Chapter-1

INTRODUCTION

CHAPTER 1

INTRODUCTION

1.1 Background Information

Aquaculture is one of the fastest growing food producing sectors of the world. It plays an important role in the socio-economic development of many countries in view of its potential contribution to national income, nutritional security, social objectives and sustainable large export earnings. In context of present world's nutritional security aquaculture is evolving as one of the solution. Aquaculture continues to be the fastest growing animal food producing sector and to outpace population growth. Per capita supply from aquaculture increased from 0.7 kg in 1970 to 7.8 kg in 2008, an average annual growth rate of 6.6 percent. From a production of less than one million tons per year in the early 1950s, production in 2006 was reported to be 51.7 million tones and 52.5 million tones in 2008 (FAO, 2010). The contribution of aquaculture to the total production of capture fisheries and aquaculture continued to grow, rising from 34.5 percent in 2006 to 36.9 percent in 2008. In 2008, the total world production of fisheries was 142 million tones of which aquaculture contributed 52.5 million tons (FAO, 2010). With increase in population expansion, the demand for high quality protein is rising dramatically and is expected to be fulfilled by aquaculture. The value of the world aquaculture harvest, excluding aquatic plants, is estimated at US$98.4 billion in 2008 (FAO, 2010).

1.2 Contribution of Nepalese in Aquaculture

In Nepal, fisheries have been practiced for a long time and have a strong tradition in the country. But aquaculture is fairly a new activity and was started in early 1950's. At present the total fish production in Nepal is 48,230 metric tons including 26,730 tones from aquaculture practices and 21,500 tons from capture fisheries (DoFD, 2009/10). This production is not sufficient to meet demand of Nepalese fish market, so, there is a large portion of fish import to Nepal. Also a high portion from capture fisheries creates a doubt to the role of fish in future nutritional security. Therefore, improvement of aquaculture practice and production of a healthy fish has become necessary. To achieve this goal improvement in the technologies is must.

1.3 Statement of the Problem

Larviculture is the backbone of aquaculture production. The primary problem of larviculture is food and its digestion, especially in the transition period (endogenous to exogenous feeding larvae). Artificial food is indispensible for the early developmental stages of many aquacultural species. High larval mortalities in nursery ponds of Rainbow Trout (*Oncorhynchus mykiss*) have resulted due to non availability of natural food organism and insufficient supply of appropriate artificial food. As protein utilization is fundamental to growth, proteolytic enzymes have an important role to play in the larval fish as well as adult ones. Characterization and identification of protein is essential along with quantitative estimations for the better understanding of health and growth status of the cultured species. Therefore, the study of nucleic acids and protein contents during early stage is most essential. This will help to develop suitable artificial diet which can truly replace live food. This will finally ensure the aquaculturists to develop proper feeding strategies for Rainbow Trout especially in the least developed country like Nepal.

1.4 Justification

The Southern Terai region of the country is the main area for warm water aquaculture where pond culture of Indian major carps and other carps have been well established. However, cold water fish in the mid-hills is at the beginnings although a new venture shows that it is a profitable enterprise. Since, Nepal is a mountainous country and bestowed with rich cold water resources, aquaculture of cold water fishes can be best thrived. Cool and cold water streams and rivers in Nepal extending from the Himalayas offer excellent habitat to 76 native fish (Rajbanshi, 2002). The native cold water fisheries resource offers vast scope for development of cold water aquaculture but only few indigenous species have been domesticated and propagated for cultivation purposes. Their culture has yet to be adopted by the private sector. Among the exotic species, Rainbow Trout (*Oncorhynchus mykiss*) formerly known as *Salmo gairdeneri* is widely cultivated cold water salmonid throughout the temperate world (Bardach *et al.*,1972).

In Nepal, recently considering the viability of the technology and its potential role in trout farming commercialization in hills and mountains, NARC has initiated its scaling-up of technological activities. Consequently, Government of Nepal has selected Nuwakot and Rasuwa as the “Trout Districts” under “One Village One Product (OVOP)” program (MoF, 2006). Despite this policy recognition and prioritization, we have limited

information and empirical studies that have rarely been undertaken to study food value of trout for successful scaling-up and commercialization of trout farming.

1.5 Objectives

1.5.1 General Objective

The main objective of this study is quantitative estimation of nucleic acids and protein profile in *Oncorhynchus mykiss* larvae to understand the nutritional condition and growth rate.

1.5.2 Specific Objectives

- Quantitative estimation of nucleic acids and study of RNA: DNA ratio to understand the growth performance of *Oncorhynchus mykiss*
- Quantitative estimation of total protein of *Oncorhynchus mykiss* to understand the food conversion rate with regard to food quality and feeding condition
- To study the albumin: globulin ratio in tissue of fish
- To have a better understanding of dietary requirement and nutrient composition of Rainbow Trout (*Oncorhynchus mykiss*) for practical culture conditions
- Analysis of water quality to understand the proper physico-chemical environment
- To study the growth rate of fish

1.6 Rationale of the Study

Aquaculture has expanded, diversified, intensified and technologically advanced and its contribution to aquatic food production has increased significantly in the past four decades. As civilizations almost always developed in the association with rivers or other suitable water resources, it is therefore to be expected that the hunting instincts of human beings were used effectively to harness the fish resources of such waters, from time immemorial, as often seen in old inscriptions. But the most effective exploitation of fish resources, however, occurred in the post second world war period (Bots-ford *et al.*, 1997) when fish became an increasingly important component of our daily animal protein intake and the calorie supply. With the increasing awareness of the positive effects of fish on health and well-being, the importance of aquaculture in the food sector has been realized. Obviously with a growing concern for improvements in the health and human well being

and with no further increases to the aquatic food supply from capture fisheries and the envisaged marginal increase in agriculture production, aquaculture may have an increasing role to play in the next decade and beyond. Thus, application of modern technologies in aquaculture to raise the production has become essential. Effect of different factors on growth and survival of fish larvae is important in the rearing process of larval fish and thus in aquaculture system.

1.7 Limitation

Research studies face many problems, so obviously have limitations to the study. The present study no doubt, bears the following limitations:

- This academic study has been carried out for the partial fulfillment of the requirements for the Master's Degree in Zoology at Tribhuvan University, Kathmandu, Nepal.
- Due to the lack of sophisticated instruments measurement of biochemical parameters was done for a smaller number fish so the sample size was also small.
- The research has limitations regarding finance and time constrains.

1.8 Significance

A detailed knowledge of the protein profile and growth rate in the trout larvae has a double interest. It can be utilized to estimate the limitations to the use of artificial diets and some biochemical profile can be useful indicators of the nutritional condition and viability of the larvae. The formulation of inexpensive artificial diets for larvae needs a comprehensive analysis of the ontogenic changes occurring during the early life stages of the commercial fish.

1.9 Scope

Fish culture is a recent activity in Nepal. There is, however, considerable scope for expansion of aquaculture development to increase fish production. Natural lakes and rivers in the country provide an estimated 400,000 ha of water area for development. With the steadily increasing trend in the construction of irrigation dams and hydro-electric power stations, several additional water bodies for aquaculture development are rapidly becoming available.

Considering the nature of resources and the need for increased availability of protein-rich food, Government of Nepal has accorded high priority to development of aquaculture in the country. The fifth five-year plan (1975-80) envisages a target of 5,240 tons production of fish per year, aimed at increasing per capita consumption of fish to 382 g by 1980. This objective was to be achieved through intensification of fish culture in public and private farms, in village tanks, reservoirs, lakes and paddy fields, as well as by the development of the natural water fisheries (rivers).

The results of fish culture in artificial fish ponds and paddy fields in the private sector have been encouraging and also in recent days the production of Rainbow Trout in barren and difficult areas of hilly region of Nepal has encouraging result. On the basis of results to date, it is expected that a considerable part of the animal protein needs of the population could be met if modern fish culture practices were to be gradually extended to other parts of the country after the necessary feasibility studies have been undertaken.

In this context, this study was initiated to understand production constraints of Rainbow Trout in Nepal. The specific objectives were to study the protein profile and growth pattern of Rainbow Trout which may explore the market potentials and market system, trend, assess production, and identify current constraints, risks and potentials in production and policy options in trout farming promotion in Nepal.

Chapter-2

LITERATURE REVIEW

CHAPTER 2

LITERATURE REVIEW

2.1 Trout Culture in Nepal

Rainbow Trout (*Onchorhynchus mykiss*) is the native fish of the rivers of North-West America. Living within a temperature range of 0°C-25°C with best growth at the range of 10°C-20°C, is the best suited exotic fish for commercially cultivation in cold waters (Bardach *et al.*, 1972; Huet, 1975; Shetty *et al.*, 1989). Trout was successfully introduced in Nepal in 1988 to meet many needs including substitution of import, use of cold water resources for aquaculture and promotion of fishing tourism in hill streams (Gurung & Basnet, 2003). It was first time introduced in Nepal from United Kingdom in 1960, then from Japan in 1965, from India in 1970 and again from Japan in 1970 (Gurung and Basnet, 2003). Now the breeding and culture technology of Rainbow Trout is well developed. Currently, culture of Rainbow Trout is practiced in small scale around Kathmandu, Nuwakot, Rasuwa, Sindhupalchowk, Dhading and Government Farms in Godawari and Trishuli. Recently, its culture has gained some momentum under One Village One Product (OVOP) Programme. At present there are altogether 55 farmers involved in Rainbow Trout farming utilizing 4000 m^3 of land producing 80 metric tons of fish (DoFD, 2009/10).

Rearing fish larvae successfully is one of the most important parts of aquaculture enterprise, as its sustainability highly depends on survival of larvae (Bardach *et al.*, 1972; Huet, 1975). Carp require natural food when larvae first start its feeding. However, larvae of Rainbow Trout *(Oncorhynchus mykiss)* in hatcheries exclusively depend on external feed for their growth from the time larvae commence first feeding (Bardach *et al.*, 1972). Therefore, trout larval rearing highly depends on quality feed. In many countries, high quality commercial branded dry pellet known as crumble for larvae are available (Hinshaw, 1999). However, in Nepal enterprises for commercial trout diets are not yet available. Thus, to initiate sustainable trout farming methodologies use of local feeds becomes essential. Rainbow Trout being carnivorous obtain limited amount of energy from fat and carbohydrates; thus, need diets rich in animal protein (Nomura, 1993; Hinshaw, 1999). Besides, the amount of water and seed the success of trout farming depends on the types of feed on which they are cultivated (Ghittino, 1972; Maruyama 1983). Earlier, Japanese feed was used as initial feed for early fry in Nepal. Dry feed

obtained from abroad was not only expensive but always bears risk upon unavailability. In earlier years before the methodologies were available for pellet feed, Rainbow Trout were usually fed raw feed of animal origin (Bardach *et al.,* 1972; Sedgwick 1985).

2.2 Growth Performance of Rainbow Trout

Rainbow Trout is a carnivorous species that requires high protein feed and well oxygenated water. Depending on quality of the diet and temperature, Rainbow Trout can reach marketable size (200-300 g) within 12-14 months from free-swimming larvae. It is more economical to reach marketable size as soon as possible (Huet, 1975). Studies on growth rate and effects of various factors on growth performance of Rainbow Trout have been carried out.

