Principle

The procedure involves two steps: (a) digestion of the sample to convert the N to ammonium sulphate and (b) determination of nitrogen in the digest sample through distillation process.

$$\text{Sample} - N + H_2SO_4 \xrightarrow[\text{Digestion}]{\text{Heat}} (NH_4)_2SO_4$$

$$(NH_4)_2\,SO_4 + NaOH \xrightarrow[\text{Heat}]{\text{Distillation}} NH_3 + H_2O + Na_2SO_4$$

$$NH_3 + H_3BO_3 \longrightarrow NH_4BO_3$$

$$NH_4BO_3 + \quad HCl \longrightarrow NH_4Cl + H_3BO_3$$

Apparatus

- Kjeldahl Nitrogen digestion unit (model No. OSK 8408A, OGAWA SEIKI CO. Ltd.)
- 100 ml Kjeldahl flask
- Kjeldahl Nitrogen distillation unit (model No. OSK 8416A, OGAWA SEIKI CO. Ltd.)
- Conical flask
- Measuring cylinders
- Pipette
- Burette
- Burette stand

Sample: 0.2 to 0.5 g

Reagents

- Resolvent: 2 g approximately

To mix and crush Potassium Sulfate and Mercuric oxide in the ratio of 10: 0.7 and preserve in colored bottle.

- Sulfuric acid (con.): 5 ml approx.
- Boric acid 2 % solution: 10 to 20 ml

To dilute 20 g boric acid in the distilled water and make the solution 1 liter with distilled water.

- Indicator (Tashiro's indicator): 2 to 4 drops

To dissolve 80 g methyl red and 20 mg methylene blue in 95 % ethanol and make up to 100 ml with 95 % ethanol.

- Sandy zinc: 0.5 g approx.
- 33 – 40 % caustic soda: 25 ml

Dissolve 400 g NaOH with distilled water and make up to 1liter solution.

- Hydrochloric acid (HCl): 0.1 N

Procedure

About 0.2-0.5 g sample was taken in a Kjeldahl flask and about 2 g resolvent was added into the flask. About 5 ml of concentrated sulfuric acid was added in to the flask slowly. Then the flask was kept on to a Kjeldahl nitrogen digesting apparatus and heated until a clear digestion mixture appeared. After digestion, the flask was removed from the digestion unit and kept at room temperature until cooling. After cooling the digestion mixture, 70 ml distilled water was poured slowly along the flask's inner wall and 0.5g of sandy zinc was also added to the mixture. About 25 ml of 33-40% sodium hydroxide (NaOH) solution (containing 1% potassium sulfide) was poured slowly along the flask's inner wall and washed down the remaining solution on the mouth with a small amount of water. The flask was then placed to the Kjeldahl nitrogen distillation apparatus (model no. OSK 8416A, OGAWA SEIKI CO. Ltd.). And turned the switch on. Before connecting the sample flask with the distillation plant, 10-20 ml of 2% boric acid was taken in another Erlenmeyer flask (conical flask) and 2-4 drops of Tashiro's indicator was added into it. Then the distillation tube was poured into the Erlenmeyer flask so that

the end of the glass tube was immersed in the boric acid solution. Distillation procedure was continued for about 30 minutes to obtain about 30 ml distilled solution. After completion of distillation, the solution was titrated against 0.1 N hydrochloric acid (HCl).

Calculation:

$$\% \text{ Total nitrogen (\% N)} = \frac{\text{Ttitrant (ml)} \times \text{Strength of titrant} \times 0.014}{\text{Weight of sample (g)}} \times 100$$

% Crude protein = % N × 6.25 (Pearson, 1976)

Annexure- B

Crude Lipid

Procedure

About 70 ml of acetone was poured in the round bottom flask of the extractor. About 2-5 g of sample was taken into a thimble paper. The thimble paper was placed in into chamber of the Soxhlet fat extractor (model no. OSK 8407A, OGAWA SEIKI CO. Ltd.) . The extractor was placed on the heater and the switch of the extractor was turned on. After 4 -5 hours, acetone extract with lipid was collected in a cleaned beaker from the extractor and acetone was evaporated by placing the beaker in an oven (model no. N 400, Philip Haris. Ltd., London) at 65-70° C for over night. The weight of residue in beaker was taken by using an electronic balance (model no. B303 - S, Metter Toledo,Switzerland).

