

**RNA: DNA RATIO, PROTEIN PROFILE AND GROWTH RATE OF
RAINBOW TROUT (*Oncorhynchus mykiss*) LARVAE FED WITH
VARIED PROPORTION OF DIETARY PROTEIN**



A Thesis Submitted

In partial fulfillment of the requirements for the award of the degree of
Master's 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

Submitted by

Rahul Ranjan
T.U. Registration No: 5-2-12-683-2004
T.U. Examination Roll No: 5862
Batch: 064/065

November, 2011

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 or institutions.

Date:

.....

Rahul Ranjan

T.U. Registration No: 5-2-12-683-2004

T.U. Examination Roll No: 5862

Batch: 064/065

RECOMMENDATION

This is to recommend that the thesis entitled “RNA: DNA RATIO, PROTEIN PROFILE AND GROWTH RATE OF RAINBOW TROUT (*Oncorhynchus mykiss*) LARVAE FED WITH VARIED PROPORTION OF DIETARY PROTEIN” has been carried out by Rahul Ranjan 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.

.....
Dr. Shyam Narayan Labh

(Co-Supervisor)

Associate Professor

Department of Zoology

Amrit Science Campus

Lainchaur, Kathmandu

.....
Dr. Archana Prasad

(Supervisor)

Lecturer

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu

Date:

LETTER OF APPROVAL

On the recommendation of supervisor Dr. Archana Prasad this thesis submitted by Rahul Ranjan entitled “RNA: DNA RATIO, PROTEIN PROFILE AND GROWTH RATE OF RAINBOW TROUT (*Oncorhynchus mykiss*) LARVAE FED WITH VARIED PROPORTION OF DIETARY PROTEIN” is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Masters’ Degree of Science in Zoology with special paper Fish and Fisheries.

Date:

.....
Prof. Dr. Ranjana Gupta
Head of Department
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

CERTIFICATE OF APPROVAL

This thesis work submitted by Rahul Ranjan entitled “RNA: DNA RATIO, PROTEIN PROFILE AND GROWTH RATE OF RAINBOW TROUT (*Oncorhynchus mykiss*) LARVAE FED WITH VARIED PROPORTION OF DIETARY PROTEIN” has been approved as a partial fulfillment for the requirements of Master’s Degree of Science in Zoology with special paper Fish and Fisheries.

EVALUATION COMMITTEE

.....
Supervisor
Dr. Archana Prasad
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

.....
Head of Department
Prof. Dr. Ranjana Gupta
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

.....
External examiner

.....
Internal Examiner

Date:-

Dedicated to my Grand Parent
Late Shree Rameshwar Nidhi
Srimati Saraswati Nidhi

ACKNOWLEDGEMENT

*First, I would like to express my sincere gratitude to honorable supervisor **Dr. Archana Prasad**, Lecturer, Central Department of Zoology, Tribhuvan University, for her constant inspiration, encouragement, continuous support, valuable suggestions and constant guidance during the entire period of this research work.*

*I express my sincere gratitude to **Prof. Dr. Ranjana Gupta**, the Head of Department for her kind administrative support.*

*I am extremely indebted to my Co-supervisor **Dr. Shyam Narayan Labh**, Associate Professor, Amrit Science Campus for his continuous encouragement, valuable suggestions and help throughout the long duration of this research work.*

*I would like to express my deepest thanks to **Mr. Daman Lama**, Fall and Trout Village Fish Farm, Nuwakot for providing the experimental setup and fish larvae as well continuous help during culture period.*

*I would also like to thank **Mr. Indra Kunwar**, MV Polyclinic and Diagnostic Center, Sinamangal for allowing me to use the lab and helping me in the instrumental works.*

I would also like to express my thanks and best regards to all the staffs of CDZ, T.U. for their help from respective sectors.

*I would like to thank my intimate colleagues **Khagendra Gautam, Madhu Nepal, Ranju Paudyal, Robin Rana, Sunil Poudel and Hemant Dhakal** for their kind support throughout my dissertation work. I am really blessed to have all of them and wish good luck for their future.*

I would like to express my deepest gratitude to my family members without whose support and inspiration I could not have gained the Masters' Degree.