Nepal *et al.* (1997) in their study about the economics of Rainbow Trout farming in Nepal has shown that the SGR of fish was 1.92% per day to 2.00% per day while FCR was found to be 2.04- 2.00 during the period of study. They have studied the fish growing from 70 gm to 161 gm. Mulmi and KC (2007) studied the growth rate and survival of Rainbow Trout in two private farms in Jitpur Phedi, Kathmandu and Okharpauwa, Nuwakot. In the farm of Jitpur Phedi, Kathmandu they studied the growth rate of fish cultured for 223 days reared from 7.25 gm to 194.0±26.6 gm. The growth rate was found to be highest in January (1.74gm/day) and lowest (0.208 gm/day) in July and the mortality rate was 11.84% during study period. In a similar study (Mulmi and KC, 2008) the highest growth rate (1.54 gm/day) was found in January and lowest (0.51 gm/day) in October with mortality rate 2.5%. This study was also carried out in Okharpauwa, Nuwakot rearing fish from 44.5 gm to 234.5±28.37 gm within 149 days.

A technical report (FRD, 2001) has described the growth rate and FCR of Rainbow Trout larvae with different levels of total crude protein. According to the report, the growth rate of free swimming stage (60-80 mg) was found to be 0.020 gm/day, 0.026 gm/day and 0.031 gm/day with crude protein 25%, 35% and 45% respectively, while the FCR was found to be 4.97, 2.45 and 3.14 respectively.

A study by Chung and Segnini (1998) has shown that SGR of Rainbow Trout fed ad libitum six times per day was 9.8% per day while that of fed three times per day was 8.5% per day. Similarly, the SGR of fasted fish was found to be decreasing i.e. -1.96% per day. The group of fish which were starved for previous six days recovered SGR after

three days of feeding. Kayim *et al.* (2004) found SGR 0.915, Condition factor 1.55 and FCR 1.63 in an experiment carried on Rainbow Trout fed with ad libitum pellet feed number four in net cages in Almus Dam Lake (Tokat) for a period of two months.

There are different factors which affects the growth of Rainbow Trout. Jurss *et al.* (1987) studied the effects of temperature, food deprivation and salinity on growth of Rainbow Trout. They have shown that these fish grow best at a temperature of 11°C and salinity 20% with a good supply of food while the growth is lowest at 1°C with same salinity.

Feed efficiency in trout in Japan ranges from 60-80% (Tasiro *et al.,* 1974) but it has been found lower (43-46%) in Nepal (FRD, 1999/'00). This suggests that there is still need of research on trout feed efficiency improvement.

2.3 Biochemical Indices

Biochemical analysis, which determines the quantities of chemical constituents serving as energy substrates, could be one of the indicative measures to show changes of nutritional condition. Among the biochemical indices, the ratio of RNA: DNA has been proven as a useful and reliable indicator of nutritional condition and growth of fishes (Buckley, 1979; Zhou *et al.*, 2000; Buckley *et al.,* 2008). Similarly, the A: G ratio in fishes reflects the health status of fishes (Rao *et al.,* 2005).

2.3.1 Growth Performance and Nucleic Acid

Nucleic acids play a major role in growth and development. The amount of DNA, the carrier of genetic information, remains stable under changing environmental situations and has been used as an indicator of biomass (Holm-Hansen *et al.*, 1983). The concentrations of RNA in a tissue provide an estimate of ribosome numbers. The changes in nutritional status lead to alterations in ribosome numbers and thus the amount of RNA (Bulow, 1970). Larval fish respond to favorable growth conditions by increasing RNA content relative to DNA (Buckley, 1979). Growth in fish as in most other organisms is accomplished by protein synthesis which is directly related to ribosome number. That is, the protein synthetic machinery per cell is increased with the increase in RNA content on commencement of suitable condition (Wang and Stickle, 1986).

Chung and Segnini (1998) has shown that SGR of Rainbow Trout fed ad libitum six times per day was 9.8% per day while that of fed three times per day SGR was found to be 8.5% per day and concentrations of RNA and DNA increased from similar to 960 µg/mg and similar to 960 µg/mg respectively for both groups during the study period. Similarly, the SGR of starved fish was found to be decreasing i.e. -1.96% per day and DNA and RNA concentrations of starved fish increased from similar to 450 µg/mg and similar to 460 µg/mg respectively. The group of fish which were starved for previous six days recovered SGR after three days of feeding and their DNA and RNA concentration increased from similar to 600 µg/mg and similar to 840 µg/mg respectively.

2.3.2 Growth Performance and RNA: DNA Ratio

The RNA: DNA ratio is considered an instantaneous measure of growth rate in the field (Buckley, 1984) because it responds to changes in feeding conditions and growth after periods as short as 1–3 days in a variety of marine species. There is usually a significant correlation between nutritional status, RNA: DNA ratio and rates of growth (Lied *et al.*, 1983; Loughna and Goldspink, 1984). RNA: DNA ratio serves as an indicator of the protein synthesizing potential of a cell (Bergeron, 1997; Buckley *et al.*, 1999) and can be successfully used as an indicator of growth rate in fish larvae. Several workers have shown the relation between RNA: DNA ratio and A: G ratio with the growth and health status of different fishes. A correlation between RNA-DNA ratio and growth rate is expected since RNA comprises a large part of the cell's protein synthesizing machinery and DNA is an index of cell number. Growth in fish as in most other organisms is accomplished by protein synthesis. Larval fish respond to favorable growth conditions by increasing RNA content relative to DNA (Buckley, 1979). That is, the protein synthetic machinery per cell is increased. Similar relationships between RNA: DNA ratio (and RNA concentration) and growth rate have been observed for a wide variety of organisms (Leick, 1968; Bulow, 1970; Sutcliffe, 1970).

Changes in the biochemical values such as RNA: DNA and Protein: DNA reflects yolk absorption, feeding, morphogenetic changes, growth and environmental conditions in fish larvae (Park *et al.*, 2008). Comparative studies on growth and biochemical analyses among the fed and starved larvae have also been carried out which strengthens the relation between nutritional status and RNA: DNA ratio of fish.

Chung and Segnini (1998) have shown a relation between SGR of Rainbow Trout fed and fasted with their RNA: DNA ratio in an experiment carried on 17 day old fry of Rainbow Trout at 10° C for 13 days. Four groups of fishes were kept in different tanks. SGR of Rainbow Trout fed ad libitum six times per day was 9.8% per day and the RNA: DNA ratio changed from 1.8 to 3.4, while that of fed three times per day SGR was 8.5% per day and RNA: DNA ratio changed from 1.8 to 3.3. SGR of fasted fish was found to be decreasing i.e. -1.96% per day and the RNA: DNA ratio changed from 1.72-0.73. The group of fish which were starved for previous six days recovered SGR after three days of feeding and RNA: DNA ratio changed from 1.96-1.91 during study period.

Similarly a study in Pakistan (Ali *et al.*, 2006) on effect of feed cycling on *Labeo rohita* showed the SGR 0.12731±0.8269; RNA: DNA ratio 3.6745±0.2572 and Condition Factor 0.8168±0.0936 in control condition i.e. fed continuously with 24% protein at the rate of 3% by the body weight. This experiment also showed that there is significant effect of starvation on RNA: DNA ratio on *Labeo rohita*.

2.3.3 Nutrition and RNA: DNA Ratio

The RNA: DNA ratio gives a measure of the synthetic capacity of the cell and usually correlated with nutritional status (Buckley *et al.*, 1999). RNA is directly involved in protein synthesis and therefore increase in RNA content is observed during periods of rapid growth whereas DNA content is usually stable (Bulow, 1970) making the RNA: DNA ratio an indicator of protein synthesis capacity per cell. Different workers have worked on the nutritional status of fish and correlated it with RNA: DNA ratio in fish.

According to a study in Japan on the larvae of Pacific Bluefin Tunna (*Thunnus orientalis*), RNA: DNA ratio is a useful and reliable indicator of the nutritional status of fish larvae and juveniles and thus can be used to study the nutritional condition of fish of any area (Tanaka *et al.*, 2008).

Nutrition and farm management strategies play critical roles in fish health and disease outbreaks within intensive farming system and, to a lesser extent, in semi-intensive farming systems (Tacon, 1997; Hasan, 2001). Trout nutrition and feed formulation research has received significant attention on the use of plant and animal by-products as fishmeal and shrimp meal substitutes in Nepal (Igarashi & Roy, 1999; Pradhan, 1999).

2.3.4 Temperature and RNA: DNA Ratio

Buckley *et al.* (1984) has shown an agreement between growth estimates based on the relation between RNA: DNA ratio, temperature, and growth of sand lance and the general model is particularly good at intermediate temperatures (5 to 9ºC). Growth estimates based on the relation between RNA: DNA ratio, temperature and growth of sand lance are slightly higher at low temperatures and lower at high temperatures. They showed that the effect of feeding level on larval growth and biochemical composition was generally greater than the effect of either temperature alone or the interaction of temperature and feeding level. In the combined data matrix no correlation was observed between temperature and RNA: DNA ratio of larval Sand Lance.

In an earlier study (Buckley, 1982), a weak correlation was observed between rearing temperature and RNA: DNA ratio of Winter Flounder. What little effect temperature has on larval RNA: DNA ratio may be indirect, acting through alteration of metabolic rate, and therefore, food requirements. This and other studies with larval fish (Buckley, 1979, 1980, 1982) have clearly demonstrated a role for supply of RNA in acclimation to various feeding levels and a role of temperature in controlling growth rate. Temperature, however, does not appear to be acting through adjustments in the supply of RNA. Haschemeyer (1978) has studied the role of the supply of either messenger RNA or ribosomal RNA in temperature acclimation in poikilothermic organisms.

2.3.5 RNA: DNA Ratio, A: G ratio and Health Status of Fish

Several authors have shown a clear relation between the RNA: DNA ratio, A: G ratio and health status of fish. Rao *et al.*, (2004) studied the relation of RNA: DNA ratio and protein content of *Labeo rohita* with the immunity level of the fish. In this experiment the fish were fed with *Achyranthes aspera* and the potentiation of antibody production was checked, which was found to be increasing with the increasing level of RNA: DNA ratio and total protein content of fish. In a similar study in India (Rao *et al.*, 2005) with Common Carp (*Cyprinus carpio*) fed with the seeds of *Achyranthes aspera* has shown the increase in immunity level with the increase in RNA: DNA ratio and A: G ratio in the experimental group of fish.

Similarly, an experiment carried on *Labeo rohita* fed with Baker's Yeast (*Saccharomyces cerevisae*), the RNA: DNA ratio was found to be significantly (P

<0.005) increasing in experimental feed with respect to control feed while the A: G ratio was found to be significantly (P <0.005) decreasing after an experimental period of 60 days (Tewary and Patra , 2011). A study (Del *et al.*, 2010) on biochemical changes in Rainbow Trout with Rainbow Trout Gastroenteritis (RTGE) has shown that there is a reduction in A: G ratio owing to the selective loss of albumin of fish infected with RTGE compared with the healthy ones.

2.4 Feed of Rainbow Trout

Rainbow Trout is a carnivorous fish, so it needs high protein content in feed. Making feed of high protein content is much more expensive and raises the production cost too much. So, many studies are carried out to replace the animal protein with plant protein in the feed of Rainbow Trout.

