GROWTH RESPONSE OF RAINBOW TROUT (*Oncorhynchus mykiss*) ON SUBSTITUTION OF SHRIMP MEAL BY DIFFERENT PROTIEN SOURCES

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T.U Res. No. 5-1-20-24-2003

Symbol No. 13087

Batch: 2066/2067

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Zoology with special paper Fish and Fisheries

Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

September, 2013

RECOMMENDATIONS

This is to recommend that the thesis entitled "**GROWTH RESPONSE OF RAINBOW TROUT (*Oncorhynchus mykiss*) ON SUBSTITUTION OF SHRIMP MEAL BY DIFFERENT PROTIEN SOURCES** has been carried out by Suman Dheke for the partial fulfillment of Master's Degree of Science in Zoology with special paper Fish and Fisheries. This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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LETTER OF APPROVAL

On the recommendation of supervisor "Prof. Dr. Surya Ratna Gubhaju" this thesis submitted by Suman Dheke entitled "**GROWTH RESPONSE OF RAINBOW TROUT (*Oncorhynchus mykiss*) ON SUBSTITUTION OF SHRIMP MEAL BY DIFFERENT PROTIEN SOURCES**" is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Fish and Fisheries.

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This thesis work submitted by Suman Dheke entitled "**GROWTH RESPONSE OF RAINBOW TROUT (*Oncorhynchus mykiss*) ON SUBSTITUTION OF SHRIMP MEAL BY DIFFERENT PROTIEN SOURCES**" has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Fish and Fisheries.

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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the authors and institutions.

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ACKNOWLEDGEMENTS

I extend my sincere gratitude to my respected supervisor Prof. Dr. Surya Ratna Gubhaju, Central Department of Zoology, Tribhuvan University, Kirtipur for his valuable guidance, timely suggestion, motivations, inspiring endurance, critical comments and dynamic supervision throughout this research work and thesis preparation. I am greatly privileged to articulate my special gratitude to Prof. Dr. Ranjana Gupta, Head, Central Department of Zoology, Tribhuvan University, Kirtipur for administrative support. I am grateful to Dr. Usha Lohani, Professor and Dr. Archana Prasad, Lecturer, Center Department of Zoology for their constructive suggestion and consistent encouragement throughout the preparation of thesis.

I owe a special gratitude to Fisheries Research Division, Godawari for giving me opportunity to work on this project. My sincere thanks goes to Mr. Nand Kishwor Roy, senior technical officer, Fisheries Research Division, for co-supervising the thesis. Mr. Santa Kumar Shrestha, technical officer, FRD receives sincere thanks for guiding field work. I am greatly indebted to Chief of Fisheries Research Division, Mr. Suresh Kumar Wagle and senior scientist Mrs. Neeta Pradhan for valuable guidance, comments and providing supporting materials.

I am grateful to Agro Top Industries, Co. Nepal (P). Satghumti, for providing experimental site. My heartily thanks goes to Mr. Sadhuram Basnet for providing appropriate environment and genuine help during feeding trial period in the field and to Mr. Ganga Ram Pradhan for providing supporting information about Agro Top Industries. I am extremely thankful to Mr. Mehar Singh Tamang and all the staffs of Agro Top Trout farm, Saatgumti for their constant co-operation.

I'll fail in my duty if I don't express special thanks to Mr. Jagat Shrestha, Research Assistant, Molecular Biotechnology Unit, NAST for assisting in data analysis and statistical interpretation. I am also indebted to library staffs of different institutions such as Tribhuvan University Central Library, Central Department of Zoology and FRD.

At last, but not the least, I am very grateful to my family members for their incessant support, encouragement and affection shown throughout the whole course of my study including present research work.

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Suman Dheke

ABSRTACT

Nepal is rich in cold water resource, commercial rearing of indigenous cold water fish species is not yet in practice. Rainbow trout (*Oncorhynchus mykiss)* had been introduced in Nepal to expedite the comparative advantage of available suitable climatic conditions. As its farming is expanding, requirement of nutritionally formulated balanced feed has appeared as major bottleneck for sustainable trout production. Till now, shrimp meal (SM) is one of the major ingredients for protein content in trout feed in Nepal. The present work is aimed to develop low cost trout feed replacing SM by alternate locally available ingredients. The four diets were prepared iso-nitrogenous with 44.44 ($\pm$0.5) % protein. The feeds were tested in eight weeks experiment trial to determine the efficacy of different feeds on growth and survival of trout fry. Trout fry were kept in twelve cages (0.5m) ^{3} in a raceway pond with three replications for each diet in random block design. Fry of average 0.31g were stocked in cages in a density of 2000 fry m^{-2} and fed to satiation 8 to 10 times a day. Feed containing SWP showed significantly higher weight gain ($p<0.5$) and the highest mortality rate (77%) was observed in synthetic amino acid. Mortality rates of fry fed with feed containing SM, SPM and SMM were found statistically insignificant ($p>0.05$). Lowest feed conversion ratio (FCR) was 1.33 recorded in feed containing SWP. However, FCR was calculated statistically insignificant ($p>0.05$) in all four diets. Study concluded silkworm meal could be a proper substitute in rainbow trout feed if it is locally available.

CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviated form	**Details of abbreviations**
AA	Amino acid
AGDP	Agriculture Gross Domestic Product
ANOVA	Analysis of Variance
CBS	Central Bureau of Statistics
CF	Condition Factor
DO	Dissolved Oxygen
DWG	Daily Weight Gain
EAAs	Essential Amino acids
FAO	Food and Agriculture Organization
FCE	Food Conversion Efficiency
FCR	Food Conversion Ratio
FRC	Fisheries Research Center
FRD	Fisheries Research Division
NARC	Nepal Agriculture Research Council
NCFR	Nonconventional Feed Resource
Ppm	Parts per million
PWG	Percent Weight Gain
RBD	Random Block Design
SAA	Synthetic Amino acids
SGR	Specific Growth Ratio
S/m	Seimen per meter
SM	Shrimp meal
SPM	Silkworm pupae meal
SMM	Silkworm moth meal
TDS	Total dissolved solid

1. INTRODUCTION

Nepal, a Himalayan kingdom situated at an altitude ranging from 60 m to 8848 m, has diverse agro-ecological regimes. Nepal is an agrarian society and agriculture shares 40% and about 88% of the population depends on agriculture for livelihood. Nepal is one of the richest countries in the world, possessing 2.27% of world water resources (CBS, 2005); approximately 5 % of total area of the country is occupied by different fresh water aquatic habitat (Bhandari, 1992). Inland water bodies of Nepal is the home ground for 217 indigenous fish species comprising about 2% of the global fish diversity. The contribution of fishery sector to national AGDP (Agriculture Gross Domestic Product) is far less, that is, 2%.

1.1 Fishery resources

Fish is an important source of animal protein in human diet. Capture fishery has old history in Nepal. Exploitation of inland water bodies is the only way for aquaculture development in Nepal. Warm water fishes like Indian major carp and Chinese carp are usually practiced in commercial production in Terai plain of Nepal. Cold water fisheries is also being targeted for further development in the rivers, lakes and reservoirs of mid hill region of Nepal as abundant non polluted cold water river/ streams in hills offer excellent habitat for cold water species (Rajbanshi, 2002). Out of fifty-nine coldwater indigenous species *Neolissocheilus hexagonolepis*, *Schizothoraichthys* spp, *Schizothorax* spp , 1838 and *Tor* spp, 1834 are the most economically important table and sport fishes of Nepal (Shrestha, 1985). Native cold water fisheries resource offer vast scope for development of cold water aquaculture. Some cold water species are successfully bred to culture i.e. *Labeo dero*, *Tor putitora*, *Tor tor*, Katle (*Neolissochilus hexagonolepsis)*, Asala (*Schizothorax* sps.) in experimental works in FRC (Fishery Research Center) under NARC (Nepal Agricultural Research Council). Due to slow growth rate of these species, commercial production has not yet been started.

1.2 Trout introduction in Nepal

Considering the suitable climatic condition and abundance of cold water, pilot scale testing of trout was ventured in Nepal by the first time introduction of juvenile of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) brought from India and United Kingdom in 1969 and 1971 respectively and was re-introduced in 1988 from Japan (Swar,2008). Rainbow trout was bred for the first time in Nepal in 1990 and its culture was initiated experimentally in 1993. The works lead to the development of a complete package of trout farming by 1997 suited to the condition of Nepal. Finally, rainbow trout is taken as an alternative to commercial production of cold water fishes in this country and technical package of trout farming was disseminated to fish farmers and entrepreneur.

1.3 Characteristic features of trout (*Oncorhynchus mykiss*)

Rainbow trout is an exotic cold water carnivorous fish requiring specific water quality parameters and high protein rich feed.

1.3.1 Optimum water quality parameters

Availability of adequate cold (below 20^{o}C) throughout the year is pre-requisite when selecting the sites. Feed consumption decreases when it rises above 20^{o}C (Yamazaki, 1991). Trout require high levels of dissolved oxygen for survival and growth, growth decreases when this level falls below 7 ppm (Gibon's, 1998). The pH of water can range from 6.5-8.0 but optimum range is 7.0 - 7.5. At higher pH levels relatively low levels of ammonia can be dangerously toxic (Bromage and Shepherd, 1990; Sedgewick, 1985). A minimum rate of 500 m^3 per day of water flow is necessary per ton of trout produced. Therefore the site should have a slope of 1-3 % which permits good water flow and helps maintain good water quality.

