

Dedicated

To

To my beloved parents

&

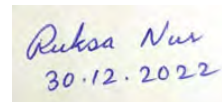
Jethu (Lt. Mr. Md Zainuddin)

DECLARATION

I declare that the thesis entitled '**Study on protein requirement vis-à-vis the digestive enzyme patterns in Mahseer fingerlings, *Neolissocheilus hexagonolepis* (McClelland)**' has been prepared by me under the guidance of Prof. (Dr.) Sudip Barat and Prof. (Dr.) Min Bahadur, Department of Zoology, University of North Bengal.

No part of the thesis has formed the basis for the award of any Degree or Fellowship previously.

Date:



Ruksa Nur
30.12.2022

Ruksa Nur
Aquaculture and Limnology Research
Unit
Department of Zoology
University of North Bengal
District Darjeeling
West Bengal, India
Pin-734013

UNIVERSITY OF NORTH BENGAL

ACCREDITED BY NAAC WITH GRADE B++

DEPARTMENT OF ZOOLOGY

T-FIST & UGC SAP Sponsored



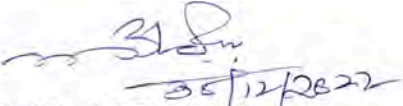
সমানো মন্ড্র: সন্মিতি: সন্মানী


P.O. North Bengal University
Raja Rammohunpur, Dist. Darjeeling
West Bengal, India, PIN - 734013

Date: 29th December, 2022

CERTIFICATE

We certify that **Ms. Ruksa Nur, M.Sc.**, has completed the thesis entitled 'Study on protein requirement vis-à-vis the digestive enzyme patterns in Mahseer fingerlings, *Neolissocheilus hexagonolepis* (McClelland)' for the award of **Ph. D Degree in Zoology of the University of North Bengal**, under our guidance. She had carried out her research work at the Department of Zoology, University of North Bengal. No part of the Ph.D. thesis has formed the basis for the award or any fellowship previously.



Prof. (Dr.) Min Bahadur
(Co-Supervisor)


Prof. (Dr.) Sudip Barat
(Supervisor)

Document Information

Analyzed document	Ruksa Nur_Zoology.pdf (D154425957)
Submitted	12/23/2022 12:32:00 PM
Submitted by	University of North Bengal
Submitter email	nbuplg@nbu.ac.in
Similarity	0%
Analysis address	nbuplg.nbu@analysis.arkund.com

Sources included in the report

W	URL: https://moam.info/download/inland-fisheries-society-of-india_5c240663097c471e5f8b4644.html Fetched: 11/29/2021 1:17:51 PM	 2
----------	--	---

Entire Document

Title: Study on protein requirement vis-à-vis the digestive enzyme patterns in Mahseer fingerlings, *Neolissocheilus hexagonolepis* (McClelland) INTRODUCTION 1.1. Background Mahseers are famous among cold-water hill-stream fishes for fishing and tourism, and is considered a cultural icon in 11 Asian countries for economic, recreational and conservation reasons (Nautiyal, 2006). Out of the 47 mahseer species found worldwide, about 15 mahseer species have natural habitats in India (Nautiyal et al., 2013). Among these, the natural population of Copper Mahseer (*Neolissochilus hexagonolepis*, McClelland, 1839) in the water bodies of the major rivers and streams of upland of the Northeast Himalaya has markedly decreased due to over-exploitation and habitat loss in most of the regions, along with other species (Dash et al., 2021). As such, it is classified as a 'Near Threatened' fish according to the International Union for Conservation of Nature (IUCN) status (Arunachalam, 2010). However, this represents one of the dominant fish species along the Teesta River and its tributaries in the Darjeeling Himalayas, that is why the main part of this river is called the 'Valilius-Masir Snow Trout Stream' (Acharjee and Barat 2013). Additionally, the highly desirable taste of flesh, abundant amounts of n-3 (omega-3) polyunsaturated fatty acids (n3/n6 PUFA 2.21) and essential amino acids (Sarmaet, 2013). However, the rising concern regarding the decreasing population has led to the need for rehabilitation programmes through artificial propagation, ranching, and building sanctuaries (Mahapatra and Vinod, 2011; Mandal et al., 2012; Laskar et al., 2013; Jyrwa and Bhuyan, 2017). Further, it is considered as a potential candidate species for culture in Jhora pond system of Darjeeling Himalaya (Ayyappan et al., 2001; Dash et al., 2021; Laskar et al., 2009; Laskar et al., 2013, Nur et al., 2010;). But, its aquaculture is not getting momentum because of non-availability of suitable feeds that are readily consumed, efficiently digested and utilized for growth and survival. The nutritional requirements of this fish have not been studied for any life stages which are essential for formulating feeds for its optimum growth at the lowest cost.