Last but not the least I would like to acknowledge all those persons who help me directly or indirectly to complete this work.

.....
Rahul Ranjan

T.U. Registration No: 5-2-12-683-2004

T.U. Examination Roll No: 5862

Batch: 064/065

CONTENTS

Topic	Page no.
Declaration	i
Recommendation	ii
Letter of Approval	iii
Certificate of Approval	iv
Dedication	v
Acknowledgement	vi
Contents	vii
List of tables	x
List of figures	x
List of photographs	xi
List of abbreviations	xii
Abstract	xiii
1: INTRODUCTION	1-6
1.1 Background Information	2
1.2 Contribution of Nepalese in Aquaculture	2
1.3 Statement of the Problem	3
1.4 Justification	3
1.5 Objectives	4
1.5.1 General Objective	4
1.5.2 Specific Objectives	4
1.6 Rationale of the Study	4
1.7 Limitation	5
1.8 Significance	5
1.9 Scope	5
2: LITERATURE REVIEW	7-15
2.1 Trout Culture in Nepal	8
2.2 Growth Performance of Rainbow Trout	9
2.3 Biochemical Indices	10
2.3.1 Growth Performance and Nucleic Acid	10
2.3.2 Growth Performance and RNA: DNA Ratio	11
2.3.3 Nutrition and RNA: DNA Ratio	12

2.3.4 Temperature and RNA: DNA Ratio	13
2.3.5 RNA: DNA Ratio, A: G ratio and Health Status of Fish	13
2.4 Feed of Rainbow Trout	14
3: MATERIALS AND METHODS	15-23
3.1 Experimental System	16
3.2 Experimental Feed	17
3.3 Study of Growth Parameters	17
3.4 Study of Water Quality Parameters	18
3.5 Biochemical Estimation	18
3.5.1 Tissue Preparation	19
3.5.2 Estimation of Protein	19
3.5.3 Estimation of Albumin	20
3.5.4 Nucleic Acid Isolation	21
3.5.5 Estimation of DNA	21
3.5.6 Estimation of RNA	22
3.6 Statistical Analysis	23
4: RESULTS	24-33
4.1 Growth Performance	25
4.1.1 Average Length	25
4.1.2 Average Weight	26
4.1.3 Specific Growth Rate (SGR)	28
4.1.4 Feed Conversion Ratio (FCR)	28
4.2 Biochemical Analysis	28
4.2.1 Total Protein	28
4.2.2 Albumin Content	29
4.2.3 Globulin Content	30
4.2.4 Albumin: Globulin Ratio (A: G Ratio)	31
4.2.5 RNA Content	31
4.2.6 DNA Content	32
4.2.7 RNA: DNA Ratio	33
5: DISCUSSION	34-38
5.1 Growth of Rainbow Trout larvae	35
5.2 Protein profile of Rainbow Trout larvae	36
5.3 RNA: DNA of Rainbow Trout larvae	36

6: CONCLUSION AND RECOMMENDATIONS	39-41
6.1 Conclusion	40
6.2 Recommendations	41
7: REFERENCES	42-55
ANNEXES	
ANNEX I: Photographs of Feed and Making Materials	56
ANNEX II: Photographs of Field Work	57
ANNEX III : Photographs of Instruments used and Lab work	58
ANNEX IV : Record of Water Quality Parameters during Study Period	59
ANNEX V : Individual Length (cm) of fish larvae during different samplings	60
ANNEX VI : Individual Weight (g) of fish larvae during different samplings	60
ANNEX VII : Individual Protein Content ($\mu\text{g}/\text{mg}$) of fish larvae during different samplings	61
ANNEX VIII : Individual Albumin Content ($\mu\text{g}/\text{mg}$) of fish larvae during different samplings	61
ANNEX IX : Individual RNA Content ($\mu\text{g}/\text{mg}$) of fish larvae during different samplings	62
ANNEX X : Individual DNA Content ($\mu\text{g}/\text{mg}$) of fish larvae during different samplings	62
ANNEX- XI: Biochemical Estimation Flow Chart	63-64