1.3.2 Nutrition, Feeds and Feeding

The commercial culture of trout began as early 1853 in the United States and possibly even earlier in Europe (Bardach *et al.*, 1972). In natural habitats trout depends on aquatic insects, small crustaceans and small larvae. However, stages of trout from larvae to adult can be grown feeding upon artificial food. Unlike pond cultured carps for which natural food stuff constitute major feed with supplementary feeding, trout farming is mostly characterized by intensive farming system using high-cost nutrient inputs in the form of "nutritionally-complete formulated diets". This feed should constitute proper amount of protein, lipids, energy, vitamins and minerals in their diet for growth, reproduction and other normal physiological functions. Hence nutritionally complete, manufactured feeds are commonly used in trout culture. Nutritionally complete feed are typically multi-ingredient pelleted feed produced either a steam pelleting or extrusion process.

1.3.2.1 Protein and Amino acids

Proteins compose approximately 70% dry weight of the organic material in fish tissue; therefore, protein content is one of the most important nutritional compounds of fish feeds. Aquaculture feeds contain a higher percent of protein than feeds used in agriculture for poultry, swine and beef. Higher protein levels in fish feed is incorporated into fish tissue because fish require less energy for maintenance of normal body functions than warm blooded animal.

Fish require a balanced combination of the 20 amino acids that make up proteins. Fish utilize dietary proteins by digesting them into free amino acids, which are absorbed into the blood and distributed to tissues throughout the body where they are then reconstituted into new specific proteins of the fish tissues. Protein in fish tissues is formed from all 20 major amino acids. Fish can synthesize some of these amino acids in their body, but others cannot be synthesized and must be consumed. The 10 amino acids that fish cannot synthesize are the "essential amino acids" that must be provided in proper amounts in their diet. Qualitatively essential amino acids required by *Oncorhynchus mykiss* are the same as required for all fish and animals but quantitatively they are different.

Table 1. Essential Amino acid requirement of *Oncorhynchus mykiss* (Anderson and Lall 2005)

EAA	Lys	Met (Cys)	Trp	Thr	Leu	Val	Ileu	Phe+Tyr	His	Arg
% of Protein	**3.7-6.1**	**1.9-2.4**	**0.5-1.4**	**3.2-3.7**	**4.4**	**3.1**	**2.4**	**4.3-5.2**	**1.6**	**3.3-5.9**

Crude protein content is the general measure of fish feed quantity. Optimum dietary protein levels vary with fish species, stage of life, water temperature, daily feed allowance, feeding frequency, quality of protein and quantity of non protein energy. A typical commercial production diet for tilapia or catfish contains approximately 32-40 % protein while the formulated feed for trout should contain 40-50 % protein on a dry weight basis.

Feed costs are usually the major operational expense in most aquaculture typically ranging from 30 % to 50 % of the variable operating costs. Protein is typically the most costly nutrient in a formulated feed. In the formulated diets the most expensive ingredients are fish and shrimp meal which have been incorporated to meet the protein requirement of trout. The cost of fish and shrimp meal is very high in comparison to high quality plant based protein sources, such as soybean.

1.3.2.2 Silkworm Pupae

A variety of invertebrates are commonly fed to carnivorous fish, house cricket nymphs (*Acheta domesticus*), superworms (*Zophobas mori*) larvae, giant mealworm larvae, mealworm larvae and adult mealworms (*Tenebrio molitor*), waxworm larvae (*Galleria mellonella*), and silkworm larvae (*Bombyx mori* Linnaeus,1758,) etc. When the silkworm enters the pupa phase, it builds a protective cocoon made of raw silk. At the end of pupation, the pupa releases an enzyme that creates a hole in the cocoon and the moth emerges. In order to produce silk, the pupae is killed by boiling, drying or soaking in NaOH before they produce the enzyme (Datta, 2007). The spent pupae are produced in large quantities and are a major by-product of silk production (Datta, 2007). Spent silkworm pupae is a waste material often discarded in the open environment or used as fertilizer. Silkworm pupae have long been part of human food in Asian silk-producing countries, and is considered as a delicacy in regions of China, Japan, Thailand and India (Longvah *et al.*, 2011), among others. Due to its high protein content, silkworm pupae meal has been found suitable as a livestock feed.

Fresh spent silkworm pupae spoils rapidly due to its high water content and spent pupae are generally sun-dried and ground. The degradation of mulberry-fed silkworm pupae products a foul smell that has been attributed to the presence in mulberry leaves of compounds that might be sequested by silkworms, including essential oils, favenoids and terpenoids. The high protein content and well balanced amino acids are much valued component in silk worm pupae. Several investigators have demonstrated the potential of silkworm pupae inclusion in fish diet. Silkworm meal can be used to replace fish meal completely in carp diets with similar performance of growth and feed conversion. Supplementation of silk worm meal with minerals was found to improve performance also. It is no secret that Japanese breeders use silkworm pupae extensively during the summer to grow and prepare their koi (*Cyprinus carpio)* for the market.

1.4 Objectives

- To develop low cost feed for rainbow trout (*Oncorhynchus mykiss*) fry
- To study the growth response of trout fry on diets containing different protein sources**.**

1.5 Rational of the study

Trout has been introduced in Nepal to utilize huge cool fresh water resource in middle hills and high mountains. The main objective behind the introduction of trout farming are to encourage rural farmers to produce high quality protein to be consumed and to provide an alternative income generating opportunity for the people living in the hilly region that would utilize their abundant cold water resource. In trout farming, the amount and suitability of feed used determines the profitability of production (Hinshaw, 1999). Removal of fishmeal from the diet of rainbow trout and other carnivorous fish species has had wide interest globally. The field of trout nutrition and feed formulation is critical for sustainable trout production in Nepal. To fulfill the high protein requirement of this fish, shrimp (*Macrobrachium spp.*) is being used as major animal protein source that are being imported from India. Because of increasing cost and relative scarcity of such feed stuffs, it is necessary to develop dietary formulations based on the use of high inclusion levels of locally available ingredients. Hence, the present research aims to open up the new arena for the development of novel alternate low cost protein source in trout feed.

2. LITERATURE REVIEW

Nepal has immense scope for the expansion of aquaculture in this country. The fish production from capture fisheries comprises 43 percent of total production in Nepal (DOFD, 2007). But the production has become static; moreover, wild catch is declining in our nation and worldwide due to environment degradation and/or over exploitation of the resources. Decline in capture fishery may also be due to increasingly vulnerable effects of global climate change affecting rural livelihoods in the tropics and subtropics badly with sudden profound changes in social and economic systems. To substitute capture fishery, aquaculture practices had been started in Nepal since last four decades. Warm water fishes like carps are the major items of aquaculture in the plain areas of Nepal. The wealth of coldwater resources bears a great prospect for fish farming in Nepal and the development of sustainable cold-water fishery has been gaining increased importance in recent years through three interrelated aspects - production technology, social/cultural and environmental aspects.

Rainbow trout (*Oncorhynchus mykiss*) is a cold water carnivorous sport fish, native to North America. Rainbow trout is a typical high protein and oxygen demanding carnivorous fish. The commercial culture of trout began as early 1853 in the United States and possibly even earlier in Europe (Bardach *et al.*, 1972). In natural habitats trout depends on aquatic insects, small crustaceans and small larvae. However, stages of trout from larvae to adult can be grown feeding upon artificial food. Its farming is mostly characterized by intensive farming system elsewhere, using high-cost nutrient inputs in the form of "nutritionally-complete formulated diets". In the formulated diets the most expensive ingredients are fish and shrimp meal which have been incorporated to meet the protein requirement of trout.

There is a great prospect for commercial farming of exotic Rainbow trout in Nepal. The primary requirement for trout culture is an abundant supply of clean and cold water. Rainbow trout culture requires a permanent supply of water with a temperature range of 10 to 20°C, and optimum temperature of 15-18°C (Yamazaki, 1991). The water should be clear, not turbid. A pH value of 6.5 - 8.0 and dissolved oxygen above 8 mg/l are considered suitable for trout culture (Huet, 1975). Water supply of at least 5.0 l/sec is necessary to produce a ton of trout, although less may be sufficient, when temperature decreases (Huet, 1975). Detail technical package of trout farming was given in simple forms for trout farmers in Matsya Palan Shrinkhala 13 and 14 (DOFD, 2007/8). FRC Trishuli has confirmed that water temperature range of 10-20°C is suitable for rainbow trout culture, if volume of supply is adequate and the water has a level of dissolved oxygen (DO) of 7-10 mg/l. Spring water is recommended for rearing alevins up to swim-up stage, because its temperature is warmer than that of a river. If the temperature of spring water is higher than 20°C it lowers the concentration of DO, therefore it should be mixed with cold water of snow and ice melt origin to adjust the temperature and DO. Water temperature should never exceed more than 23°C for rainbow trout culture (Sedgwick, 1985). Trout should not be kept for longer period in water temperature above 21°C as it stops feeding. It also stops feeding at temperatures less than 10°C. Sedgwick (1985) reported that a temperature of 18°C is regarded as the optimum for metabolism in rainbow trout. Higher temperature would assist higher level of metabolism and growth as well.