Experiments are also carried out to supply with the easily and locally available materials in the feed of Rainbow Trout as well experiments to raise the flesh quality of Rainbow Trout has been also carried out. According to a study on effect of protein source on the growth efficiency of Rainbow Trout showed that the growth rates of Trout fed with fish meal free diets, using conventional and concentrated plant protein ingredients, are good but some limitation to growth exists in the fish meal free diets (Barrow *et al.,* 2007; Overturf *et al.,* 2009). Similarly a study in Norway (Hensen *et al.,* 2007) suggests that inclusion of cellulose in pellets feed does not have any effect on the digestivity and digest ability of main nutrients; rather it increases the durability and hardness of feed.

Pandey and Shuichi (2008) have proven that certain organic acids such as citric acid and lactic acid may replace the need of phosphorous in the fish meal diet for Rainbow Trout. Similarly, a study in China (Yu *et al.,* 2009) has shown that incorporation of Black Soldier Fly (*Hermetia illucens*) into the feed stuff of Rainbow Trout is possible and can be used as protein rich (42-44%) source.

Also, a study has shown that feather meal, poultry by-product meal, blood meal and meat and bone meal have good potential for the use in Rainbow Trout at high levels of incorporation (El-Haroun *et al.,* 2009). Similarly, use of macro algae meal might help to increase lipid content in the final product due to the beneficial effects of PUFAs for human health. But the inclusion of macro algae in diet of Rainbow Trout does not have any effect on protein and lipid content at muscle level (Dantagnan, 2010).

Chapter-3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental System

The experiment was conducted with the Eight week old larvae of Rainbow Trout, *Onchorhynchus mykiss,* during February–March, 2011. Larvae were procured from "Fall and Trout Village Fish Farm, Doman, Nuwakot" and cultured at same place and the biochemical analyses were done at the Laboratory of "MV Polyclinics and Diagnostic Centre, Sinamangal, Kathmandu".

Fish were cultured in outdoor natural conditions under three feeding regimes in order to find out the effects of different proportion of protein content in food on the growth performance, RNA: DNA ratio and protein profile of the fish. Culture was done in small netted cages (50cm X 50cmX 50cm) which were previously used for hatchery purpose by the farmer. The stocking density was 320 fish/m^3 i.e. 40 fish in each cage. These cages were maintained in the raceways of fish farm. Water from a natural

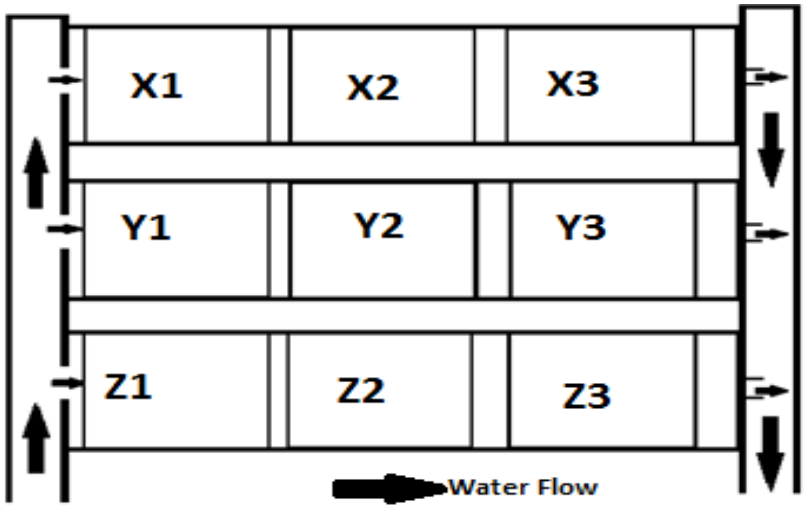

Figure 1 Experimental Design

source is passed through these raceways. Three raceways were used for the experimental purpose each with three cages set as three replicates for each feeding regime (Figure 1). Raceways were coded as X, Y and Z while cages were tagged as 1, 2 and 3. Fish in raceway X were fed with control diet (C) that is used by farmer, fish in raceway Y were fed with 40% protein diet (D1) while fish in raceway Z were fed with 50% protein diet

(D2) each twice daily. Size of pellet for all type of feed was 60 microns for the whole study period. Sampling was done after each week and samples were coded as St, S1, S2, S3, S4 and S5 for the sample during stocking, sample after 1st week, after 2nd week, after 3rd week, after 4th week and after 5th week respectively. Figure 1 shows the experimental design.

3.2 Experimental Feed

Commercial feed which was previously used by farmer was used as control diet I. Two experimental diets were formulated using Pearson's Square method. Diet I contained

Diet / Ingredients	C (Control Diet)	D_1 (40% Protein)	D_2 (50% protein)
Dried Fish	40%	48%	74%
Mustard oil Cake	10%	25%	12%
Wheat Flour	17%	25%	12%
Rice Bran	30%		
Vitamins and Minerals	2%	1%	1%
Salt	1%	1%	1%

Table 1 Ingredients composition of different diets

40% protein (D_1) while Diet II contained 50% protein (D_2). The constituents used for preparing different diets are shown in table 1 (Fig. Annex I).

Calculation of amount of feed to be provided to trout larvae were done after each sampling and fed with 3% by body weight twice per day, at 9.00 AM and at 4.00 PM.

3.3 Study of Growth Parameters

Fish were collected after each week and data about length were taken instantly while for weight data sample fish were taken to laboratory packed in vials in ice-box. For this purpose fish were collected after blotting in pre-dried and tarred vials. These sample fish were carried to laboratory in an Ice-box, since these were more vulnerable to decomposition.

Analysis of growth parameters were done using following formulae:-

Length Gain (∂L) = $L_t - L_o$

Where L_t = Length during survey (after time t)

L_o= Length during previous survey

Weight Gain (∂W) = $W_t - W_o$

Where W_t = Weight during survey (after time t)

W_o = Weight during previous survey

Specific Growth Rate (SGR) = $100 (\ln W_t - \ln W_o)/t$

Also feed conversion ratio was calculated using following formulae:-

Feed Conversion Ratio (FCR) = $F_k / (W_t - W_o)$

Where F_k = Amount of feed consumed by each fish during time period t, which was calculated by calculating the amount of feed given during certain time period divided by number of fish.

3.4 Study of Water Quality Parameters

Water quality parameters about following parameters were taken using following methods:-

- Temperature – Using Laboratory Celsius thermometer
- pH – Using digital pH meter
- Dissolved Oxygen (DO) – Using Winkler's Method

3.5 Biochemical Estimation

Biochemical analyses about total protein content, albumin content, RNA content and DNA content estimation were done using appropriate methods. For this purpose fish samples were carried to the laboratory packed in vials in ice-box. Total protein content analysis was done using Biuret's method developed by Doumas (1975) followed by Garry and Williams (1977). Albumin content was measured using Bromo-Cresol Green (BCG) Method developed by Doumas et al (1971) followed by Cheesbrough (1987). Similarly,

Isolation of nucleic acids from the fish larvae were done by the method developed by Schneider (1945) and followed by Mustafa (1982). Estimation of RNA and DNA in fish larvae were done by method developed by Buckley and Bulow (1987). All the spectrophotometric readings were taken in Beltronix Digital Photo Colorimeter (BDPC-4637).

3.5.1 Tissue Preparation

Fish larvae were collected at 9 AM after first feeding and blot dried. Single sample was taken from each cage. These samples were taken to the laboratory in vials carried in ice-box to prevent the temperature raise and to keep the sample in cold condition. All fish larvae were weighed and fine crushed using small mortar and pestle. 100 mg of this fine crushed sample were taken in the vials and dissolved (homogenized) in 2 ml of ice-cold double distilled water. From this 2 ml of homogenate sample, 1 ml was taken for protein analysis and another 1 ml was taken for nucleic acid analysis.

3.5.2 Estimation of Protein

1 ml of homogenate which was previously separated for protein analysis was centrifuged at 10000 rpm for 10 minutes at 4°C. Supernatant was collected for measurement and sediment was discarded.

Tubes	Biuret's Reagent (ml)	BSA (ml)	Test Sample (ml)	Distilled Water (ml)
Blank	2.0	-	-	0.05
Standard I (40µg)	2.0	0.01	-	0.04
Standard II (80µg)	2.0	0.02	-	0.03
Standard III (160µg)	2.0	0.04	-	0.01
Sample	2.0	-	0.02	0.03

Table 2 Reagents for total protein test and its standard

Estimation of total protein was done by Biuret Method (Doumas B.T., 1975). For this 0.02 ml of protein supernatant was taken in a tube and made upto 0.05 ml adding distilled water. To this 2 ml of Biuret's Reagent from the Readymade Kit was added. A blank determination was also done, in which 0.05 ml of distilled water was used without

any protein supernatant. Both were kept at room temperature for 15 minutes and the absorbance was read at 550 nm in the Spectrophotometer.

Calibration of spectrophotometer was done using Bovine Serum Albumin (BSA) which is available with the test kit. This readymade kit has a concentration of 4 g/dl. From this three standards were made. For the preparation of three standards respectively 0.01 ml, 0.02 ml and 0.04 ml were taken in three different tubes. Volume in these tubes was made upto 0.05 ml adding appropriate amount of distilled water. In all tubes 2 ml of Biuret's Reagent was added and kept at room temperature for 15 minutes. Now, the absorbance at 550 nm was taken in the Spectrophotometer and calibrated accordingly. Table 2 shows the amount of reagents to be added in different tubes.

3.5.3 Estimation of Albumin

Estimation of albumin was done by Bromo-Cresol Green (BCG) Method (Doumas et al, 1971). For this 0.02 ml of protein supernatant was taken and made upto 0.05 ml adding distilled water. To this 2 ml of BCG Reagent was added. A blank determination was done, in which only 0.05 ml distilled water was instead of protein supernatant. This was then kept at room temperature for 15 minutes and thereafter absorbance was read at 630 nm in the Spectrophotometer.

Tubes	BCG Reagent (ml)	BSA (ml)	Test Sample (ml)	Distilled Water (ml)
Blank	2.0	-	-	0.05
Standard I (40µg)	2.0	0.01	-	0.04
Standard II (80µg)	2.0	0.02	-	0.03
Standard III (160µg)	2.0	0.04	-	0.01
Sample	2.0	-	0.02	0.03

Table 3 Reagents for albumin content and its standard

BSA was used as standard for the calibration of spectrophotometer. In Albumin Test Kit the standard solution of BSA comes in a concentration of 4 g/dl. From this three standards were made. For the preparation of three standards respectively 0.01 ml, 0.02 ml and 0.04 ml were taken in three different tubes. Volume in these tubes was made upto

0.05 ml adding appropriate amount of distilled water. In all tubes 2 ml of BCG Reagent was added and kept at room temperature for 15 minutes. Now, the reading at 630 nm was taken for the calibration of Spectrophotometer and calculation was done. Table 3 shows the amount of reagents to be added in different tubes for the estimation of Albumin content.