Ruksa Nur
Signature of the Candidate
30.12.2022

[Signature]
30/12/2022
Signature of the Supervisor(s)

[Signature]
30.12.2022

PREFACE

The future strategies for increasing fish production mainly from coldwater sector should be directed towards horizontal and vertical growth of the enterprise. Several components hold the key to success of these strategies subjected to suitability of culture practices according to location and productivity of the coldwater. Study of carrying capacity of hill streams to evolve stocking policies and to make sport fishery more attractive by exploring the possibilities of liberation (ranching) of the coldwater fish mainly Mahseer in some of the streams after evaluating the physico-chemical and biological characteristics of streams are required. The productivity and production of food in high altitudes is comparatively less. Therefore, the major objective of the development of aquatic resources in this region should be increasing productivity, production from aquatic resources to ensure nutritional food security at higher altitudes. There is tremendous scope for augmenting the fish production from the jhora fish pond by manipulating fish combinations of indigenous species like Mahseer (*Tor putitora*), Snow trouts (*Scizothorax sp.*), Katli (*N. hexagonolepis*). *N. hexagonolepis* commonly known as Copper Mahseer, are predominantly available in the foothills of Darjeeling hills of Eastern Himalayas. It has been widely culture in Jhora Ponds in Darjeeling Districts of West Bengal. However, its aquaculture is not getting momentum because of non-availability of suitable feeds that are readily consumed, efficiently digested and utilized for growth and survival. Further, nutritional requirements and feeding strategies of this fish have not been studied for any life stages which are essential for formulating feeds for its optimum growth at the lowest cost. Therefore, the present investigation was aimed to study the basic feeding strategy (stocking density, feeding frequency and feeding rate) and understand the nutrient (macronutrient) requirement of fingerling stage of *N. hexagonolepis* using semi-purified (artificial) diets. The information thus generated will be able to provide prerequisite information for the formulation of practical diets using the locally available farm resources in lowest possible cost. A brief Introduction is provided in Chapter 1, Chapter 2 comprises of Review of Literature and Objectives. The materials and methods were provided in Chapter 3, Chapter 4 contains the Results and Chapter 5 contained detailed discussion of the findings of the present study. Chapter 6 include Conclusion and Chapter 7 contains Summary followed by the list of research articles presented under Bibliography, Index and Appendix comprising the list of publication and papers presented at different conferences and seminars.

Acknowledgement

I am indebted to a large number of people for their support, encouragement and assistance in the completion of my thesis.

At first, it is a pleasure for me to implore reverting gratitude and indebtedness to my cogent and perspicacious Supervisor Dr. Sudip Barat, Former Professor, Department of Zoology, University of North Bengal for his unequivocal motivation, benevolent guidance, affectionate encouragement and constant supervision during the entire period of investigation and preparation of the manuscript. I immensely thank him for introducing me to the field of fish nutrition and retaining belief in the enterprising worth of this study.

I would also especially like to acknowledge my deepest gratitude to one of the reverend person I have been blessed with in my life as my Co-Supervisor, Prof. Min Bahadur. I am earnestly obliged for his unfailing support, empathy, valuable guidance, critical discussions and continued advice throughout this study.