Rainbow trout was bred for the first time in 1990, culture experiments were initiated in 1993, and production of rainbow trout was started from 1995 and 1998 by government and private farms respectively in Nepal (Aryal *et. al.*, 2007 and Rai *et. al.* 2007). Hence trout farming in Nepal has a fairly short history. The research works carried out by NARC during past 10 years have demonstrated appreciable achievements in trout farming practices by developing suitable trout farming system in the country. Rainbow trout (*Oncorhynchus mykiss*) is a suitable fish for intensive aquaculture, sport fishery and possess high economic prospect providing ample of opportunities for commercial production in mid and high hill area (Singh, 2008). In Trisuli fish farm, trout reached 200-500 g after 14-15 months of rearing from free swimming larval stage. Comparative study on Rainbow trout *Oncorhynchus mykiss* breeding and table fish production management (Govt.& Private farm) and various aspects of rainbow trout farming in Nepal was done by Basnet *et al.*, (2007). Basnet *et al.*, (2008) had carried scaling up of Rainbow Trout Farming in farmer field. Basnet et al. (2008) had described that supplement feed took about 70 percent weight age of cost for trout fish farming.

2.1 Nutrition, Feeds and Feeding

Cultured fish require protein, lipids, energy, vitamins and minerals in their diet for growth, reproduction and other normal physiological functions. A typical commercial production diet for tilapia or catfish contains approximately 32-40 % protein while one formulated for trout contains as much as 40-50 % protein, on a dry weight basis.

Qualitatively essential amino acids (AA) required by *Oncorhynchus mykiss* are the same required for all fish and animals. It is discovered that test diets for AA requirements of fish could be improved by simulating the AA profile of the whole body tissue of the species under investigation (Ketola, 1982; Wilson and Cowey, 1985). Amino acids profiles of whole body tissue of a given species of fish resemble those of the dietary requirements of the fish (Arai, 1981; Ogata *et al*., 1983; Wilson and Poe, 1985; Mambrini and Kaushik, 1995). Limited differences in the AA profile of salmonids, chickens and pigs have been observed. Lysine is the most abundant essential amino acid in the whole body of salmonids and its concentration is higher compared with chicken and pigs.

Methionine deficiency resulted in reduced growth and feed deficiency in rainbow trout (Poston et al., 1977). Dietary methionine requirement estimated ranging from 1.8 to 4.0 % of dietary protein for commonly cultured species of fish (Halver and Hardy 2002). The quantitative methionine requirement of rainbow trout ranges between 1.0% and 2.35% of dietary protein bases (Walton *et al.,* 1982). Lysine is not the first-limiting amino acid in either natural food or a formulated diet based on fish meal / shrimp meal for carnivorous fish. The lysine requirement for fish is between 3.7 and 6.1 % of dietary protein (NRC 1993). Lysine is one of the ten essential amino acids. It has only one major function in the animal body and that is for protein tissue deposition. Lysine is usually most limiting amino acid.

Protein is typically the most costly nutrient in a formulated feed. Feed costs are usually the major operational expense in most aquaculture typically ranging from 30 % to 50 % of the variable operating costs. Protein in the majority of formulated fish diets depends greatly on fish meal, which is more costly than high quality plant based protein sources, such as soybean.

2.2 Silkworm Pupae

These Silkworm pupae are a high source of protein which contributes to the rapid growth of the Fish. Feeding silkworm provides a better slime coat protection to resist parasite and bacterial infections resulting in a better, bigger healthy fish. Silkworm is naturally high in protein, calcium, nutrients, vitamins & minerals and other essential fatty acids.

Silkworm of different butterfly species such as *Bombyx mori*, *Samia ricinii*, *Antherae assama*, 1837 and *Anthera paphia*, 1758 are raised for silk fiber production (Longvah *et al.*, 2011). The spent pupae are produced in large quantities and are considered as major by-product of silk production (Datta, 2007) in silk producing countries. The high protein content and well balanced amino acids are much valued (Longvah *et al.*, 2011). Several investigators have demonstrated the potential of silkworm pupae inclusion in fish diet. Silk worm meal can be used to completely replace fish meal in Carp diets with similar performance of growth and feed conversion. (Nandeesha *et al.*, 1990) Digestibility of crude protein in Silkworm meal was found to be similar to fish meal (Borthakur *et al.*, 1998) when fed to *Clarius batrachus.* Dried silkworm meal could replace completely the soybean meal in balanced diets for growing rabbits, without any problems (Carregal and Takahashi, 1887). It was shown silk worm meal could totally replace fishmeal without altering pigs blood parameters (Mehdi, 2011). It is no secret that Japanese breeders use silkworm pupae extensively during the summer to grow and prepare their koi for the market. Likewise Japanese hobbyists use silkworm pupae to provide growth and improve body shape, color and gloss in preparing their koi for shows.

3. MATERIALS AND METHODS

The experiment was conducted from 5th March, 2012 to 14th May, 2012 as part of research project carried out by Fisheries Research Division, Godawari. A private Trout Farm, Agro Top Industries, Co. Nepal (P) at Mulkhola, Thakre, Dhading was selected as experimental site.

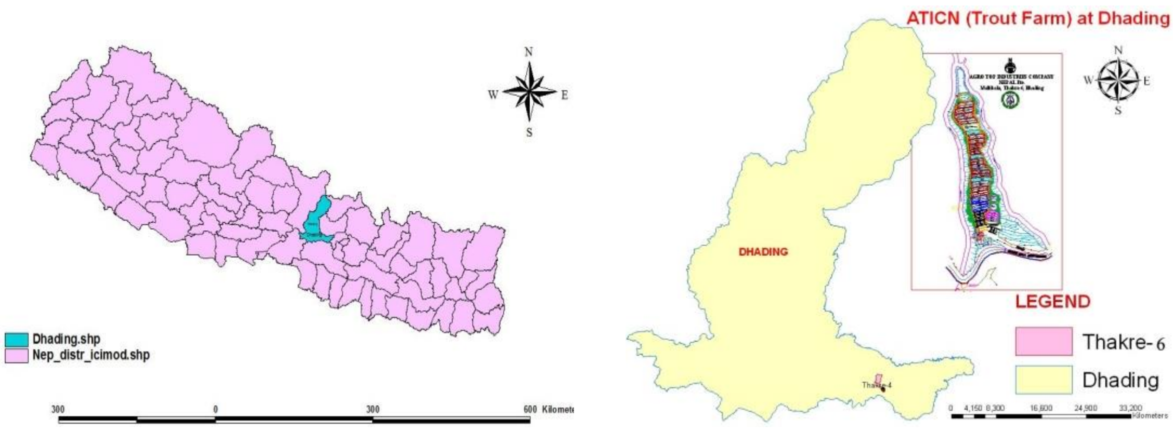

Fig 1. Experimental site

3.1 Experimental fish

Oncorhynchus mykiss fry of average 0.31g and 2.9 cm were used in present experiment. Fry for experiment were selected or graded from indoor rearing pond two days before the start of trial to obtain aforementioned sized trout fry.

3.2 Culture unit and stocking density

Twelve cages (0.5m x 0.5m x 0.5m) were used in this experiment. Each cage had wooden frame and nylon net of mesh 2mm. The selected raceway pond (8m x 1.25m 0.9m) was drained and cleaned thoroughly before experiment started. Fry were stocked in each cage at the density of 2000m^{-2}.

Fig 2. Diet-1, Shrimp meal (SM)

Fig 3. Diet-2, Synthetic amino acid (SAA)

Fig 4. Diet-3, Silkworm pupae meal (SPM)

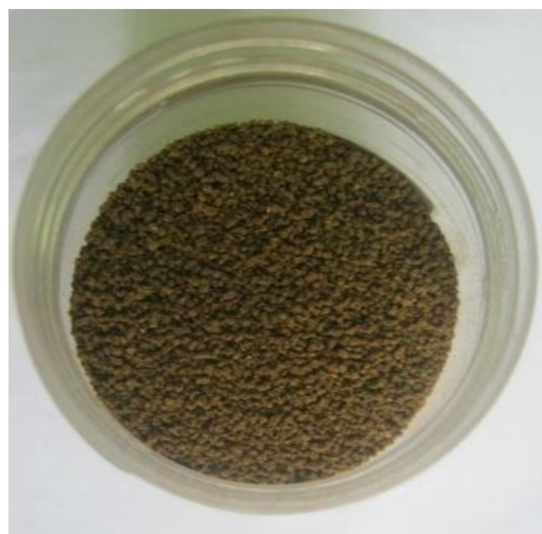

Fig 5. Diet-4, Silkworm moth meal (SMM)

Fig 2, 3, 4 and **5** showing Experimental diets

Fig 6. Experimental fish, *Oncorhynchus mykiss*

Fig 7. Feeding to fry

Fig 8. Weight measurement

Fig 9. Length measurement

3.3 Dietary Ingredients

Big shrimp used in diet-1 (control) is imported source. Mithionine and Lysine were synthetic amino acids used in diet-2. Silkworm (*Bombax mori)* pupae and silkworm moth were used in diet-3 and diet-4 respectively. Locally available soybean and wheat were supplementary ingredients in feed formulation. Vitamin premix, mineral premix and vitamin C were used as additives in all diets. Proximate analysis of dietary ingredients is shown in appendix I.

3.4 Experimental Design

Four diets had three replicates of each. The cages were allocated as Randomized Block Design (RBD) in a selected raceway pond.