3.5.4 Nucleic Acid Isolation

1 ml of homogenate was taken in a centrifuge tube and 2.5 ml of 10% Trichloroacetic Acid (TCA) was added and incubated at 4°C for 30 minutes to remove acid soluble components. After incubation, the sample was centrifuged at 10000 rpm for 20 minutes. The supernatant was again washed in 2.5 ml of 10% cold TCA and kept for 30 minutes at 4° C. Again it was centrifuged at 10000 rpm for 20 minutes, supernatant discarded and the sediment was washed with 5 ml of ethanol to remove the lipid components and kept at room temperature for 30 minutes. Again centrifugation was done at 10000 rpm for 20 minutes and the supernatant was discarded and sediment washed with 5 ml of 95% ethanol. This was again centrifuged at 10000 rpm for 20 minutes. The supernatant was discarded and sediment was washed with 2.5 ml of 5% TCA. Now the vials were kept at 90° C for 15 minutes in a boiling water bath. After cooling, these vials with sample were kept in refrigerator at 4° C for 30 minutes. Now, centrifugation at 10000 rpm for 20 minutes was done and the supernatant was collected in a separate tube. The sediment was again washed with 2.5 ml of 5% TCA and centrifuged at 10000 rpm for 20 minutes. Now, the supernatant was collected and mixed with the earlier collected supernatant. This supernatant contains nucleic acid mixture and is used for nucleic acid estimation.

3.5.5 Estimation of DNA

Estimation of DNA was done spectrophotometrically by Diphenylamine method. For this purpose 1 ml of nucleic acid mixture supernatant was taken and to that 2 ml of diphenylamine reagent was added and kept in a boiling water bath for 10 minutes. After boiling, the samples were cooled and the absorbance was recorded at 600 nm. In blank 1 ml of distilled water was used instead of nucleic acid mixture.

Pure DNA isolated from calf thymus (Sisco Research Laboratories, Mumbai) was used for the calibration of spectrophotometer. In 1 mg of DNA 10 ml of 10% TCA was

added and kept in boiling water bath for 10 minutes to dissolve DNA, and cooled to room temperature.

Standard concentrations from 10 µg to 100 µg were taken and made upto 1 ml with distilled water. For this purpose solution was taken from 0.1 ml to 1.0 ml and made upto 1 ml adding distilled water. In all tubes 2 ml of Diphenylamine reagent was added and kept in boiling water bath for 10 minutes and then cooled to room temperature. The

Tubes	Diphenylamine Reagent (ml)	Calf Thymus DNA (ml)	Test Sample (ml)	Distilled Water (ml)
Blank	2.0	-	-	1.0
Standard I (10µg)	2.0	0.1	-	0.9
Standard II (40µg)	2.0	0.4	-	0.6
Standard III (70µg)	2.0	0.7	-	0.3
Standard IV (100µg)	2.0	1.0	-	-
Sample	2.0	-	1.0	-

Table 4 Reagents for DNA content and its standard

absorbances were taken in Spectrophotometer at 600 nm for calibration. Table 4 shows the amount of reagents to be added in different tubes.

3.5.6 Estimation of RNA

Estimation of RNA was done spectrophotometrically using Orcinol method. For this purpose, 1 ml of nucleic acid mixture supernatant was taken and to that 0.5 ml of distilled water and 1.5 ml of Orcinol reagent was added. This was kept in boiling water bath for 20 minutes. After boiling, this was cooled and the absorbance was read at 660 nm.

Pure RNA from calf thymus (Sisco Research Laboratories, Mumbai) was used for calibration purpose. In 2 mg of RNA 10 ml of 10% TCA was added and kept in a boiling water bath for 15 minutes to dissolve the RNA and cooled to room temperature. Standard concentrations from 20 µg (equivalent to 0.1 ml) to 200 µg (equivalent to 1.0 ml) were taken in a series of test tubes and made upto 1.5 ml with distilled water. A control sample was also made using 1.5 ml of distilled water instead of RNA mixture solution. Now, to

each test tube 1.5 ml Orcinol reagent was added and kept in boiling water bath for 20 minutes and then absorbance is read at 660 nm in the Spectrophotometer. Table 5 shows

Tubes	Orcinol Reagent (ml)	Calf Thymus RNA (ml)	Test Sample (ml)	Distilled Water (ml)
Blank	1.5	-	-	1.5
Standard I (20µg)	1.5	0.1	-	1.4
Standard II (80µg)	1.5	0.4	-	1.1
Standard III (160µg)	1.5	0.8	-	0.7
Standard IV (200µg)	1.5	1.0	-	0.5
Sample	1.5	-	1.0	0.5

Table 5 Reagents for RNA content and its standard

the amount of reagents to be added in different tubes

3.6 Statistical Analysis

The data obtained from the study was investigated by ANOVA to evaluate the effect of different diet on growth and Feed Conversion Ratio of fish and the relation between RNA: DNA ratio and protein content of different Feed fed fish. The critical probability was set at $p = 0.05$. Correlation coefficient was also calculated among different variables such as length and weight; RNA: DNA ratio and SGR. For ANOVA different hypotheses were set wherever needed.

Analysis was also done by representing with the table, bar diagram.

Chapter-4

RESULTS

CHAPTER 4

RESULTS

The experiment was conducted with the larvae of Rainbow Trout, *Onchorhynchus mykiss,* during February–March, 2011. Larvae were procured from "Fall and Trout Village Fish Farm, Doman, Nuwakot" and cultured at the same place. Fish were cultured in outdoor conditions under three feeding regimes in order to find out the effects of varied proportion of protein content in food on the growth performance, RNA: DNA ratio and protein profile of the fish. The stocking density was 320 fish /m^3. In the first group, fish were fed with commercial artificial diet used by farmer (considered as control diet, C), the second group of fish was fed with artificial diet containing 40% protein (D1) while the third group was fed with artificial diet containing 50% protein (D2). Fish were fed 2 times daily at the rate of 3% of body weight. Three replicates were used for each feeding scheme. Water temperature ranged from 8° C to 12° C throughout the study period; pH ranged from 7.0 to 7.6. Dissolved oxygen level ranged from 6.8 to 7.1 mg/l. Sample fish were collected after each week and data on length were taken instantly, while data on weight and biochemical parameters were taken in the laboratory. For this purpose fish were taken in vials in ice box to the laboratory.

4.1 Growth Performance

4.1.1 Average Length

There was no significant (P>0.05) difference in the average length among the fish fed with different experimental diets at the beginning of the study (2.57±0.09, 2.63±0.09 and 2.60±0.06 cm). The final average length was significantly higher in all the fish. Fish fed with diet C grew to 4.20±0.06 cm, that fed with diet D1 grew to 4.13±0.03 cm and those fed with diet D2 grew to 4.30±0.06 cm after the experimental period of five weeks. Table 6 shows the average length of trout larvae during the study period. Figure 2 shows the average length gain pattern of experimental fish fed with different diets. The average length gain in trout larvae after 1st week (S1)

Diet	C	D1	D2
Sample	Mean ±SE	Mean ±SE	Mean ±SE
St	2.57±0.09	2.63±0.09	2.60±0.06
S1	2.80±0.06	2.73±0.03	3.10±0.06
S2	3.17±0.03	3.13±0.03	3.33±0.03
S3	3.27±0.03	3.37±0.03	3.60±0.06
S4	3.80±0.06	3.77±0.07	3.90±0.06
S5	4.20±0.06	4.13±0.03	4.30±0.06

Table 6 Average length of trout larvae

was 0.23, 0.10 and 0.50 respectively for fish fed with diets C, D1 and D2 while it was 0.37, 0.40 and 0.23 respectively after 2nd week (S2). Similarly, average length gain was 0.10, 0.23 and 0.27 after 3rd week (S3), and 0.53, 0.40 and 0.30 after 4th week (S4) for

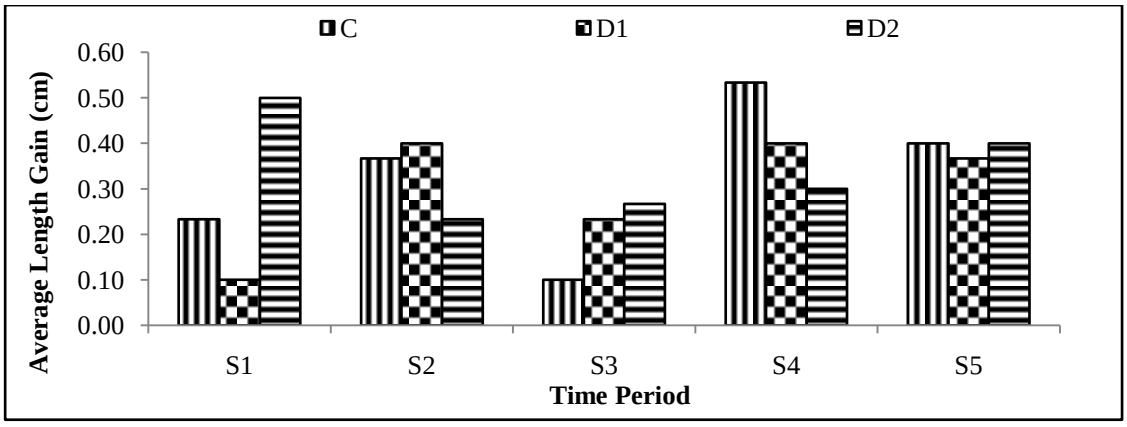

Figure 2 Bar diagram showing average length gain of trout larvae

fish fed with diets C, D1 and D2 respectively. During final sampling i.e. after 5th week (S5) the average length gain was 0.40, 0.37 and 0.40 respectively for fish fed with diets C, D1 and D2.

4.1.2 Average Weight

There was no significant (P>0.05) difference in the average weight among the fish fed with different experimental diets (C, D1 and D2) at the beginning of the study (0.1824±0.0056, 0.1850±0.0071 and 0.1863±0.0058 g). From the beginning of the experiment to fifth week of feeding trial a direct relationship had been found between the protein contents of diets and final average body weight of trout. The final average weight was significantly higher in all the fish fed with diets C, D1 and D2 (0.6030±0.0020, 0.6094±0.0010 and 0.7027±0.0022 g) of fifth week of feeding trial. The average weight of trout larvae from the beginning of experiment up to the final weeks are shown in the table 7. The final average weight was 1.06% higher in the fish fed diet D1 while it was 16.53% higher in larvae fed with diet D2

Diet	C	D1	D2
Sample	Mean ±SE	Mean ±SE	Mean ±SE
St	0.1824±0.0056	0.1850±0.0071	0.1863±0.0058
S1	0.2280±0.0021	0.2309±0.0008	0.2399±0.0007
S2	0.2878±0.0018	0.2975±0.0024	0.3120±0.0013
S3	0.3792±0.0006	0.3875±0.0019	0.4191±0.0022
S4	0.4801±0.0005	0.4873±0.0046	0.5420±0.0058
S5	0.6030±0.0020	0.6094±0.0009	0.7027±0.0022

Table 7 Average Weight of trout larvae

as compared to the fish fed with diet C. Finally it was 15.31% body weight of larvae increased in larvae fed with diet D2 as compared to larvae fed with diet D1.