Calculation

$$\% \text{ lipid} = \frac{W - w}{\text{Weight of sample (g)}} \times 100$$

Where,

W = weight of residue + beaker

w = weight of empty beaker

Annexure- C

Ash

Procedure

About 2-5 g sample was taken in a porcelain crucible. The crucible with the sample was placed in a Muffle furnace for ignition for about 6 hours at 550°C. After ignition the crucible was taken out from the furnace and kept in a desiccator for an hour. The weight of the ash in the crucible was taken by an electrical balance (model no. B 303 - S, Metter Toledo,Switzerland).

Calculation

$$\text{Ash content (\%)} = \frac{W - w}{\text{Weight of sample}} \times 100$$

Where,

W = weight of residue + crucible

W = weight of crucible

Annexure- D

Crude Fiber

Material

- Analytical balance
- Muffle
- Porcelain crucible
- Oven
- Sample with out fat
- 5 % Sulfuric acid (50 ml Sulfuric acid in every 1 litre distilled water)
- 1 % HCl (10 ml concentrated HCl in every 1 litre distilled water)
- 40 % NaOH (400 g in every 1 litre distilled water)
- 1 % Phenolphthalein indicator in ethanol
- Boiled water

Procedure

1 to 2g of dry sample, filter paper (541) and porcelain crucible were weighed and the dry sample was placed in 800 ml beaker. 50 ml 5% sulfuric acid and 150 ml of distilled water were added into the beaker. The beaker was connected with a condenser, refluxed it for 30 minutes and the temperature was decreased. Then 2 to 3 drops of Phenolphthalein indicator and 10 ml 40 % NaOH were added into the beaker and the mixture was refluxed for another 30 minutes. The solution was collected and filtered with Whatman (541) through a funnel. The residue from the beaker was rinsed with boiling water, 1 % HCl and again by the boiling water to remove HCl. Then the funnel was rinsed with methylated sprit. The filter paper with the sample was transferred to the porcelain crucible and placed in the oven at 105°C to a constant weight. After cooling down, the porcelain crucible was weighed and placed into the muffle at 550°C for about 5 hours and left until a white ash. Finally the crucuble with residue was weighed.

Calculation

$$\% \, Crude \, fiber = \frac{W2 - W3}{W1}$$

where,

W_1= Weight of sample taken

W_2= Weight of dried sample + filter paper + crucible

W_3 = Weight of residue + filter paper + crucible

Annexure-E

Temperature

Every one week water temperature was determined once in the morning and evening by using a mercury–filled centigrade thermometer. The temperature measurements were made in the morning time between 10 A.M. to 11.00 A.M and in the evening time between 5.0 P.M. to 6.0 P.M

Annexure-F

Dissolve Oxygen

Principle

The iodometric test is the major precise and reliable titrametric method for DO analysis. It is based on the addition of divalent Manganese solution, followed by strongly alkali, to the sample in a glass-stopped bottle. Dissolve oxygen rapidly oxidizes an equivalent amount of the dispersed divalent manganese hydroxides to higher valiancy states.in the presence of iodine ions in an acidic solution, the oxidized manganese reverts to the divalent state with the liberation of iodine equivalent to original dissolve content. The iodine is then titrated with a standard solution of thiosulphate. The titration end point can be detected visually with a starch indicator.

$$4Mn(OH)_2+O_2+2H_2O \longrightarrow 4Mn(OH)_3$$

$$Mn(OH)_3+I^- +3H^+ \longrightarrow Mn^{+2}+1/2I_2+3H_20$$

$$2S_2O_3^{2-}+I_2 \longrightarrow S_4O_6^{2-}+2I^{2-}$$

Apparatus

- BOD bottles (300 ml)
- Burette(25 ml)
- Pipette (10ml)
- Funnel
- Conical flask (250 ml)
- Measuring flask (100 ml)
- Beaker
- Burette stand

Chemicals

- Manganese Sulfate ($MnSO_4$-$4H_2O$)
- Concentrated sulfuric acid
- Starch indicator
- Standard sodium thiosulphate, Na_2SO_3 (0.025N)