3.5 Experimental Diet

Four iso-nitrogenous diets were formulated to contain 44.44 (±0.5)% protein. Compositions of experimental diets are shown in table 2. The proximate composition of these diets is shown in appendix I.

Table 2. Composition of experimental diets.

S.N.	Ingredients (%)	SM, diet-1	SAA, diet-2	SPM, diet- 3	SMM, diet- 4
1	Big shrimp powder	50	-	-	-
2	Silk worm pupae	-	-	50	-
3	Silk worm moth	-	-	-	50
4	Lysine	-	30	-	-
5	Mithionine	-	10	-	-
6	Soybean powder	35	35	35	35
7	Wheat powder	15	25	15	15
	Additives	-	-	-	-
8	Vitamin pemix	1	1	1	1
9	Mineral premix	1	1	1	1
10	Vitamin-c	0.1	0.1	0.1	0.1
11	Crude protein	44.44	44.29	44.89	44.44
	Cost/kg	153.5	234	148	141

Source: FRD, Godawari

3.5 Feeding Rate

The maximum protein required for rainbow trout fry (0-5gm) used in present experiment is 28.5 gm protein Kg^{-1} body weight day^{-1} . The feed prepared in the laboratory contained 44.44 % protein and the basic protein requirement was calculated to be 6.4% of the body weight. Fish were fed by hand feeding to satiation 8 to 10 times a day at an interval of hour.

3.7 Analytical Methods

3.7.1 Growth Response of Fish

Growth performance of experimental fish and feed utilization were evaluated biweekly. Measured weights were used to adjust the feeding rate for upcoming two weeks.

A. Percent weight gain (%)

$$= \frac{mean\ final\ wt - mean\ initial\ wt}{mean\ initial\ fish\ wt} \times 100$$

B. Daily weight gain

$$= \frac{mean\ final\ wt - initial\ wt}{days}$$

C. Specific Growth Rate SGR ($\%/day$)

$$= \frac{log_e W - log_e w}{T - t} \times 100$$

Where W is the weight at the time T and w the weight at the time t. This gives the average percentage increase in body weight per day over any given time interval.

D. Condition Factor $= \frac{\mathrm{wt}}{\mathrm{l}^3} \times 100$

3.7.2 Feed Utilization

A. Food Conversion Ratio (FCR)

$$= \frac{feed\ intake}{wt\ gain}$$

$$= \frac{g\ dry\ food\ fed}{g\ live\ wt\ gain}$$

B. Food Conversion Efficiency

$$= \frac{wt\ gain\ (g)}{feed\ intake\ (g)}$$

3.8 Water Quality Analysis

Water quality parameters vis. dissolved oxygen, conductivity, total dissolved solid, pH and temperature of raceway pond were measured using respective instruments. A bucket was used to collect water at outlet of raceway to estimate average flow of water biweekly.

Table 3. Instruments used for recording water quality parameters

SN	Parameters	Instrument used
1	Dissolved Oxygen	*HANNA* oxy-check
2	Conductivity	Conductivity Meter Model-621 E (India)
3	Total Dissolved Solid	TDS Meter Model-661 E (India)
4	pH	*HANNA* pH-ep
5	Temperature	Glass thermometer

3.9 Statistical Analysis

Statistical analysis was carried out to evaluate growth response of rainbow trout fry on feeding different protein source. One way Analysis of Variance, ANOVA followed by Tukey Multiple range test using SPSS software was used to evaluate the significant difference at 95% confident level for feeding regime.

4. RESULTS

4.1 Fish Growth

4.1.1 Mean Body Weight

The fry of 0.31 g, average body weight, were stocked at the start of experiment. Mean weight gained by fish fed with silkworm pupae was 2.34 g and the value was found significantly superior ($p<0.05$) among all the experimental diets used. Mean weight gained by fish fed on synthetic amino acids was recorded lowest (0.55g). The performance on fish diet of shrimp meal showed best after silkworm and it was found significantly higher ($p<0.05$) in mean weight from those reared on silkworm moth (Table 4).

4.1.2 Total length

Average length of fry was 2.9 cm at the beginning of the experiment. Later, the average length of fishes fed with shrimp meal, silkworm pupae and silkworm moth reached 5.82cm, 6.21cm and 6.22cm respectively. But the length gained on these three different fish diets were recorded statistically insignificant ($p>0.05$). However, length of fish fed on synthetic amino acids was significantly different and lowest (3.99cm) among all the experimental fish diets (Table 4 and Fig. 11).

4.1.3 Specific growth rate (SGR)

Highest specific growth rate (SGR) was found in fishes fed with silkworm pupae (1.26 %/day/fish) followed by shrimp meal (1.11 %/day/fish) and silkworm moth (0.99 %/day/fish). But the lowest specific growth rate was recorded in synthetic amino acid (0.41 %/day/fish). SGR was recorded significantly different in all the test protein diets ($p<0.05$) (Table 4). Linear growth of fishes was observed in all diets (Fig 10).

4.1.4 Percentage of weight gain (PWG)

Percentage of weight gain (PWG) of fishes was recorded significantly different ($p<0.05$) among all the four test diets. PGW was found highest (707 %/fish) in fish fed with silkworm pupae followed by shrimp meal (531 %/fish) and silkworm moth (418 %/fish) and lowest in fish fed with synthetic amino acids (96 %/fish) (Table 4).

4.1.5 Daily Weight Gain (DWG)

Daily weight gain (DWG) was also recorded significantly different ($p<0.05$) among all the four test diets. DGW was found highest (0.0339 g/fish/day) in fish fed with silkworm pupae followed by shrimp meal (0.028 g/fish/day) and silkworm moth (0.0234 g/fish/day) and lowest in fish fed with synthetic amino acids (0.0045 g/fish/day) (Table 4).

4.1.6 Condition Factor (CF)

The value of Condition Factor for experimental fishes were found in sequence order of silkworm pupae, shrimp meal, silk worm moth and synthetic amino acid at the values of 1.12, 1.08, 1.02 and 0.95 respectively. CF of fish fed with silkworm pupae was recorded highest and significantly different from other fish diets. On the other hand, CF of fish fed with shrimp meal and silkworm moth were found statistically insignificant (Table 4).

4.1.7 Mortality Rate

The highest mortality rate (77%) was observed in synthetic amino acid. Mortality rates were recorded 4%, 15% and 16% in diets of shrimp meal, silkworm pupae and silkworm moth respectively and the mortality rates were found statistically insignificantly ($p>0.05$) among later three. But the mortality rates were recorded lower in all test diets than synthetic amino acid (Table 4, Fig 12 and Appendix VI).

4.2 Feed Utilization

4.2.1 Feed Conversion Ratio (FCR)

Mean FCR of fish reared on shrimp meal, synthetic amino acid, silkworm pupae and silkworm moth was recorded 1.46, 3.75, 1.33 and 2.46 respectively (Table 4 and fig 5). The mean FCR of all test experimental diets was found statistically insignificantly at 95 % confidence limit. FCR for diet 4 was observed highest (8.16) in 1st growth check (Fig 13).

4.2.2 Feed Conversion Efficiency (FCE)

FCE of fishes reared on synthetic amino acid was recorded significantly lower (0.27) at 95% confidence limit than all other test diets. FCE was found highest in silkworm pupae (0.77) followed by shrimp meal (0.75) and silkworm moth (0.59) (Table 4).

Table 4. Growth response and feed utilization of *Oncorhynchus mykiss* fry reared on different diets.

Growth Response	SM	SAA	SPM	SM
Mean Initial Weight (g/fish)	0.32^a	0.28^a	0.29^a	0.34^a
Mean Final Weight (g/fish)	2.02^a	0.55^b	2.34^c	1.76^d
Mean Initial Length (cm/fish)	2.9^a	2.9^a	2.9^a	2.9^a
Mean Final Length (cm/fish)	5.82^a	3.99^b	6.21^a	6.22^a
Specific Growth Rate (%/day/fish)	1.11^a	0.41^b	1.26^c	0.99^d
Percent Weight Gain (%/fish)	531^a	96^b	707^c	418^d
Daily Weight Gain (g/fish/day)	0.028^a	0.0045^b	0.0339^c	0.0234^d
Condition Factor (CF)	1.08^{ab}	0.95^a	1.12^b	1.02^{ab}
Mortality%	4^a	77^b	15^a	16^a
Feed Utilization				
Food Conversion Ratio (FCR)	1.46^a	$*3.75^a$	1.33^a	2.46^a
Food Conversion Efficiency (FCE)	0.75^a	$*0.27^b$	0.77^a	0.59^a

*negative value of FCR was neglected and its value in 1st growth measurement was considered as mean value. Values in a same row having the different superscript are significantly different ($p < 0.05$: Tukey multiple range test).