LIST OF TABLES

Table no.	Title of table	Page no.
Table 1	Ingredients composition of different diets	17
Table 2.	Reagents for total protein test and its standard curve	19
Table 3.	Reagents for albumin content and its standard curve	20
Table 4	Reagents for DNA content and its curve	22
Table 5	Reagents for RNA content and its standard curve	22
Table 6	Average length of trout larvae	25
Table 7	Average Weight of trout larvae	26
Table 8	Average A: G ratio of trout larvae	31
Table 9	RNA: DNA ratio of trout larvae	33

LIST OF FIGURES

Figure no.	Title of figures	Page no.
Figure 1	Experimental Design	16
Figure 2	Bar diagram showing average length gain of trout larvae	26
Figure 3	Bar diagram showing average weight gain of trout larvae	27
Figure 4	Bar diagram showing SGR of trout larvae	27
Figure 5	Bar diagram showing FCR of trout larvae	28
Figure 6	Bar diagram showing average protein content of trout larvae	29
Figure 7	Bar diagram showing average albumin content of trout larvae	30
Figure 8	Bar diagram showing average globulin content of trout larvae	30
Figure 9	Bar diagram showing Average RNA content of trout larvae	31
Figure 10	Bar diagram showing DNA content of trout larvae	32

LIST OF PHOTOGRAPHS

Photograph No.	Title of photograph	Page no.
Photograph 1.	Photographs of Feed and its Ingredients	56
	Dried Fish	56
	Wheat Flour	56
	Vitamin	56
	Mustard Oil Cake	56
	Rice Bran	56
	Control Diet (C)	56
	40% Protein Diet (D1)	56
	50% Protein Diet (D2)	56
Photograph 2.	Photographs of Field Work	57
	Raceway dried before stocking	57
	Stocking of Trout Larvae	57
	Larvae in Cage	57
	Researcher getting information from farmer	57
	Researcher collecting water sample for analysis	57
	Length measurement of Trout Larvae	57
Photograph 3.	Photographs of Instruments used and Lab work	58
	Researcher testing Water Quality	58
	pH meter	58
	Digital Photo Colorimeter	58
	Researcher using Colorimeter	58
	Researcher preparing solution	58
	Variable Volume Automatic Pipette	58

LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
µg	microgram
A: G ratio	Albumin: Globulin ratio
BCG	Bromo-Cresol Green
BSA	Bovine Serum Albumin
dl	deciliter
DNA	Deoxy-ribonucleic Acid
DoFD	Department of Fisheries Development
FAO	Food and Agriculture Organisation
FCR	Feed Conversion Ratio
FRD	Fisheries Research Division
g	gram
mg	milligram
ml	milliliter
MoF	Ministry of Agriculture
nm	nanometer
PUFA	Poly-unsaturated fatty acid
RNA	Ribonucleic Acid
SGR	Specific Growth Rate
TCA	Tri-chloro Acetic Acid
UV	Ultra Violet

ABSTRACT

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.

The present study was carried out to determine the effects of dietary protein on growth performance, protein profile and RNA: DNA ratio of the Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792) larvae. Eight week old Rainbow Trout larvae were used for the experiment and divided in three fed with : commercial diet; diet containing 40% protein and diet containing 50% protein respectively twice (at 9:00 am and 4:00 pm) a day. Forty fish weighing 0.1846 ± 0.0055 g were placed in each of the three experimental raceways and were fed with one of the experimental diets for 6 weeks consisting of three replicates of each. All records were taken at seven days interval. Protein profile and RNA: DNA ratio was analyzed spectrophotometrically. The results showed higher growth and better feed conversion ratio in experimental group fed with 50% protein diet. The results also confirmed that the protein content and RNA: DNA ratio were all higher in larvae fed with 50% protein diet compared to larvae fed with other two diets indicating that dietary protein concentration has a great influence on growth, protein profile and RNA: DNA ratio as physiological index in Rainbow Trout larvae.

Keywords: growth performance, biochemical indices, protein profile, RNA: DNA ratio