- Alkali iodide – azide reagent

Procedure

- The sample was taken into a 'BOD bottle' in proper way.
- 1 ml. $MnSO_4$ solution was added into the sample followed by 1 ml. Alkali iodide – azide reagent added into the sample.
- Then the sample was stoppered carefully to exclude air bubbles, mixed by inverting bottles and then was kept for 10 minutes.
- When the precipitate was settled sufficiently to leave clear superannuate above the Manganese Hydroxide floc, 1 ml. conc. H_2So_4 was added to dissolve the precipitate.
- Restopper and mixing by inverting bottle well until dissolution was completed.
- Then the 50-ml. sample from the bottle was taken in a conical flask.
- The burette was rinsed with distilled water then rinsed with Na_2SO_3 up to mark.
- Then in the 50-ml. sample, few drops of starch were added.
- The sample was titrated with Na_2SO_3 solution until the 'blue' color disappeared

Calculation

1 ml of 0.025N $Na_2S_2O_3$ is equivalent to 0.2 mg of O_2.

Since the volume of the sample is 50 ml

Dissolve oxygen (in mg/l) = Y ×200/ X

Where,

Y =ml of the titration

X=ml of the sample

Annexure-G

Alkalinity

The alkalinity of water measurements were measured once a week with the use of procedure cited by APHA, 1992.

Principle

Hydroxyl ions present in a sample as a result of dissociation or hydrolysis of solutes react with additions of standard acid which neutralized the sample at the end point.

$H_2SO_4 = 2H^+ + SO_4^{-2}$

$NaOH = Na^+ + OH^-$

$H_2SO_4 + NaOH = NA_2SO_4 + H_2O$

Apparatus:

- Burette(25 ml)
- Pipette (10ml)
- Funnel
- Conical flask (250 ml)
- Measuring flask (100 ml)
- Beaker
- Burette stand

Chemicals

- Carbon dioxide-free distilled water
- Standard sulfuric acid 0.1 N titrant
- Sodium Carbonate 0.02 N solution
- Phenolphthalein indicator
- Methyl orange indicator

Procedure

- At first the burette was rinsed with distilled water and then rinsed with 0.1 N H_2SO_4 by pipette.
- The burette was taken clamped and filled with 0.1N H_2SO_4 solution up to mark.
- 50 ml of the sample was taken into conical flask and 3 drops of Methyl orange indicator solution was added into it.
- Finally, the sample was titrated with 0.1N H_2SO_4 until the color becomes 'light pink.'

Calculation

Alkalinity, mg $CaCo_3/L = A \times N \times 50000/$ ml sample

Where,

A= ml titrant

N= Normality of titrant

Annexure -H

Table shows different water quality parameters observed during the culture period

Parameter	Treatments				
	T_1	T_2	T_3	T_4	T_5
Temp Morn (^OC)	$28.38^a \pm 1.96$	$28.25^a \pm 2.22$	$28.44^a \pm 2.06$	$28.40^a \pm 2.05$	$28.38^a \pm 2.01$
Temp Even (^OC)	$29.35^a \pm 2.15$	$29.25^a \pm 2.17$	$29.42^a \pm 2.15$	$29.38^a \pm 2.16$	$29.33^a \pm 2.20$
pH Morning	$7.95^a \pm 0.21$	$7.98^a \pm 0.18$	$7.92^a \pm 0.19$	$7.91^a \pm 0.16$	$8.02^a \pm 0.15$
pH Evening	$8.08^a \pm 0.21$	$8.10^a \pm 0.17$	$8.05^a \pm 0.17$	$8.00^a \pm 0.32$	$8.16^a \pm 0.15$
DO(mg/l)	$6.87^a \pm 0.46$	$7.00^a \pm 0.30$	$7.01^a \pm 0.19$	$6.93^a \pm 0.41$	$6.98^a \pm 0.50$
Alkalinity (mg $CaCO_3/l$)	$180.29^a \pm 1.83$	$189.12^b \pm 1.12$	$188.13^{ab} \pm 1.71$	$203.58^c \pm 10.84$	$208.04^c f \pm 6.19$

Data are mean values of two replicates. Values are mean ± SD. Values within the same row having the same superscripts shows no significant difference (P>0.05).

Annexure- I

Water temperature

TEMP.MO

Duncan

FEED	N	Subset for alpha = .05
		1
2.00	12	28.2500
1.00	12	28.3750
5.00	12	28.3750
4.00	12	28.3958
3.00	12	28.4375
Sig.		.846

Means for groups in homogeneous subsets are displayed.
A Uses Harmonic Mean Sample Size = 12.000.