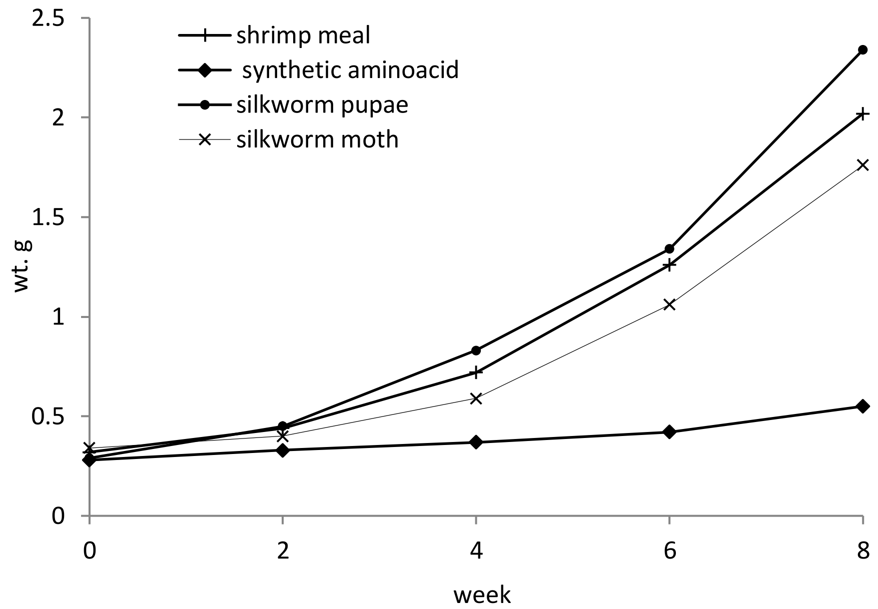

Fig 10. Growth curve of *O. mykiss* fry reared on different diets

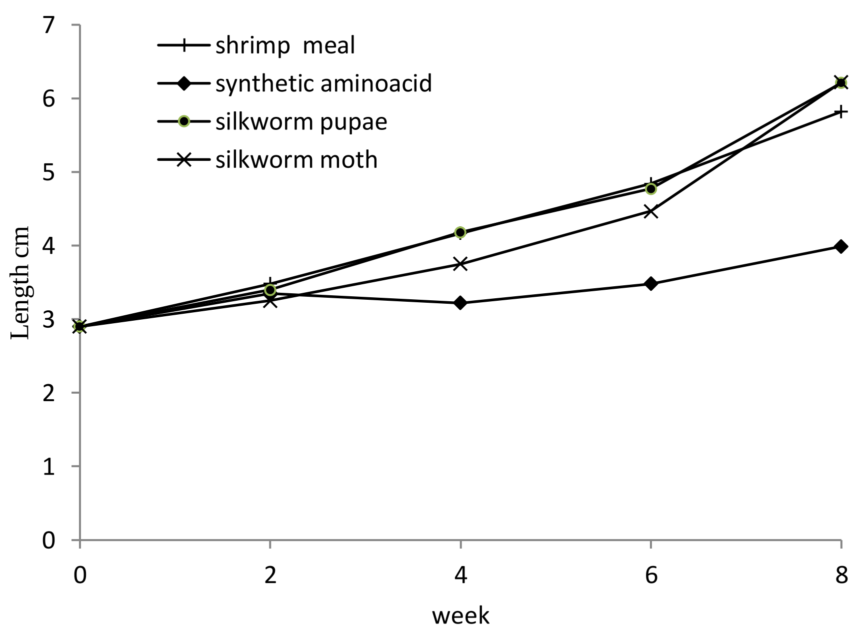

Fig 11. Mean Length of fry (cm/fish)

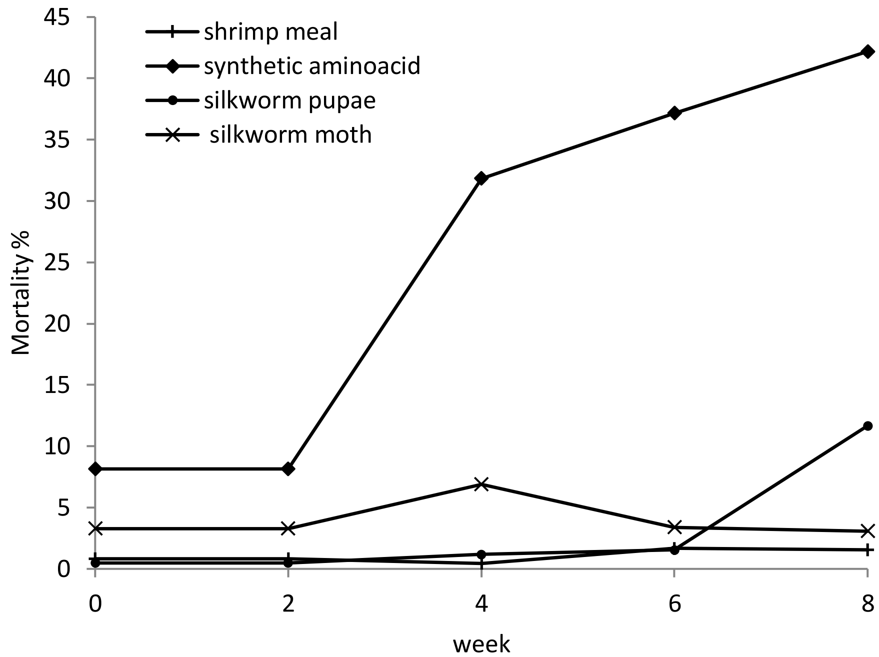

Fig 12. Mortality % of *O. mykiss* fry reared on different diets.

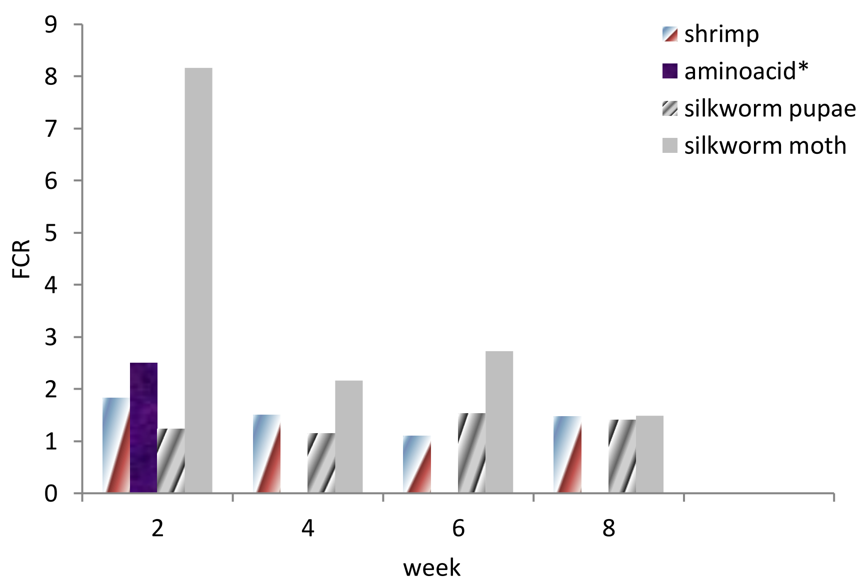

Fig 13. FCR of the fry reared on different diets calculated at interval two week.

*negative value of FCR for synthetic amino acid in 2nd, 3rd, 4^{rth} growth check is not mentioned.

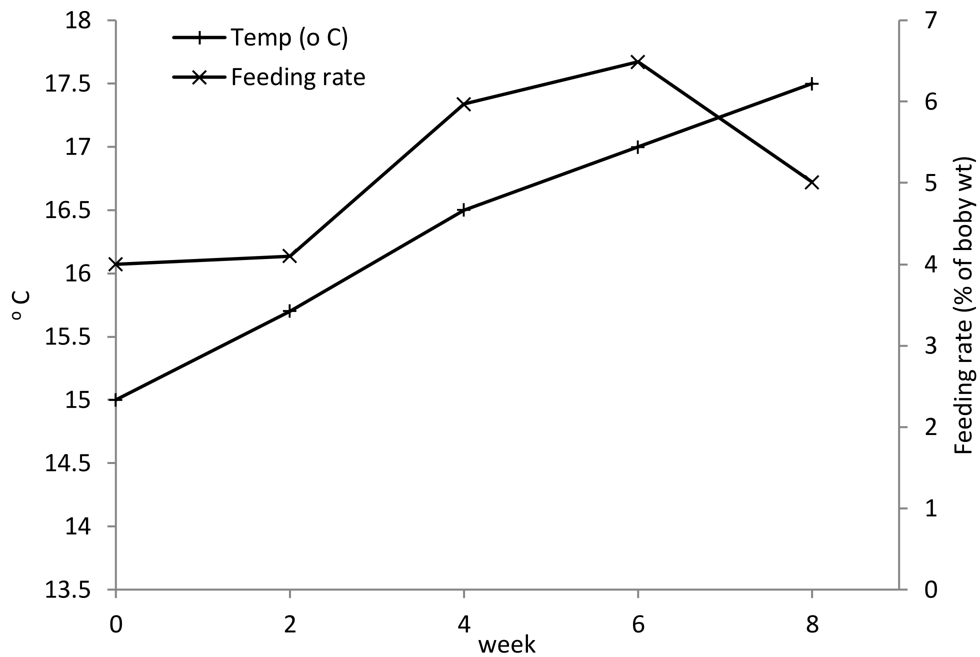

Fig 14. Temperature curve against feeding rate*

*The value of feeding rate is mean for experimental diets.

4.3 Water quality Parameter

Dissolved Oxygen was found decreased greatly from 9.4 mg/l to 7.4 mg/l in inlet and outlet respectively. The value of pH was also recorded slightly decreased from 7.5 to 7.2 in between inlet and outlet and pH value was also noticed slightly decreased afterwards during the progress of the experiment. The values of conductivity and total dissolved solid (TDS) were recorded very slightly increased from inlet to outlet. Water temperature was found gradually increasing with the progress of experiment from 15 -17.5^{0}C (Fig. 14). However, water flow could not be maintained static but recorded erratic at different periods of the experiment (Table 5).