Average weight gain was significantly higher in fish fed with diet D2 as compared to other two groups. Average weight gain of fish fed with diet C was 0.0456, 0.0598, 0.0913, 0.1009 and 0.1229 respectively in samples S1, S2, S3, S4 and S5. Similarly, the

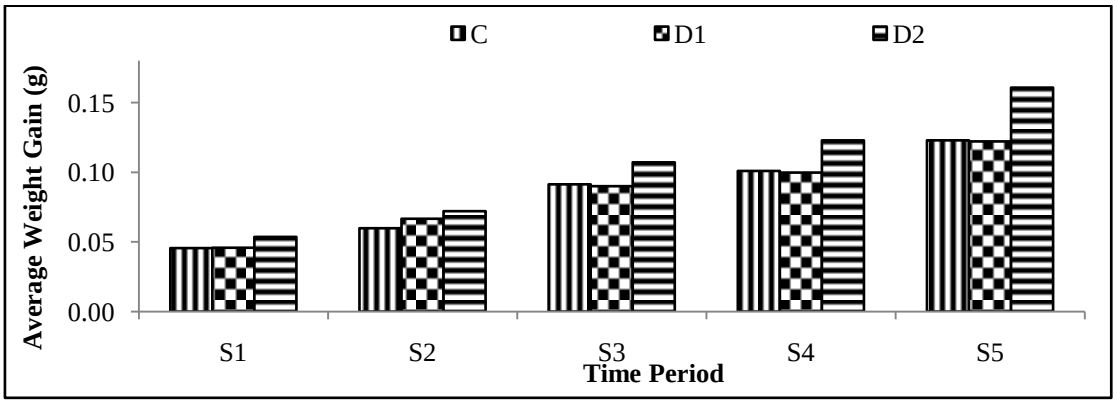

Figure 3 Bar diagram showing average weight gain of trout larvae

average weight gain of fish fed with diet D1 was 0.0458, 0.0666, 0.0900, 0.0998 and 0.1221 in S1, S2, S3, S4 and S5 respectively. While it was recorded as 0.0536, 0.0721, 0.1071, 0.1229 and 0.1607 respectively in S1, S2, S3, S4 and S5 in fish fed with diet D2.

4.1.3 Specific Growth Rate (SGR)

Similar result for SGR was found as with average weight gain in trout larvae. The SGR after 1st week (S1) was 3.18, 3.16 and 3.62 for fish fed with diets C, D1 and D2 respectively. It was 3.33, 3.62 and 3.75 after 2nd week (S2) and 3.94, 3.78 and 4.22 after

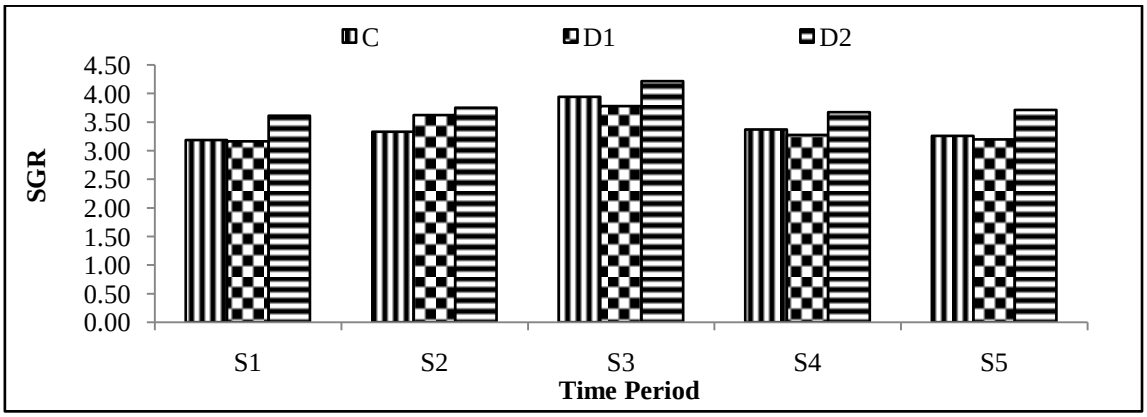

Figure 4 Bar diagram showing SGR of trout larvae

3rd week (S3) respectively for fish fed with diets C, D1 and D2. After 4th week the SGR was found to be 3.37, 3.27 and 3.67 while it was 3.26, 3.20 and 3.71 after 5th week (S5) for fish fed with diets C, D1 and D2 respectively. Relatively a higher SGR was recorded for the fish fed with diet D2 compared to other two groups.

4.1.4 Feed Conversion Ratio (FCR)

An inverse relation between the protein content of diet and feed conversion ratio was observed during study period in the trout larvae. FCR of fish fed with diet D2 was

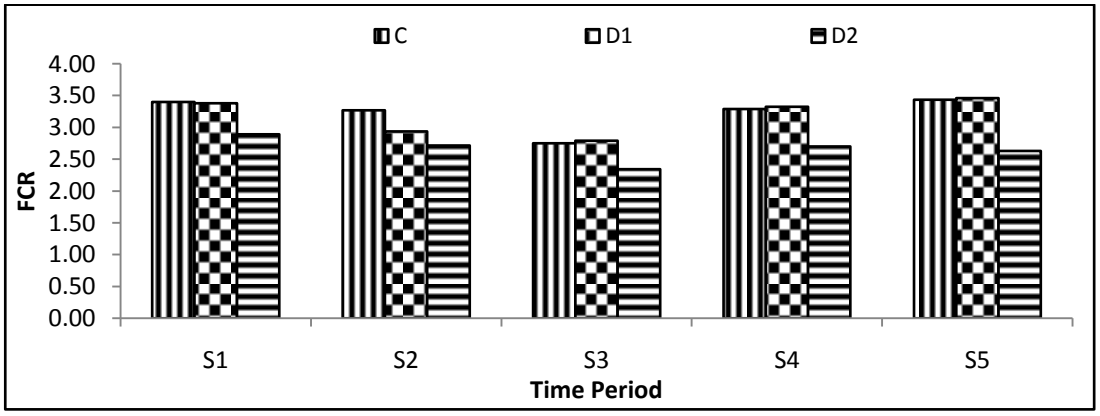

Figure 5 Bar diagram showing FCR of trout larvae

found to be significantly lower (P>0.05) as compared with other two groups of trout larvae. FCR of trout larvae fed with diet C was 3.40, 3.27, 2.75, 3.29 and 3.44 respectively in sample S1, S2, S3, S4 and S5. Similarly it was 3.38, 2.93, 2.79, 3.33 and 3.46 respectively in sample S1, S2, S3, S4 and S5 for fish fed with diet D1. While for fish feed with diet D2 it was 2.89, 2.72, 2.35, 2.70 and 2.63 after 1st week (S1), after 2nd week (S2), after 3rd week (S3), after 4th week (S4) and after 5th week (S5).

4.2 Biochemical Analysis

4.2.1 Total Protein

A significant (P>0.05) difference among the average total protein concentration in trout larvae was found after 1st week (S1) of feeding (124.50±0.43, 125.16±0.31 and 130.20±0.28 µg/mg). The similar trend proceeded on following weeks and finally the average total protein content was significantly (P>0.05) higher in fish fed with different diets (144.52±0.59, 145.76±0.51 and 167.23±0.55 µg/mg) compared to the initial protein content which was 120.86±0.48 µg/mg during stocking (St). The final average total

protein was 0.86% higher in the fish fed with diet D1 and 15.72% higher in larvae fed with diet D2 as compared to the fish fed with diet C. Finally it was 14.73% increase in average total protein content in larvae fed with diet D2 as compared to larvae fed with

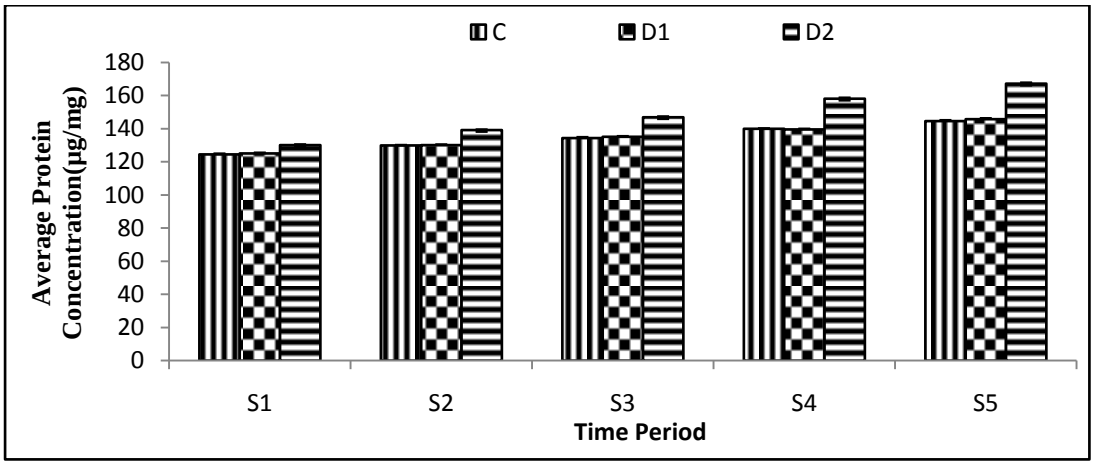

Figure 6 Bar diagram showing average protein content of trout larvae

diet D1. As a whole an increase of 19.57%, 20.60% and 38.37% in average total protein in fish larvae fed with diets C, D1 and D2 respectively was found after 5th week (S5) as compared to average protein content during stocking (St). Average total protein content of trout larvae after 2nd week (S2) was 129.91±0.20, 130.04±0.38 and 139.29±0.19 for fish fed with diets C, D1 and D2 respectively. Similarly, it was 134.38±0.50, 135.04±0.43 and 147.00±0.35 after 3rd week (S3) and 139.96±0.20, 139.54±0.37 and 158.17±0.52 after 4th week (S4) for trout larvae fed with diets C, D1 and D2 respectively.