TEMP.EV

Duncan

FEED	N	Subset for alpha = .05
		1
2.00	12	29.2500
5.00	12	29.3333
1.00	12	29.3542
4.00	12	29.3750
3.00	12	29.4167
Sig.		.869

Means for groups in homogeneous subsets are displayed.
A Uses Harmonic Mean Sample Size = 12.000.

Annexure-J

PH.MO

Duncan

FEED	N	Subset for alpha = .05
		1
3.00	12	7.9083
4.00	12	7.9417
1.00	12	7.9667
2.00	12	7.9675
5.00	12	8.0208
Sig.		.169

Means for groups in homogeneous subsets are displayed.
A Uses Harmonic Mean Sample Size = 12.000.

PH.EV

Duncan

FEED	N	Subset for alpha = .05
		1
4.00	12	8.0042
3.00	12	8.0500
1.00	12	8.0833
2.00	12	8.0958
5.00	12	8.1583
Sig.		.119

Means for groups in homogeneous subsets are displayed.
A Uses Harmonic Mean Sample Size = 12.000.

Annexure-K

ALKA

Duncan

FEED	N	Subset for alpha = .05		
		1	2	3
1.00	12	180.2917		
3.00	12	188.1250	188.1250	
2.00	12		189.1250	
4.00	12			203.5833
5.00	12			208.0417
Sig.		.052	.800	.262

Means for groups in homogeneous subsets are displayed.
A Uses Harmonic Mean Sample Size = 12.000.

Annexure- L

DO

Duncan

FEED	N	Subset for alpha = .05
		1
1.00	6	6.8700
4.00	6	6.9325
5.00	6	6.9804
2.00	6	6.9992
3.00	6	7.0108
Sig.		.590

Means for groups in homogeneous subsets are displayed.
A Uses Harmonic Mean Sample Size = 6.000.

Annexure-M

FCR

Duncan

FEED	N	Subset for alpha = .05		
		1	2	3
1.00	2	3.2136		
4.00	2	3.6684	3.6684	
2.00	2	3.7043	3.7043	3.7043
3.00	2		3.9912	3.9912
5.00	2			4.4282
Sig.		.145	.306	.052

Means for groups in homogeneous subsets are displayed.
A Uses Harmonic Mean Sample Size = 2.000.

Annexure-N

PER

Duncan

FEED	N	Subset for alpha = .05	
		1	2
5.00	2	.8349	
3.00	2	.9420	.9420
2.00	2	.9943	.9943
4.00	2	1.0456	1.0456
1.00	2		1.1848
Sig.		.083	.055

Means for groups in homogeneous subsets are displayed.
A Uses Harmonic Mean Sample Size = 2.000.

Annexure- O

SGR

Duncan

FEED	N	Subset for alpha = .05
		1
4.00	2	3.1350
3.00	2	3.1500
1.00	2	3.1550
2.00	2	3.1950
5.00	2	3.2100
Sig.		.218

Means for groups in homogeneous subsets are displayed.
A Uses Harmonic Mean Sample Size = 2.000.

Annexure- P

SUR

Duncan

FEED	N	Subset for alpha = .05		
		1	2	3
3.00	2	73.5300		
5.00	2	76.4700	76.4700	
2.00	2		81.3700	81.3700
4.00	2			83.3300
1.00	2			85.2900
Sig.		.261	.088	.161

Means for groups in homogeneous subsets are displayed.
A Uses Harmonic Mean Sample Size = 2.000.

Annexure- Q

M.I.WT

Duncan

FEED	N	Subset for alpha = .05
		1
3.00	2	28.7300
2.00	2	30.2500
4.00	2	31.4350
1.00	2	32.3600
5.00	2	34.0900
Sig.		.078

Means for groups in homogeneous subsets are displayed.
A Uses Harmonic Mean Sample Size = 2.000.

Annexure- R

Y.DM.107

Duncan

FEED	N	Subset for alpha = .05	
		1	2
3.00	2	1.4050	
2.00	2	1.6350	1.6350
5.00	2		1.7400
4.00	2		1.7450
1.00	2		1.8400
Sig.		.060	.093

Means for groups in homogeneous subsets are displayed.
A Uses Harmonic Mean Sample Size = 2.000.