Table 5. Water quality parameter of the experimental pond.

Water Parameter	Stocking		2nd week	4rth week	6th week	8th week
	Inlet	Out let				
DO (mg/l)*	9.4	7.4	-	-	-	-
pH (range)	7.5	7.2	7	7	6.5	7
Conductivity (S/m)*	146	147	-	-	-	-
TDS (ppm)*	130	132	-	-	-	-
Water Temp (^oC)	15	15	15.7	16.5	17	17.5
Water flow (l/s)	4.22	4.22	4.44	3.8	5.7	3.7

* These parameters were measured only on stocking day and were not possible in following growth check due to unavailability of the instruments.

5. DISCUSSION

In present study, the growth performance was found highest, mean weight of 2.34 g per fry after the eight week, in feeding trial using silkworm pupae. Rangacharyulu *et al.,* (2003) used fermented silk worm pupae (SWP) silage while Hossain *et al.,* (1997) used boiled silkworm pupae to replace fishmeal in the formulation of fish diet. Nanda (1967) carried out a pilot study, on this and indicated that the processed silkworm pupae an excellent source of protein. Nataraj and Basavanna (1969) reported that the protein concentration of pupae can be used to supplement the fish feed and this meal might be an important substitute for fish meal. Panda and Rao, (1975), replaced 10 per cent of maize and entire portion of fishmeal with deoiled silkworm pupae meal. Singh *et al.,* (1992) reported that dead silkworm pupae and moths can be used as fish feed. Fish fed with pupa showed significant increase in body weight against the control fed with fishmeal. Swamy (1994) reported superior fat and protein content in silkworm pupae to plant proteins.

Mean body weight fed on silkworm pupae was recorded highest (2.34g) (Table 4). Mean FCR of fish reared on shrimp meal, synthetic amino acid, silkworm pupae and silkworm moth was recorded 1.46, 3.75, 1.33 and 2.46 respectively showing lowest Mean FCR value for silk worm pupae (Table 4 and Fig 13). Highest specific growth rate (SGR) was found in fishes fed with silkworm pupae (1.26 %/day/fish) followed by shrimp meal (1.11 %/day/fish) and silkworm moth (0.99 %/day/fish). But the lowest specific growth rate was recorded in synthetic amino acid (0.41 %/day/fish) (Table 4). In general, growth performance and feed utilization (Table 4 and Fig 10) by fish reared on silkworm pupae meal suggest silkworm pupae as reliable feed ingredient in trout feed. Partial or complete replacement of fish meal by silkworm pupae meal showed better results in other species as suggested by several workers. In supplementation of fermented silkworm pupae silage in feed for carps, Rangacharyullu et al. found better FCR (2.1) and SGR (2.39) compared to FCR (3.16) and SGR (2.2) by fish meal. When silkworm meal replaced a portion or all of the fish meal in carp and other fish diets similar performance of growth and feed conversion were observed (Jeyachandran et al., 1976; Erencin, 1976; Borthakur et al., 1998; Nandeesha et al., 1990). Compared to plant protein sources silkworm pupae was superior when fed to carp (Swamy et al., 1994). Digestibility of the crude protein in silkworm meal was found to be similar to fish meal (Borthakur et al., 1998) when fed to fish (*Clarius batrachus*).

But mortality revealed by silkworm pupae meal (15%) was found higher than that of shrimp meal (only 4%). This might be due to some experimental error, as Rangacharyullu et al. found better survival rate (84.2%) in fish feed containing silkworm pupae compared to survival rate (67.5%) with fish meal. Since mortality imparts great effect in trout production, diet selection very much rely on survival rate.

Because optimum temperature for food metabolism and growth ranges between 16-18^{o} C, higher feed consumption and relative growth rate is expected with linear increase in water temperature from 15 ^{o}C at the time of stocking to 17.5 ^{o} C at the time of harvesting (Fig 14). In contrast, feed consumption and relative growth rate did not increase as expected (Fig 10). Increasing mass density and constant space (0.5m^3, the size of experimental cage) may account for this.

Reduced growth and high mortality was reported accompanied with excess methionine and lysine in different experiments (Steffens and Albrecht, 1979). High mortality and poor growth were also reported incorporated with 30 % lysine and 10 % methionine (Yvonne et al., 1957). The result of study revealed that excess lysine causes lysine toxicity and so then depress growth and efficiency of feed utilization. The normal quantity of methionine should be around 1.0- 2.35 % in rainbow trout (Walton et al. 1982). Only plant protein like soybean and wheat powder with deficient animal proteins might be responsible for high mortality, reduced growth and poor feed efficiency. The feed is considered incomplete diet due to deficiency of essential amino acids or rainbow trout relied much on natural animal proteins.

Yamazaki (1991) reported water temperature in between from 10 to 18ºC suitable for trout suitable farming. DO range from 6.5 to 9 mg/l and pH from 7.0 to 8.5 and suitable for the growth of the trout larvae. The pH value of 6.5 to 8.5 and DO above 8 mg/L are reported suitable for trout culture (Huet, 1975). Water quality parameters like dissolved oxygen, pH, conductivity, total dissolved solid (TDS) and water temperature was found suitable for the rearing and growth of trout fish.

For many years water quality has been the most important limitation to fish production. Advances in life support technology have been substantial in recent years, and nutrition is increasingly regarded a key limitation to increased production efficiency as well as the growth and propagation of “new” species. Commercially prepared feeds for salmonids have been developed using a great deal of research data on specific nutritional requirements of this species, their production systems and their life stages.

6. CONCLUSION AND RECOMMENDATIONS

Growth performance and feed utilization efficiency of the fish fed with silkworm pupae meal is the best among the newly formulated three dietary compositions. Inclusion of silkworm pupae meal might reduce feed cost as transportation cost is minimized. The quantitative methionine and lysine supplemented with plant protein in diet 2 revealed unsatisfactory growth and survival rate. Hence, Silkworm meal can be used to completely replace shrimp meal in trout feed.

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APPENDIX I

Table 6 : Proximate Composition of Dietary Ingredients

S.N.	Ingredients	Moisture(%)	Ash(%)	Crude fats(%)	Crude protein(%)	Crude fiber(%)
1	Big shrimp powder	10.59	10.09	3.21	64.80	3.25
2	Silk worm pupae powder	6.65	4.01	31.29	57.21	2.39
3	Silk worm(moth) powder	11.84	4.74	29.05	54.55	4.84
4	Wheat(whole) powder	12.40	7.00	1.30	11.90	-
5	Soybean(roasted) powder	8.70	5.10	21.00	37.00	-

Source: FRD, Godawari

APPENDIX II

Table 7: Proximate Composition of Diets

S.N.	Crumbles feeds	Moisture(%)	Ash(%)	Crude fats(%)	Crude protein(%)	Crude fiber(%)
1	Control diets-1	8.97	11.67	8.62	38.27	2.65
2	Synthetic amino-acids diets-2	11.54	5.06	6.48	48.47	1.86
3	Silk worm pupae diets- 3	10.35	5.65	17.01	42.68	2.80
4	Silk worm moth diets- 4	3.66	7.13	20.85	46.01	3.11

Source: FRD, Godawari

APPENDIX III

Fish Count

Diet	*Replicate*	*Stocking*	*2 Week*	*4 Week*	*6 Week*	*8 Week*
1 (shrimp meal)	1	454	451	449	440	438
	2	468	460	459	453	451
	3	465	465	462	454	439
2 (synthetic amino acid)	1	393	358	249	159	97
	2	396	365	258	150	79
	3	403	372	239	159	95
3 (silkworm pupae)	1	486	482	473	465	389
	2	481	480	477	472	409
	3	487	485	479	470	445
4 (silkworm moth)	1	430	418	383	370	362
	2	440	420	394	382	377
	3	436	425	399	384	362
	Total	**5339**	**5181**	**4721**	**4358**	**3943**

APPENDIX IV

Fish Weight (g/50 fry)

	Diet 1			*Diet 2*			*Diet 3*			*Diet 4*		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Stocking	16.2	16.2	16.2	14.1	14.1	14.1	14.5	14.5	14.5	17	17	17
2 Week	22.8	23.7	20.2	14.1	17.7	17.3	22.8	21.9	22.1	19.5	18.5	21.6
4 Week	32.7	37.3	37.7	16.5	19	19.5	42.2	43	39.7	27	32.8	28.4
6 Week	60.5	63.9	64.8	21.6	20.6	20.9	69.1	63.4	67.8	48.2	54.2	56.2
8 Week	103.5	103.8	95	30.7	25.9	26	123	115.6	112.4	92.3	90	69

APPENDIX V

Feed Consumption (g/replicate/day)