4.2.2 Albumin Content

Similar trend of average albumin content was recorded for trout larvae during study period as seen in case of average total protein content. Average albumin content of trout larvae during stocking was 81.06±0.41 µg/mg. The average total albumin concentration was 85.79±0.26, 86.33±0.07 and 89.73±0.27 for trout larvae fed with diets C, D1 and D2 respectively after 1st week. After 2nd week (S2) average albumin content was 88.31±0.36, 89.11±0.31 and 94.60±0.36 and 95.45±0.72, 95.98±0.51 and 98.94±0.24 after 3rd week (S3) respectively for trout larvae fed with diets C, D1 and D2. Similarly, after 4th week (S4) average albumin content was 98.84±0.41, 99.01±0.20 and 101.99±0.01 respectively for trout larvae fed with diets C, D1 and D2. After 5th week (S5) the average total albumin content was found 102.95±0.53, 103.78±0.39 and 107.49±0.08

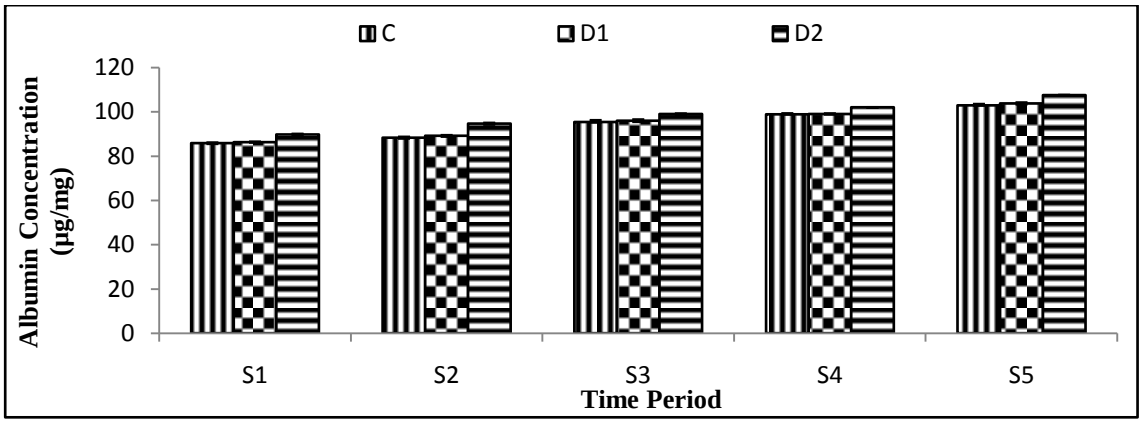

Figure 7 Bar diagram showing average albumin content of trout larvae

in fish fed with diets C, D1 and D2 respectively. An increase of 27.00%, 28.02% and 32.60% was found after 5th week compared to stocking in fish fed with diets C, D1 and D2 respectively. Protein concentration was found 4.41% higher in fish fed with 50% protein diet compared to fish fed with control diet after 5th week.

4.2.3 Globulin Content

A direct relation between the diet and globulin content could be established for fish fed with different diets but there was no significant difference (P>0.05) in average

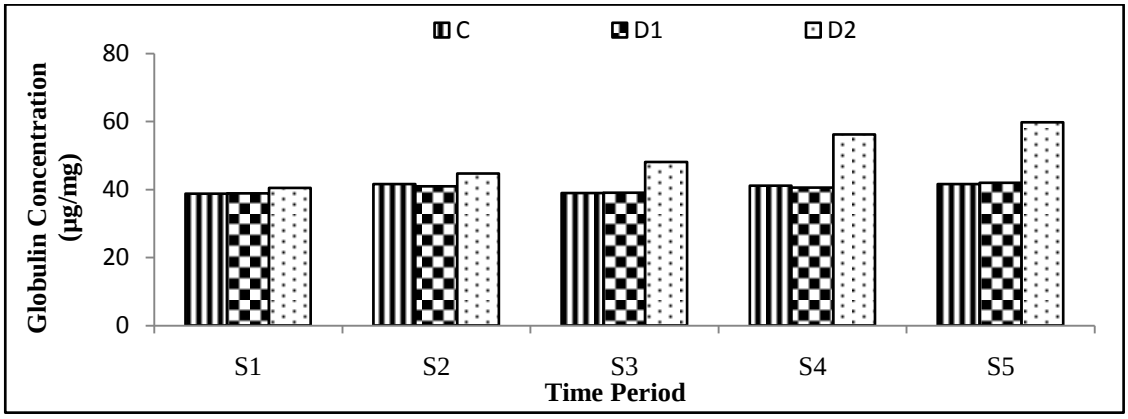

Figure 8 Bar diagram showing average globulin content of trout larvae

globulin content. Average globulin content during stocking was 39.80±0.37 µg/mg. A gradual increase in globulin concentration was found among all groups of fish but there was no significant difference. In trout larvae fed with diet C average albumin content was 38.71±0.27, 41.61±0.36, 38.93±1.21, 41.11±0.51 and 41.57±1.07 after 1st week (S1), after 2nd week (S2), after 3rd week (S3), after 4th week (S4) and after 5th week (S5)

respectively. Similarly, it was 38.83±0.30, 40.94±0.34, 39.06±0.76, 40.54±0.48 and 41.99±0.23 for fish fed with diet D1 in S1, S2, S3, S4 and S5 respectively. While it was 40.47±0.38, 44.69±0.29, 48.07±0.12, 56.17±0.53 and 59.75±0.51 respectively in S1, S2, S3, S4 and S5 for trout larvae fed with diet D2.

4.2.4 Albumin: Globulin Ratio (A: G Ratio)

Table 8 shows the average A: G ratio of trout larvae fed with different diets during the study period. During stocking the A: G ratio was 2.04±0.02. The A: G ratio of trout larvae fed with different diet has no significant (P > 0.05) difference after 1st week (S1). The average A: G ratio was 2.22±0.02, 2.22±0.02 and 2.22±0.03 after 1st week. No similar trend was found in the change of A: G ratio of fish fed with different diets during the study period. Highest A: G ratio (2.48) was found for trout larvae fed with diet C after 5th week (S5) while the lowest value was found 1.80 for trout larvae fed with diet D2 after 5th week (S5).

Diet	C	D1	D2
Sample	Mean±SE	Mean±SE	Mean±SE
S1	2.22±0.02	2.22±0.02	2.22±0.03
S2	2.12±0.03	2.18±0.02	2.12±0.02
S3	2.46±0.09	2.46±0.06	2.44±0.03
S4	2.41±0.04	2.44±0.03	1.82±0.02
S5	2.48±0.08	2.47±0.01	1.80±0.01

Table 8 Average A: G ratio of trout larvae

4.2.5 RNA Content

There was significant (P > 0.05) difference in the RNA concentration (µg/mg) of trout larvae among the trout larvae fed with different diets. During stocking (St) average

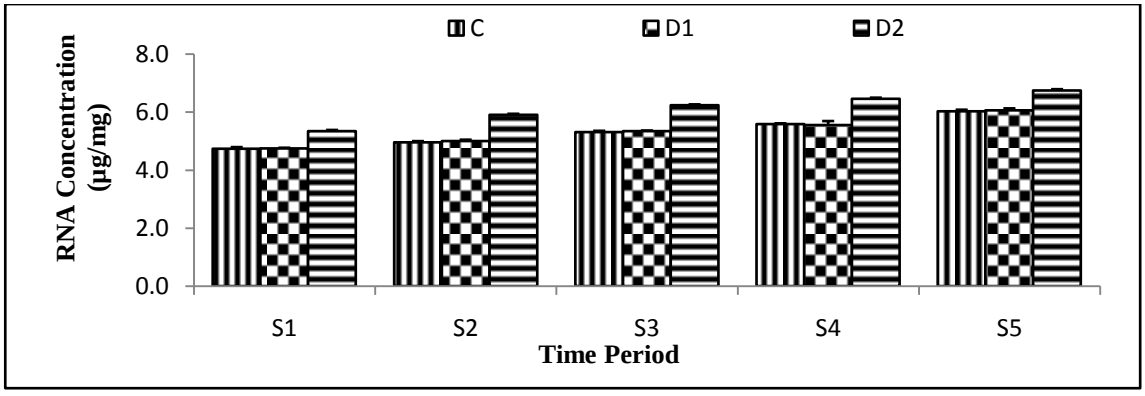

Figure 9 Bar diagram showing average RNA content of trout larvae

RNA content of trout larvae was 4.40±0.02 µg/mg. After 1st week the RNA content was

found to be 0.470±0.008, 0.473±0.008 and 0.511±0.029 for trout larvae fed with diets C, D1 and D2 respectively. RNA content showed a gradual significant (P>0.05) increase during the study period. After 2nd week (S2) average RNA content was 4.923±0.058, 4.993±0.050 and 5.900±0.036 in trout larvae fed with diets C, D1 and D2 respectively. It was 5.307±0.042, 5.340±0.020 and 6.233±0.027 after 3rd week (S3) and 5.580±0.027, 5.547±0.139 and 6.453±0.038 after 4th week in fish fed with diets C, D1 and D2 respectively. Finally, after 5th week (S5) average globulin content was 6.027±0.050, 6.053±0.069 and 6.743±0.039 respectively for trout larvae fed with diets C, D1 and D2.

4.2.6 DNA Content

There was no significant (P>0.05) difference in average DNA content among trout larvae fed with different diets from 1st week to 5th week. During stocking the average DNA content of trout larvae was 2.919±0.011 µg/mg while the average DNA content of trout larvae after 1st week (S1) was 2.932±0.007, 2.924±0.007 and 2.927±0.007 for fish fed with diets C, D1 and D2 respectively.

A gradual increase in DNA content was found among all groups of trout larvae but no significant (P>0.05) difference was found among trout larvae fed with different

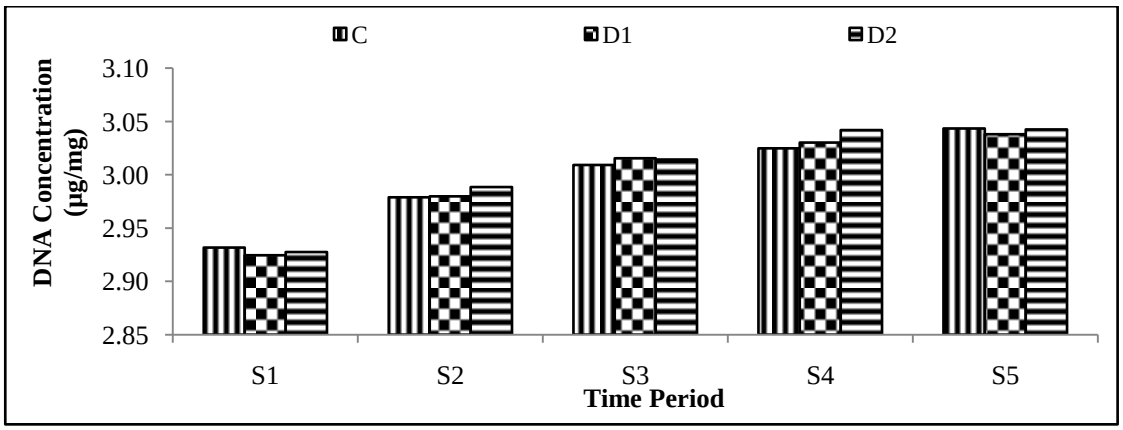

Figure 10 Bar diagram showing DNA content of trout larvae

diets. After 2nd (S2) the average DNA content was 2.979±0.005, 2.980±0.007 and 2.989±0.008 while it was 3.009±0.004, 3.015±0.003 and 3.014±0.002 after 3rd week (S3) respectively for trout larvae fed with diets C, D1 and D2 respectively. Similarly, after 4th week (S4) average DNA content was 3.025±0.006, 3.030±0.010 and 3.042±0.003 respectively. Final average DNA content of trout larvae after 5th week (S5) were 3.043±0.003, 3.038±0.003 and 3.042±0.008 for larvae fed with diets C, D1 and D2 respectively.

4.2.7 RNA: DNA Ratio

During stocking the average RNA: DNA ratio was found to be 1.509±0.005 which increased significantly (P>0.05) after 1st week (S1). After 1st week (S1) it was 1.616±0.014, 1.621±0.011 and 1.824±0.018 respectively for trout larvae fed with diets C,D1 and D2 respectively. A gradual and significant increase in RNA: DNA ratio was found during the study period for fish fed with different diets. Significant (P>0.05) difference in RNA: DNA ratio was found among three groups of fish was found after 5th Week (S5) compared to 1st week (S1). It was recorded as 1.980±0.016, 1.993±0.025 and 2.216±0.015 in trout larvae fed with diets C, D1 and D2 respectively after 5th week.