I express my indebtedness to Dr. Arun Kumar Ray, Former Professor, Department of Zoology, Visva Bharti University, Santiniketan, West Bengal for his kind concern, encouragement and necessary help during my visit to his research laboratory and many insightful advice during my course of study.

I am very much thankful to Dr. Guru Prasad Mandal and his team of Department of Animal Nutrition, West Bengal University of Animal and Fishery Sciences, Kolkata for extending necessary laboratory support for proximate analysis of most of the fish feed samples during the course of the study.

I would like to express my profound regards to Dr. Kaushik Pal, Subject Matter Specialist (Animal Nutrition) Ashoknagar Krishi Vigyan Kendra under West Bengal University of Animal and Fishery Sciences, Kolkata.

I am grateful to Mr. Kripan Sarkar, owner of Rainbow Ornamental Fish Farm, Raninagar, Jalpaiguri, for providing me the fingerlings of Copper Mahseer required for the study.

I am grateful to Dr. Rudra Prasad Roy for his generous guidance in the Aquaculture and Limnology Research Unit, Department of Zoology, University of North Bengal.

I pay my eternal regards to my beloved senior Dr. Soma Mukherjee, who has continuously boosted me up during the entire span of my research work and writing the thesis.

I would be failing if I do not acknowledge the assistance of the many fish farmers of Darjeeling Hills of Pemling village, Kalimpong, Phuguri and Bungkulung village, Mirik, West Bengal. It is from all those discussions that the idea for this research work was proposed, and upon which the selection of objectives had been based.

Over and above, from volunteering me in academics to thriving in my career, I would like to genuinely acknowledge all the efforts, blessings, support and encouragements from my respected teachers

Prof. Joydeep Pal, Prof. Ananda Mukherjee, Prof. Tapas Kumar Chaudhuri, Prof. Soumen Bhattacharjee, Prof. Dhiraj Saha and Dr. Tilak Saha.

It is my pleasure to express sincere thanks to all the Heads, Department of Zoology, University of North Bengal from time to time for providing necessary facilities to carry out the research work.

I convey my sincere gratitude to all the respected teachers of the Department of Zoology, University of North Bengal, Dr. Sourav Mukherjee, Dr. Ritwick Mondal and Dr. Arpan Kumar Maiti for their necessary advice.

I am sincerely thankful to Mr. Sukumar Barman, Deep Narayan Chaudhary and Arup Ghosh for their time-to-time assistance in the fieldwork.

I wish to acknowledge the efforts of Mr. Bappaditya Ghosh, Senior Research Scholar for assisting in statistical analysis, critical review, useful comments and constant motivation. I extremely appreciate his patience under the circumstances, which has been quite remarkable.

A special note of appreciation to my wonderful juniors, Mr. Debojit Dutta and Miss Arpita Ray for their kind cooperation and extensive help for thorough proofreading of the thesis.

Further, I extend my sincere thanks to all the staff members (Mr. Surath Kundu, Mr. Ram Krishna Ghosh, Mr. Samarendranath Ghosh, Mr. Subrata Ghosh, Late Mr. Biswajit Barman, Mrs. Deepa Das and Mrs. Sudam Das of Department of Zoology, University of North Bengal for their kind cooperation.

It is a glorifying pleasure for me to record my thanks to all the Honourable Vice-Chancellors, University of North Bengal, Dean, Faculty of Science, Registrar and other officials of the University during my research tenure for the academic and administrative facilities extended to me.

I express my sincere gratitude to Staffs of the Ph.D. section particularly Mr. Dilip Singha and Central Library, University of North Bengal, for their services and kind help.

I like to sincerely acknowledge the University Grants Commission (UGC), New Delhi, for providing the financial assistance to carry out my research work.

I express my humble and whole hearted gratitude to the Almighty for bestowing upon me with the people like Dr. Nishika Jaishee, Dr. Nita Tamang and Mrs. Tulshi Thapa, Mrs. Swati Singh who played very vital role in facing the complexities of life and completing this work successfully.