	Diet 1			Diet 2			Diet 3			Diet 4		
	1	2	3	1	2	3	1	2	3	1	2	3
					2 week							
5-Mar	9.5	9.7	9.5	9.2	10	9.9	11	11.1	11.5	9.9	10	8.6
6	7.2	7.8	10	8.9	8.8	8.6	8.8	7.8	10.6	7.7	7.1	8.8
7	5.4	7.9	7	8.8	6.8	6.3	7.4	7.3	8.4	7.7	8.2	9.6
8	6.9	4.2	5.8	4.6	3.9	4.5	5.8	5.5	6.8	5	5.2	6.4
9	7.1	**1**	6.7	6.3	6.9	6.7	7.3	6.5	7.4	5.3	7.4	6.1
10	6	5.1	5	7	5.9	5.5	6.9	4	7.2	5.8	6	5.8
11	5.9	7	6	5	3.9	4.8	6	5	8	7.3	6.4	7
12	5	5	5.6	5.6	3.4	4.2	6.3	4.6	6	5	4.3	6.2
13	5.5	6.1	5.5	3	1.6	2.8	4.8	5.6	5.1	6	5	5
14	4.8	3.4	4	3.4	2.7	3.1	3.7	3.7	4.6	4.1	3.5	3.4
15	5.9	4.4	6.3	2.2	3.4	3.3	5.2	4.8	5.3	5.8	5.5	6.1
16	5.3	5.8	5.4	0.7	1.3	1.5	4.8	4.2	4.9	4	3.9	4.8
17	7.5	7.1	7	1.4	1.3	2.4	6.4	6.6	7.2	7	5.4	7.5
18	8.8	8.6	9.1	2.8	2.5	2.6	7.7	7.8	9.1	8.1	8.2	10.1
					4 week							
22-Mar	8.6	6.9	6.1	3.7	3.5	4	9.1	11.5	12.7	8.5	9.6	11
23	9.8	7.6	3.8	2.6	3.2	3.2	11.5	11.1	14	9	9.3	10.8
24	9.2	7.6	8.7	4.2	4	4.2	11.6	12.7	13.7	8.1	7.9	6.3
25	12.5	7.4	10.1	4.1	4.4	5	10	10.6	11.9	7.5	8.8	10.5
26	11.6	9.5	9.6	3.8	4.2	4.3	12.7	12.4	12.8	6.8	6.6	7.1
27	9.6	9.6	10	3.6	3	3	9.6	11.6	12.1	7.3	7	8
28	10	9.7	11	4	2.9	4.2	9.3	11.3	12	7.4	7.1	8.2
29	12.5	9.5	12	2.5	3	3.5	12.4	12	12.5	9.8	10	11
30	13	9.7	11	3	2.9	3.6	12.6	13	13.5	10	11	11.5
31	13	11.5	10.9	4	2.5	4.8	13.1	14.5	14.6	9.4	9.4	9.5
1-Apr	13.5	12.2	11.5	3.8	4.3	5.6	13.6	13.5	11.5	10.3	10.6	10.6
2	16.7	12	14.5	4.2	6.1	4.7	15.8	15.5	19.1	12.3	12.3	15.7
3	15	13.5	15	4.5	4.3	3.8	17	15.8	18	13.9	11.9	16
4	14.5	14	16.5	3	4	4	17.5	16	17.9	14.5	18	15.5
5	16	15	17	4	3.5	5	18	17.5	18.7	15.5	14.9	17.5
					6 week							
8-Apr	15.6	14.2	12	3	3.2	2.9	21.4	23.3	20.1	19.5	16.9	19.2
9	17.7	17.5	18.2	2.8	2.9	3.1	23.5	17.4	22.1	17.6	19.4	19.5
10	16	18.5	15.9	2.7	2.8	2.8	25	19.5	23.7	17.9	20.4	18.7
11	15.9	17.5	17.3	3.5	3.6	2.6	24.5	20.3	25.8	18.3	22.6	17.9
12	17.3	13.5	17.3	4.2	4	3.2	23.5	23.7	26.7	19.5	23.8	20.5
13	14	16.9	18.3	4	2.9	4	26.5	26	24.3	20.8	19.9	21
14	13	15.9	19	2.9	3.5	3.9	24.6	25.8	25.8	20.8	18.8	21.5
15	18.5	18.2	18.9	3.5	3.3	3.3	25.6	26.8	24.7	20.8	24.3	18.5
17	17	17.7	19.2	3.9	3.5	3.2	23.6	25.1	23.3	22.8	23.8	16.7
18	20.2	16.7	16.3	2.5	3.3	2.9	26.3	25.8	20.3	19.5	21.9	19.7
19	21.4	24.6	23.7	2.9	3.7	3.3	27	24.9	26.9	22.8	22.2	20.3
20	23	21.8	24.6	3.4	2.5	2.3	26.7	27.2	30	20.8	24	20.5
21	25	22.4	26.7	4.2	3.7	2.9	26.5	27.5	30.2	19.4	23.2	21.5
22	24.2	22.5	26	2.5	3.3	3	25.7	28.2	32.3	20.8	25.8	13.7
					8 week							
25	19.8	20	17.3	5	4.5	3.8	20.9	25.4	24.6	14.1	16.2	18.5
26	22.4	18.2	23	4.8	4.3	3.7	23	23.2	25	16.3	17	20.8
27	25.4	26.3	24.9	5.2	3.8	4.2	26.7	24.5	26.8	17.9	17	22.5
28	28.2	29	28.5	5.7	4.5	4.5	28	32	34.5	20.5	20.6	0
29	30.7	32.8	29.2	4.8	4.8	4	37.2	38.5	36.7	25	20.5	5.2
30	32	34	30.5	3.2	5	3	40	42	42	27.5	25	7.2
1-May	33.5	35	34	3.8	5.2	3.5	45	45	47	34	27	5.6
2	35.6	37.2	36.9	4	5	3.9	20.5	47.2	45.3	12.5	34	7.2
3	15	21.5	17	3.7	4.5	4.7	42	13	11.5	42	12.5	6.7
4	40.2	43	38.9	3.5	4.7	5	50	48.2	47.9	14	42	7.5
5	17.5	16.7	30	2.7	2.7	2.5	10.5	13.5	15	17	14	8.2
6	16.2	16.5	35	2.9	3.3	2.3	10.7	15	20	12.5	17.5	5.9
7	17.8	18.2	37	3	3.4	4.7	17.5	16	25	15.7	12.5	7.7
8	17.5	19.2	35	2.5	3.7	2.5	19.8	20.5	27	18	157	6.8
9	20.2	18.4	38	3.5	2.5	2.7	25	27	26.5	17.5	18	5.7
10	7.5	8	15.7	1.5	1.7	1.8	8	6.8	10.3	5.3	8.3	2.5
11	13.5	14.2	25.7	4	3.2	3.8	17.3	19.8	18.5	14.5	12.4	4.2
12	15	16.7	28.5	4	3.9	4.2	18.2	20.2	19.2	16.2	16.2	5.3

APPENDIX VI

Mortality (No/replicate)

	Diet 1			Diet 2			Diet 3			Diet 4		
	1	***2***	***3***	***1***	***2***	***3***	***1***	***2***	***3***	***1***	***2***	***3***
					2 week							
5-Mar	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	*1*	-	-	*1*	-	-	*1*	-	-	-
7	-	-	*2*	*1*	*2*	-	-	-	-	-	-	-
8	-	-	-	*1*	-	-	-	-	-	-	-	-
9	-	-	-	*2*	-	-	*1*	-	-	-	-	-
10	-	-	*1*	*1*	-	*2*	*1*	-	-	-	-	-
11	-	-	*1*	*1*	*2*	-	-	-	-	-	-	-
12	-	-	-	*2*	*1*	-	*1*	-	-	-	-	-
13	-	-	-	*1*	*2*	*3*	-	-	-	-	-	-
14	-	-	*2*	*1*	*2*	*3*	*1*	*1*	-	*2*	-	-
15	-	-	-	*2*	*6*	*3*	-	-	-	*1*	*2*	*1*
16	*1*	-	-	*6*	*3*	*3*	-	-	-	*1*	*5*	*2*
17	-	-	*1*	*2*	*4*	*4*	-	-	-	*3*	*1*	*1*
18	-	-	-	*5*	*2*	*4*	-	-	-	*1*	-	*3*
19	-	-	-	*5*	*2*	*2*	-	-	-	-	*4*	-
20	*1*	-	-	*5*	*5*	*6*	-	-	*1*	*4*	*8*	*4*
21	*1*	-	-	-	-	-	-	-	-	-	-	-
Total	***3***	***0***	***8***	***35***	***31***	***31***	***4***	***1***	***2***	***12***	***20***	***11***
					4 week							
22-Mar	*2*	*1*	-	-	-	-	*2*	*1*	*1*	*4*	*6*	*6*
23	-	-	*1*	*14*	*10*	*13*	-	*1*	*2*	*5*	*2*	*4*
24	-	-	*1*	*11*	*8*	*12*	-	-	-	*5*	*3*	*5*
25	-	-	-	*10*	*6*	*10*	-	-	-	*4*	*1*	*1*
26	-	-	-	*5*	*5*	*11*	-	-	-	*5*	*2*	*2*
27	-	-	-	*6*	*10*	*3*	*1*	-	-	*3*	*3*	*1*
28	-	-	-	*4*	*5*	*5*	-	*1*	-	-	*3*	*3*
29	-	-	-	*5*	*5*	*12*	-	-	*2*	*1*	*2*	*1*
30	-	-	-	*9*	*7*	*11*	-	-	-	-	*1*	*2*
31	-	-	-	*4*	*3*	*6*	-	-	-	*3*	*1*	*1*
1-Apr	-	-	-	*7*	*3*	*8*	-	-	*1*	*1*	*1*	-
2	-	-	-	*6*	*5*	*9*	-	-	-	-	-	-
3	-	-	-	*6*	*7*	*6*	-	-	-	-	-	-
4	-	-	-	*5*	*4*	*7*	-	-	-	-	-	-
5	-	-	-	*6*	*8*	*9*	-	-	-	-	-	-
6	-	-	-	*2*	*7*	*3*	-	-	-	-	-	-
7	-	-	*1*	*1*	-	*1*	*6*	-	-	*4*	*1*	-
Total	***2***	***1***	***3***	***109***	***107***	***133***	***9***	***3***	***12***	***35***	***26***	***26***