Diet	C	D1	D2
Sample	Mean±SE	Mean±SE	Mean±SE
St	1.509±0.005		
S1	1.616±0.014	1.621±0.011	1.824±0.018
S2	1.664±0.015	1.676±0.019	1.974±0.008
S3	1.764±0.014	1.771±0.008	2.068±0.007
S4	1.845±0.005	1.830±0.044	2.121±0.013
S5	1.980±0.016	1.993±0.025	2.216±0.015

Table 9 RNA: DNA ratio of trout larvae

Chapter-5

DISCUSSION

CHAPTER 5

DISCUSSION

The water quality parameters examined in present study showed mostly all parameters falls within the desirable limit for Rainbow Trout cultivation. This study only provides limited data on water quality parameters.

5.1 Growth of Rainbow Trout larvae

Trout including catfish require a source of nonspecific nitrogen and indispensable amino acids (Robinson & Li 1996). Rainbow Trout require rather high (40-50%) dietary protein (Bista *et al.,* 2008). Protein requirement of trout has been established to be between 42 and 48% from practical ingredients (Hardy, 2002). Dietary protein is always considered to be of primary importance in fish feeding (Jauncey, 1982), thus sufficient supply of dietary protein is needed for rapid growth (Lovell, 1989). Increases in dietary protein level have often been associated with higher growth rates in many species (Page and Andrews, 1973; Ergun *et al.,*2010). Furthermore, Wilson (1989), Pillay (1990) and El-Sayed and Teshima (1991) found that dietary protein requirements decreased with increasing fish size and age. The nutritional condition of fish larvae has been characterized morphometrically (Blaxter, 1971; Ehrlich *et al.,* 1976), histologically (Strussmann and Takashima, 1990), by measuring proteolytic enzymes (Ueberschar, 1988) or by the relative amounts of RNA and DNA (Mathers *et al.,* 1994; Bailey *et al.,* 1995; Rooker and Holt, 1996; Canino, 1997; Ch´ıcharo, 1997, 1998; Ch´ıcharo *et al.,* 1998a,b).

In present study growth has been characterized morphometrically as well by the relative amounts of RNA and DNA. The present study showed that specified protein supplemented artificial diets improved the growth performance of trout larvae. As the protein percentage in the experimental diets increased, the average weight gain (AWG) and specific growth rate (SGR) increased. A better growth was observed in the larvae fed with diet D2 (50% protein) compared to diet C (control) and diet D1 (40% protein). This is in agreement with the study of Pradhan *et al.* (2008) in which they have shown that trout larvae fed with 50% protein in feed grow better than other groups, though they have concluded that a low protein containing feed can be used during the unavailability of high protein feed.

Feed conversion rates (FCR) ranged from 2.35 to 3.56, varying inversely with observed growth rate and the dietary protein level in the study. While the SGR has been found to range from 3.16 to 4.22 and was directly related to growth rate and dietary protein level. These results were in agreement with the study of Shyong *et al.*,(1998) carried on *Zacco barbata* and concluded that the indicators of growth performance such as SGR and FCR of juvenile improved when dietary protein concentration increased.

5.2 Protein profile of Rainbow Trout larvae

Total protein and albumin levels are accepted biochemically as indicators of nutritional status of an organism (Wegwu and Omeodu, 2010). Rao and Chakrabarti (2005) has shown that increase in serum globulin level is associated with increased immune level of *Cyprinus carpio* and were directly proportional to RNA: DNA ratio. In present study a direct relationship was found between the total protein level and the dietary protein in the diets of trout larvae (highest in final week fed with 50% protein diet). Like total protein, albumin concentration also showed increasing trend as the level of dietary protein increased in the diet of fish (highest in final week fed with 50% protein diet). Direct relationship was also found between the globulin level of fish and increasing level of dietary protein. This is in agreement with Shyong *et al.*, (1998) in which it has been concluded that a significant increase in muscle protein content is seen with increasing dietary protein level in *Zacco barbata*. Similar results of dietary protein on carcass composition have been observed in other studies with common carp (Zeitter *et al.*, 1984), plaice (Cowey *et al.*, 1972), and snakehead fry (Mohanty and Samantaray, 1996).

5.3 RNA: DNA of Rainbow Trout larvae

The RNA: DNA ratio is considered as a sensitive measure for the growth rate of fish (Buckley, 1979; Love, 1980). This concept is based on the fact that the quantity of RNA varies directly with the activity of the protein synthesis in tissues undergoing a faster growth while the amount of DNA per cell remains constant within the species (Buckley, 1984). Thus the ratio of RNA to DNA, which is indicative of the amount of RNA per cell, is usually a more accurate index of protein synthetic activity than the RNA concentration alone because the ratio is not affected by the differences in the cell numbers. It has proven a useful indicator of nutritional condition as has been shown in several larval fish studies (Bulow *et al.*, 1981; Martin *et al.*, 1985). Ling *et al.*, (1995) stated that RNA: DNA ratio could be used as an indicator for evaluating the fish growth

at fingerling stage. There are some indications that size may affect the relation between the RNA: DNA ratio and the temperature and the growth rate in older fish (Buckley 1982). In present study larvae of Rainbow Trout has been used to study the effect of dietary protein on its RNA: DNA ratio.

RNA: DNA ratio is an index of the amount of protein synthetic machinery per cell (Buckley, 1984). Theilacker & Shen (1993) argued that measurement of the RNA: DNA ratio of the cells of individual tissues may provide a more accurate index of physiological condition than whole fish homogenate, since the growth rate of different organs may vary, and therefore, interpretation of the RNA/DNA ratio in whole fish homogenate as an index of physiological condition may be difficult. However, Chung and Segnini (1998) have chosen whole fish homogenate to analyze nucleic acid concentration to compare with other physiological parameters, such as specific growth rate per day, SGR, condition factor, K, and total biomass increment and the similar trend is followed in present study. In present study significant increase in RNA: DNA was recorded from the whole fish homogenate. It has been found in present study that there is a significant increase in RNA alongwith dietary protein which is supported by the findings of Brachet (1955), Leslie (1955) and Mustafa and Jafri (1977). Bouche *et al.,* (1970) too have reported the loss of protein and RNA from the liver of Common Carp, *Cyprinus carpio* subjected to starvation i.e. low protein in diet.

Different methodologies can lead to different results making direct comparisons of data impossible. Several authors have compared methodologies for extraction and quantification of nucleic acids (McGurk *et al.,* 1992; Gre´mare and Ve´tion, 1994; Canino and Caldarone, 1995) and obtained significant differences. Ch´ıcharo (1996) compared the condition of *Sardina pilchardus* larvae using the spectrophotometric method of Schmidt and Tannhauser (1945) and the fluorimetric procedure of Clemmesen (1988, 1993), and found no significant differences in both RNA and DNA contents and RNA: DNA ratios. In present study, spectrophotmetric method has been used and a significant increase in RNA: DNA ratio has been recorded alongwith dietary protein.

Correlations between RNA concentration or RNA: DNA ratio and growth rate have been observed for a wide variety of organisms (Kennell and Magasanik, 1962; Bulow, 1970; Sutcliffe, 1970). The direct positive relationship between the RNA-DNA ratio and growth rate has also been observed for adult golden shiners (Bulow, 1970) in

small and mouthbass and carp (Haines, 1973) and in the muscle of catfish (Khan and Jafri 1991). In the present study the data indicated that trout larvae fed with high dietary protein (50%) showed higher growth rate and higher RNA-DNA ratio than trout larvae fed with a low protein diet (40%). Strong correlation between the RNA: DNA ratio and growth rate have been recorded in present study. The increase in RNA concentration appears to be the result of a more efficient utilization of dietary protein intake leading subsequently to an increased protein synthesis and thus growth.

The changes in RNA concentration and protein concentration maintained same trend which can be elaborated with the help of earlier findings of Mustafa and Jafri (1977) and Mustafa (1979), emphasizing the role of RNA as the organizer of protein synthesis.

Chapter-6

CONCLUSION AND RECOMMENDATIONS

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6.1 Conclusion

Aquaculture is one of the fastest growing food producing sectors of the world. It plays an important role in the economic development of the country in view of its potential contribution to national income and large export earnings. The knowledge of nutrition and practical feeding of fish are essential for sustainable aquaculture and larviculture is the backbone of aquaculture. The primary problem of larviculture is food and its digestion, especially in the transition period. As protein utilization is fundamental to growth, characterization and identification of protein is essential along with quantitative estimations for the better understanding of health and growth status of the cultured species. The present investigation aims to study the effects of varied proportion of protein contents in food on the growth performance, RNA and DNA ratio and protein profile of the fish larvae. Fish were cultured in three feeding groups with three varied diets (control diet, 40% & 50% protein diet).

It has been observed that the fish larvae fed with 50 % protein diet showed better growth performance as compared to fish larvae fed with 40 % protein diet and control. Also, the FCR of fish larvae fed with 50% protein diet were found to be lower compared to other diets.

Similarly, the protein profile and biochemical indices of fish fed with 50% protein diet were found to be better as compared to fish fed with other two diets. In the present study strong correlation between the RNA: DNA ratio and growth rate have been recorded. So, the view of using RNA: DNA ratio as an indicator for evaluating the nutritional status of fish larvae is furthermore strengthened. The increase in RNA concentration appears to be the result of a more efficient utilization of dietary protein intake leading subsequently to an increased protein synthesis and thus growth.

This may be concluded that increase in dietary protein concentration up to 50% protein improves the growth performance, health status and nutritional value of cultured species of Rainbow Trout. Finally, the utilization of knowledge generated from the present study may help to formulate quality artificial diets for Rainbow Trout.

6.2 Recommendations

From the outcome of the present study, following recommendations are made to the concerned authorities and further researches:-

1) From present study it has been concluded that feed of Rainbow Trout larvae containing 50% protein is better and more efficient than the traditional feed. Thus, it is recommended to use this feed.
2) Further studies on developing appropriate diets for Rainbow Trout at different stages should be done with appropriate cost analysis.
3) The main protein source of Trout feed in Nepal is ‘dried prawn’ which is far expensive and is imported from other country. So, study on the probability of using local materials to make Trout feed should be done which will reduce the production cost.