I am lucky to be introduced with Dr. Abhijit Barui, Veterinary Officer, Block Animal Health Centre, Mekhliganj, Coochbehar, who helped me to overcome many logistics problems during my study.

I would also like to thank my fellow labmates Dr. Manik Lal Acharjee, Dr. Rakesh Prasad Bhagat and Dr. Arpita Dey for constant co-operation and encouragement for successful completion of my work.

A special note of thanks to my wonderful seniors, friends and junior Mr. Benoy Rai, Dr. Anjali Prasad, Dr. Sangita Khewa Subba, Dr. Ritesh Biswa, Dr. Kumar Basnet, Dr. Dawa Bhutia, Dr. Tanmay Mukhopadhyay, Dr. Sutanuka Chattaraj, Dr. Uttara Dey Bhowmick, Mr. Tanmoy Dutta, Mrs. Jayashree

Saren, Mr. Mrinal Ray, Mr. Sanjib Sarkar, Mrs. Najnin Islam, Mrs. Trisita Mazumdar, Dr. Susmita Dutta and Dr. Minu Bharati for their constant co-operation valuable suggestions and constant encouragement for successful completion of my work.

I would also like to thank my fellow Research Scholars from the Department, Mr. Subhasis Paul, Mr. Debabrata Modak, Mr. Sudipta Kumar Roy, Mr. Sourav Sarkar, Miss Farha Yasmin, Mr. Sona Sutradhar, Miss Shaswati Ghosh, Mr. Rejuan Islam, Mr. Amlan Jyoti Ghosh, Mr. Sagar Sarkar, Mr. Manas Modak, Miss Priyanka Rai, Mr. Abhishek Subba, Mr. Bhaskar Paul, Miss Prerna Bhujel, Miss Nilu Limboo, Miss Prapti Das and Miss Esha Roy, for their good wishes.

I am deeply indebted to Principal, Dr. Somnath Mukhopadhyay, and to all my colleagues, Dr. Ipsit Chakraborty, Dr. Mausumi Bhattacharjee, Dr. Sutapa Gupta, Dr. Swapna Banerjee, Mr. Joydeep Das, Mrs. Banani Bindani, Mr. Arup Kumar Ghosh and Ms. Susmita Mazumder of Dinabandhu Andrews College, Kolkata affiliated to University of Calcutta, for their kind help and support.

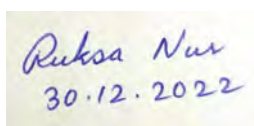
Immense thanks to Mr. Sain Shaikh, Assistant Professor, Department of Electronics & Communication Engineering, Aliah University, Kolkata for generously helping out whenever technology, somewhat predictably, conspired to defeat me (usually just before a deadline). My sincere thanks are extended to him.

I do not know how to thank my parents Mr. Md Moinuddin and Mrs. Maimuna Khatun, for the core values that they helped me inculcate, of which this work is a small reflection. May God bless them always with sound health and happiness.

And, I am thankful to my beloved brothers Md Mahebul Raheman, Md Babul Reja and Md Kabul Reja, for their love, patience, understanding and encouragement, which enabled this thesis to proceed even when sunny days and other enticing distractions beckoned.

Finally, I urge to claim the originality for the matter presented in the text and where weaknesses and errors remain, they are of course solely my responsibility.

Last but not the least, I am thankful to all of them who directly and indirectly helped me for the thesis over past couple of years.