6 week

8-Apr	3	2	1	8	13	8	4	3	2	1	2	2
9	1	-	2	7	9	4	1	1	1	1	-	-
10	1	-	-	8	10	5	-	1	-	1	1	1
11	-	-	-	6	11	4	1	-	-	1	-	-
12	-	-	1	7	10	4	-	-	1	-	2	2
13	1	2	-	6	7	11	-	-	-	2	-	-
14	-	-	-	5	5	2	1	-	-	-	1	1
15	-	-	1	3	4	3	-	-	2	3	-	-
16	-	2	-	11	5	2	-	-	-	-	1	1
17	1	-	2	7	7	4	1	-	-	1	1	1
18	-	-	-	5	7	7	-	-	-	1	-	-
19	1	-	1	3	5	5	-	-	2	-	1	1
20	-	-	-	5	6	7	-	-	-	-	-	-
21	-	-	-	4	3	8	-	-	-	2	1	1
22	1	-	-	5	6	6	-	-	1	-	2	2
23	-	-	-	-	-	-	-	-	-	-	-	1
24	-	-	-	-	-	-	-	-	-	-	-	2
Total	***9***	***6***	***8***	***90***	***108***	***80***	***8***	***5***	***9***	***13***	***12***	***15***

8 week

25-Apr	-	-	-	-	2	1	-	1	-	-	-	-
26	-	-	-	3	1	4	1	2	-	1	1	1
27	-	-	-	-	3	2	-	4	-	-	-	3
28	-	-	1	2	1	-	2	3	-	1	1	-
29	-	1	1	7	8	5	4	1	-	-	-	2
30	-	-	-	-	4	3	-	1	-	4	-	3
1-May	-	-	1	2	-	3	1	3	-	1	-	-
2	-	-	-	5	2	2	2	3	12	-	-	-
3	1	-	-	-	3	5	6	6	3	-	1	-
4	-	-	-	2	6	3	20	5	2	-	-	-
5	-	-	5	7	8	10	-	-	-	-	-	13
6	-	-	2	2	3	5	6	1	-	1	-	-
7	-	-	1	8	7	3	3	4	1	-	-	-
8	-	1	-	3	5	2	4	6	2	-	-	-
9	-	-	-	5	2	2	5	3	4	-	2	-
10	-	-	2	4	3	5	6	5	3	-	-	-
11	-	-	-	4	4	7	4	6	2	-	-	-
12	-	-	3	2	7	-	8	5	5	-	-	-
13	1	-	1	4	2	1	4	2	2	-	-	-
14	-	-	-	2	3	1	-	2	-	-	-	-
Total	***2***	***2***	***17***	***62***	***71***	***64***	***76***	***63***	***25***	***8***	***5***	***22***

APPENDIX VII

Water Temperature (^{o}C) of experimental pond

2 week

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
6:00 AM	13	14.5	14	13.5	-	13.5	14	13.5	14	14.8	14.5	14.5	-	-	-
2:00 PM	15	15	15	15.5	-	15	15.5	15	16	15	15.5	16.5	-	-	-
5:00 PM	14.5	14.5	14	14	-	14	14.5	14.5	15.5	15.5	15	-	-	-	-

4 week

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
6:00 AM	15	15	15	15	15.5	15.5	16	16	16	15.5	15.5	15	15.5	15.5	-
2:00 PM	16.5	17	17	17	17	17.5	17	18	18	17	18.5	16	18	18.5	-
5:00 PM	16	16	16	16.5	16	16	16	17	17	16.5	17	15	16	16	-

6 week

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
6:00 AM	16	16	16	16	16	16	16.5	16	16	16	16	17	17	16.5	16.5
2:00 PM	17	17.5	17.5	18	18	18.5	17.5	18	18	18	18	19	20	20	19
5:00 PM	15.5	16	17	17	17.5	17	16.5	17	17	17	16	18	18	18	17

8 week

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
6:00 AM	16.5	17	16.5	16.5	16.5	15	15.5	15.5	17	16.5	16.5	16.5	17	16.5	16.5
2:00 PM	18	19	20	18	20	19	18.5	18.5	20	19	20	20	19	19	18
5:00 PM	17	17	17	16	17.5	16	16	16	17	16.5	18	19	18	17.5	17

APPENDIX VIII

Fish Length (cm/fry)

Diet 1			*Diet 2*			*Diet 3*			*Diet 4*		
2 week											
1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
3.5	4	3.1	3.8	3.5	3	3	3.3	4.2	3	3	3.3
3.4	3.4	3.2	3.5	3.1	3.4	3.3	3.2	3	2.7	2.9	4
3.3	3.7	3.5	3	3.8	3.4	3.9	3.1	2.6	3.3	2.9	3.1
3.4	3.5	3.4	2.9	3.4	3.4	3.7	4.5	3.3	3.6	3.5	3.5
4.2	4	3.8	3.9	3.5	3.1	3.8	3.6	4.4	3.6	2.8	3.3
3.3	3.1	3.2	3.4	3.8	4	3.6	4.1	3.1	3.4	2.6	3.6
3.4	3.2	4	2.9	3.6	3.5	3.5	3.2	3.1	3.8	3.3	3.6
3.1	3.3	3.4	4	3.2	3	3.3	4	2.6	3.4	3	2.8
3.6	3.8	3.5	2.9	2.5	3.5	3	2.8	3.5	2.9	3.6	3.4
3.8	3	3.1	3.4	3	3.1	2.8	3.5	2.9	3.1	3.4	3.1
4 week											
3.1	4.2	3.8	3.6	3.5	3.6	4.2	4.7	4.7	3.5	4.5	3.3
3.7	3.8	5.5	3.1	3	3.8	3.5	3.6	4.5	3.2	4	3.8
3.5	4.2	4	2.8	2.5	3.5	4.1	2.9	3.5	3.6	4	3.5
4.6	3	3.8	3.5	2.7	2.8	4.6	3.4	4.8	3.8	3.6	3.5
4.7	4.1	3.7	3	4	2.6	3.2	4.9	5.3	3.8	3.4	4.4
3.9	3.9	4	2.5	3.1	3.5	4.3	5.1	4.1	3.1	4.5	3.4
3.7	3.8	3.9	3	3.2	2.9	4.5	4.3	3.5	4.5	3.3	3.5
4	5.6	3.7	3.8	3.1	3.5	4.5	3.3	4.4	3.9	3.9	3.5
4.4	4.2	4.5	3	3.5	3.2	4.6	4.5	4	4.8	4.2	3.3
4.1	4.7	3.9	3.1	3.5	3.4	5.2	3.9	3.2	3.8	3.3	3.7
6 week											
5.8	6.2	5.2	2.9	3.8	3.6	5.4	5.5	5.5	5.4	3.9	4.3
5	5.3	5.3	4	3.1	4.1	5	4.7	5.1	4.3	4.5	4.5
4.8	4.6	4.7	3.4	3.1	3.5	4.4	4.5	4.1	4	4.6	4.6
4.6	5.3	4.6	3.1	3.7	3.4	4.8	5	4.1	4.4	4.6	5.1
4.6	4.3	5	3.4	3.7	3.1	4.2	4.7	4.8	3.3	3.9	4.9
4.4	4.8	4.9	4	3.6	3.7	5.2	4.8	5.2	4.9	4.4	4.6
5	5	5.5	3.5	3.5	3.3	5.2	4.1	5	4	5.2	4.9
4.5	4.3	4.6	3.6	3.6	3.9	4.7	4.3	4.5	4.5	4.7	5
4.4	4.4	4.6	3.4	3	3	4.3	5.3	5.2	3.7	4.4	4.1
4.6	4.5	4.4	3	3.3	3.2	4.6	4.8	4	3.7	4.9	4.7
8 week											
4.9	5.5	6.2	4.2	4	4.5	6.4	6	7.4	6.4	5.8	6.6
5.5	5.7	5.9	4.7	4.3	3.9	6.9	6.9	6.1	5.9	5.8	65
5.4	5.6	5.9	4	3.8	3.8	6.2	6.3	6.3	5.8	5.9	6.3
4.9	5.5	5.8	3.8	4	3.8	6.2	5.7	6.6	5.7	5.8	6.2
5.2	6.2	6.4	4.1	4.2	4.3	6.1	6.1	6.3	6	6.7	7
5.3	5.5	6.3	3.9	3.8	3.9	6	6.1	6.1	6	6	6.4
4.9	7.4	5.9	4.2	4.3	3.7	6	5.9	6	5.7	6.2	6.3
5.9	6.2	5.9	4.1	3.8	3.8	6.5	6	5.9	6	6.5	6
5.8	6.4	5.7	3.7	3.8	3.7	6.2	6.4	6.1	5.7	6.5	55
7	6.1	5.7	4.3	3.8	3.7	6.6	6.9	6	5.9	6.8	6.6