Chapter-7

REFERENCES

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REFERENCES

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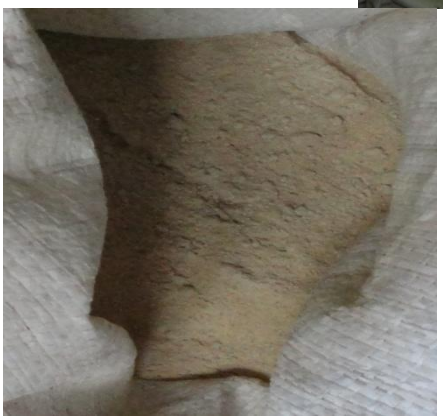
ANNEX I: Photographs of Feed and its Ingredients

Dried Fish

Wheat Flour

Vitamin



Mustard Oil Cake

Rice Bran

Control Diet (C)

40% Protein Diet (D1)

50% Protein Diet (D2)

ANNEX II: Photographs of Field Work

Raceway dried before stocking

Stocking of Trout Larvae

Larvae in Cage

Researcher getting information from farmer

Researcher collecting water sample for analysis

Length measurement of Trout Larvae

ANNEX III : Photographs of Instruments used and Lab work

Researcher testing Water Quality

pH meter

Digital Photo Colorimeter

Researcher using Colorimeter

Researcher preparing solution

Variable Volume Automatic Pipette

ANNEX IV

Record of Water Quality Parameters during Study Period

Date	Temperature (°C)	pH	Dissolved Oxygen
19-Feb 2011	8	7.4	7.1
20-Feb	8	7.4	
21-Feb	8.5	7.4	
22-Feb	9	7.5	
23-Feb	8	7.2	
24-Feb	8.5	7.4	
25-Feb	8	7.1	
26-Feb	9	7.1	7.1
27-Feb	8	7.4	
28-Feb	8.5	7.3	
1-Mar	8.5	7.5	
2-Mar	8.5	7.3	
3-Mar	9	7.4	
4-Mar	8.5	7.5	
5-Mar	9	7.6	6.9
6-Mar	9	7.3	
7-Mar	9.5	7.6	
8-Mar	10	7.3	
9-Mar	9.5	7	
10-Mar	9.5	7.3	
11-Mar	10	7.5	
12-Mar	10	7.2	7.0
13-Mar	9	7.5	
14-Mar	10	7.1	
15-Mar	10.5	7.6	
16-Mar	10.5	7.3	
17-Mar	11	7.2	
18-Mar	11	7.5	
19-Mar	10.5	7.3	
20-Mar	11	7.4	6.9
21-Mar	12	7.2	
22-Mar	11.5	7.4	
23-Mar	11.5	7.5	
24-Mar	11	7.4	
25-Mar	11	7.3	
26-Mar	12	7.3	
27-Mar	11.5	7.4	6.8

ANNEX V

Individual Length (cm) of fish larvae during different samplings

Diets	Date	19th Feb	26 th Feb	5th Mar	12th Mar	20th Mar	27th Mar
	Cage	**St**	**S1**	**S2**	**S3**	**S4**	**S5**
Control (C)	R1	2.60	2.70	3.00	3.40	3.70	4.00
	R2	2.70	2.70	3.10	3.40	3.70	4.10
	R3	2.40	2.90	3.20	3.60	3.90	4.30
40% Protein (D1)	R1	2.50	2.80	3.20	3.40	3.90	4.30
	R2	2.40	2.60	3.10	3.50	3.70	4.20
	R3	2.60	2.90	3.20	3.60	3.90	4.10
50% Protein (D2)	R1	2.60	2.80	3.30	3.70	4.00	4.40
	R2	2.50	2.90	3.30	3.60	3.90	4.20
	R3	2.70	2.70	3.10	3.50	3.80	4.30

ANNEX VII

Individual Weight (g) of fish larvae during different samplings

Diets	Date	19th Feb	26 th Feb	5th Mar	12th Mar	20th Mar	27th Mar
	Cage	**St**	**S1**	**S2**	**S3**	**S4**	**S5**
Control (C)	R1	0.1822	0.2258	0.2862	0.3781	0.4795	0.6068
	R2	0.1728	0.2260	0.2858	0.3802	0.4798	0.6024
	R3	0.1923	0.2322	0.2915	0.3792	0.4810	0.5998
40% Protein (D1)	R1	0.1988	0.2318	0.2952	0.3851	0.4812	0.6108
	R2	0.1811	0.2316	0.2950	0.3912	0.4843	0.6076
	R3	0.1752	0.2292	0.3023	0.3862	0.4963	0.6098
50% Protein (D2)	R1	0.1900	0.2398	0.3143	0.4152	0.5459	0.7067
	R2	0.1750	0.2388	0.3120	0.4192	0.5494	0.7023
	R3	0.1940	0.2412	0.3097	0.4229	0.5306	0.6991

ANNEX VII

Individual Protein Content (µg/mg) of fish larvae during different samplings

Diets	Date	26 th Feb	5th Mar	12th Mar	20th Mar	27th Mar
	Cage	**S1**	**S2**	**S3**	**S4**	**S5**
Control (C)	R1	124.560	129.630	133.640	139.640	145.650
	R2	125.210	128.810	134.340	141.890	143.650
	R3	123.730	130.300	133.150	140.340	144.250
40% Protein (D1)	R1	125.750	128.320	135.600	140.450	145.320
	R2	124.710	129.630	134.890	141.230	146.790
	R3	125.020	129.180	135.630	141.300	145.180
50% Protein (D2)	R1	129.650	135.480	141.360	145.320	151.230
	R2	130.540	135.470	139.300	144.200	150.180
	R3	130.420	134.920	141.350	144.980	150.290

ANNEX VIII

Individual Albumin Content (µg/mg) of fish larvae during different samplings

Diets	Date	26 th Feb	5th Mar	12th Mar	20th Mar	27th Mar
	Cage	**S1**	**S2**	**S3**	**S4**	**S5**
Control (C)	R1	85.500	88.650	96.210	98.560	102.310
	R2	86.320	87.590	94.010	99.650	103.990
	R3	85.560	88.680	96.120	98.320	102.540
40% Protein (D1)	R1	86.320	88.980	95.320	98.650	103.180
	R2	86.210	89.690	95.640	99.020	104.500
	R3	86.450	88.650	96.990	99.350	103.650
50% Protein (D2)	R1	89.650	94.320	99.120	101.980	107.630
	R2	89.320	95.320	98.460	102.020	107.490
	R3	90.230	94.160	99.230	101.980	107.340

ANNEX IX

Individual RNA Content (µg/mg) of fish larvae during different samplings

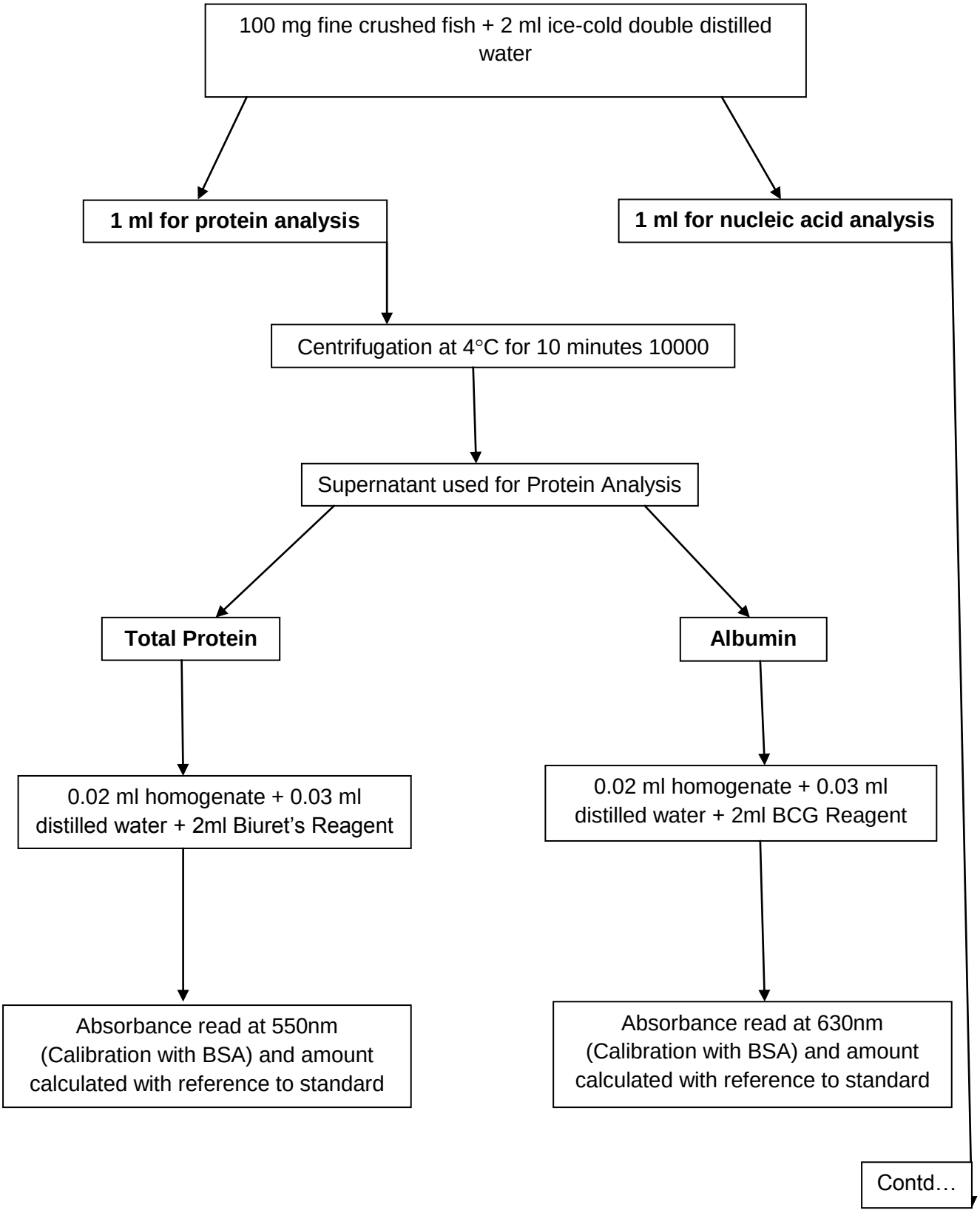
Diets	Date	26 th Feb	5th Mar	12th Mar	20th Mar	27th Mar
	Cage	S1	S2	S3	S4	S5
Control (C)	R1	4.634	4.830	5.250	5.530	5.950
	R2	4.779	5.030	5.390	5.620	6.010
	R3	4.798	4.910	5.280	5.590	6.120
40% Protein (D1)	R1	4.786	4.920	5.320	5.750	6.090
	R2	4.739	4.970	5.320	5.610	5.920
	R3	4.692	5.090	5.380	5.280	6.150
50% Protein (D2)	R1	5.396	5.970	6.180	6.520	6.720
	R2	5.260	5.850	6.270	6.450	6.820
	R3	5.359	5.880	6.250	6.390	6.690

ANNEX X

Individual DNA Content (µg/mg) of fish larvae during different samplings

Diets	Date	26 th Feb	5th Mar	12th Mar	20th Mar	27th Mar
	Cage	S1	S2	S3	S4	S5
Control	R1	2.920	2.981	3.002	3.013	3.047
	R2	2.932	2.969	3.009	3.033	3.038
	R3	2.944	2.987	3.017	3.028	3.046
40% Protein	R1	2.914	2.993	3.018	3.024	3.038
	R2	2.936	2.971	3.019	3.049	3.044
	R3	2.923	2.975	3.010	3.018	3.032
50% Protein	R1	2.915	2.999	3.010	3.039	3.029
	R2	2.937	2.973	3.018	3.047	3.042
	R3	2.931	2.994	3.015	3.040	3.057

ANNEX- XI: BIOCHEMICAL ESTIMATION FLOW CHART



Flow Chart Continued…….