Ruksa Nur
30.12.2022

(Ruksa Nur)

Department of Zoology, University of North Bengal

LIST OF TABLES

Table 3.1 Semi-purified Diets prepared for determination of Optimum Protein Requirement	22
Table 3.2 Proximate composition (g/100 g diet) of Semi-purified Diets prepared for determination of Optimum Protein Requirement	23
Table 3.3 Semi-purified Diets prepared for determination of Optimum Carbohydrate Requirement	26
Table 3.4 Proximate composition (g/100 g diet) of Semi-purified Diets prepared for determination of Optimum Protein Carbohydrate Requirement	26
Table 3.5 Semi-purified Diets prepared for determination of Optimum Lipid Requirement.	27
Table 3.6 Proximate composition (g/100 g diet) of Semi-purified Diets prepared for determination of Optimum Lipid Requirement	28
Table 3.7 Semi-purified Diets prepared for determination of Optimum Energy Requirement	30
Table 3.8 Proximate composition (g/100 g diet) of Semi-purified Diets prepared for determination of Optimum Lipid Requirement.	30
Table 4.1 Descriptive statistics, estimated Length–weight relation parameters and Condition Factor (K) of <i>N. hexagonolepis</i> fingerlings from three different sites of River Ghish, Darjeeling Himalaya, West Bengal.	42
Table 4.2 Estimated Relative Gut length (RGL) of <i>N. hexagonolepis</i> fingerlings from three different sites of River Ghish, Darjeeling Himalaya, West Bengal.	44
Table 4.3 Critical thermal maxima (CT _{max}) and critical thermal minima (CT _{min}) of <i>N. hexagonolepis</i> acclimated to seven different temperatures	46
Table 4.4 Growth performance of <i>N. hexagonolepis</i> fingerlings reared at increasing levels of stocking density	47
Table 4.5 Growth performance of <i>N. hexagonolepis</i> fingerlings reared at increasing levels of feeding frequency	48
Table 4.6 Growth performance of <i>N. hexagonolepis</i> fingerlings reared at increasing levels of feeding rate	49
Table 4.7 Growth performance of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Protein	51
Table 4.8 Growth performance of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Protein to Carbohydrate ratio	54
Table 4.9 Growth performance of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Lipid	57
Table 4.10 Growth performance of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Protein to energy levels	60

Table 4.11 Digestive enzyme activity of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Protein levels	62
Table 4.12 Digestive enzyme activity of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Carbohydrate levels	64
Table 4.13 Digestive enzyme activity of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Lipid levels	66
Table 4.14 Digestive enzyme activity of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Energy levels	68
Table 4.15 Study of physico-chemical parameters of the rearing system of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Protein	74
Table 4.16 Pearson's Correlation Coefficients matrix for physico-chemical parameters of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Protein	75
Table 4.17 Study of physico-chemical parameters of the rearing system of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Carbohydrates	81
Table 4.18 Pearson's Correlation Coefficients matrix for physico-chemical parameters of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Carbohydrate	82
Table 4.19 Study of physico-chemical parameters of the rearing system of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Lipids	88
Table 4.20 Pearson's Correlation Coefficients matrix for physico-chemical parameters of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Lipid	89
Table 4.21 Study of physico-chemical parameters of the rearing system of <i>N. hexagonolepis</i> fed with diet containing increasing levels of Dietary Energy1	96
Table 4.22 Pearson's Correlation Coefficients matrix for physico-chemical parameters of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Energy	97

LIST OF FIGURES

Figure 3.1 Sampling and Experimental sites (A. River Ghish, B. Aquaculture Unit, Dept. of Zoology, NBU, Fisheries Research & Training Centre, Dept. of Zoology, NBU) showing their respective locations (Lat and Longitude)	13
Figure 3.2 Fingerling of <i>N. hexagonolepis</i> (McClelland, 1839)	15
Figure 3.3 A Schematic representation of the Experimental Design	16
Figure 3.4 Sampling sites (1, 2 & 3) at River Ghish, Pemling Forest, Kalimpong I, Darjeeling, West Bengal, India.	18
Figure 4.1 Shows the Regression graph of Length-Weight Relationship of <i>N. hexagonolepis</i> fingerlings collected from three different sites (A-Site 1, B- Site 2 and C-Site 3).	43
Figure 4.2 Shows the linear regression graph of Total body length (cm) and Gut length (cm) of <i>N. hexagonolepis</i> fingerlings collected from three different sites (A-Site 1, B- Site 2 and C-Site 3).	45
Figure 4.3 Thermal tolerance polygon of <i>N. hexagonolepis</i> generated from the critical thermal limits (CTmax and CTmin) at seven different temperatures	46
Figure 4.4 Second-degree polynomial relationships of Specific Growth Rate (SGR) to varying levels of Dietary Protein	52
Figure 4.5 Line diagram showing relationships between Food Conversion Ratio and Feed Conversion Efficiency to varying levels of Dietary Protein	52
Figure 4.6 Second-degree polynomial relationships of Specific Growth Rate (SGR) to varying levels of Dietary Protein to Carbohydrate	55
Figure 4.7 Line diagram showing relationships between Food Conversion Ratio and Feed Conversion Efficiency to varying levels of Dietary Carbohydrate	55
Figure 4.8 Second-degree polynomial relationships of Specific Growth Rate (SGR) to varying levels of Dietary Protein to Lipid	58
Figure 4.9 Line diagram showing relationships between Food Conversion Ratio and Feed Conversion Efficiency to varying levels of Dietary Lipid	58
Figure 4.10 Second-degree polynomial relationships of Specific Growth Rate (SGR) to varying levels of Dietary Protein to Energy ratio	61
Figure 4.11 Line diagram showing relationships between Food Conversion Ratio and Feed Conversion Efficiency to varying levels of Dietary Energy	61
Figure 4.12 Relationship between digestive protease and Specific Growth Rate of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Protein	63

Figure 4.13 Pattern of digestive amylase and lipase with of <i>N. hexagonolepis</i> fingerlings fed with increasing levels of Dietary Protein	63
Figure 4.14 Relationship between digestive protease and Specific Growth Rate of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Carbohydrate	65
Figure 4.15 Pattern of digestive protease and lipase with of <i>N. hexagonolepis</i> fingerlings fed with increasing levels of Dietary Carbohydrate	65
Figure 4.16 Relationship between lipase and Specific Growth Rate of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Lipid	67
Figure 4.17 Pattern of digestive protease and amylase with of <i>N. hexagonolepis</i> fingerlings fed with increasing levels of Dietary Lipid	67
Figure 4.18 Relationship between digestive protease and Specific Growth Rate of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Energy	69
Figure 4.19 Pattern of digestive amylase and lipase with of <i>N. hexagonolepis</i> fingerlings fed with increasing levels of Dietary Energy	69
Figure 4.20 Physico-chemical parameters (Ammonium Nitrogen, Nitrite, Nitrate and Phosphate) of the rearing system of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Protein	76
Figure 4.21 Physico-chemical parameters (Ammonium Nitrogen, Nitrite, Nitrate and Phosphate) of the rearing system of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Carbohydrates	83
Figure 4.22 Physico-chemical parameters (Ammonium Nitrogen, Nitrite, Nitrate and Phosphate) of the rearing system of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Lipids	90
Figure 4.23 Physico-chemical parameters (Ammonium Nitrogen, Nitrite, Nitrate and Phosphate) of the rearing system of <i>N. hexagonolepis</i> fed with increasing levels of Dietary Energy	98

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOAC	Association of Official Agricultural Chemists
APHA	American Public Health Association
IMC	Indian Major Carp
RGL	Relative Gut Length
FCR	Food Conversion ratio
LWR	Length-Weight Relationship
FCE	Food Conversion Efficiency
SGR	Specific Growth Rate
PER	Protein Efficiency Ratio
CP	Crude Protein
DE	Digestible Energy
GE	Gross Energy
pH	Hydrogen ion concentration
TDS	Total Dissolved Solids
DO	Dissolved Oxygen
CO ₂	Free Carbondioxide
TA	Total Alkalinity
TH	Total Hardness
cm	Centimeter
@	At the rate
no.	Number
M	Mean
SE	Standard Error
p	Probability
°C	Degree Celcius
%	Per cent
mg/l	milligram per litre
µS cm ⁻¹	micro simens per centimeter