Dedicated to

my parents

Mr. Mir Murshed Ali

and

Mrs. Benajir Ahmed

Declaration

I declare that the thesis entitled **"Role of gut melatonin and dietary nutrients on the major digestive enzymes and management of oxidative stress in early and late juvenile female carp, *Catla catla* (Hamilton, 1822)"** has been prepared by me under the supervision of Dr. Sourav Mukherjee, Assistant Professor of Zoology, University of North Bengal. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

Farha Yasmin 16/08/2024

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ACCREDITED BY NAAC WITH GRADE B++

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CERTIFICATE

16th August, 2024

This is to certify that research work embodied in the thesis entitled **"Role of gut melatonin and dietary nutrients on the major digestive enzymes and management of oxidative stress in early and late juvenile female carp, *Catla catla* (Hamilton, 1822)"** has been carried out by **Ms. Farha Yasmin** under my supervision and guidance in the Fish Biology and Endocrinology Laboratory, Department of Zoology, University of North Bengal, Raja Rammohunpur, Siliguri, Darjeeling, West Bengal, India, PIN: 734 013. The work incorporated in this thesis is original and has not been submitted by her for any diploma and degree to this or any other University. She has followed all the rules and regulations led by University for the fulfillment of requirements for the degree of Doctor of Philosophy in Science. She had delivered the Pre-Ph.D. Seminar on 12th August, 2024 before the members of the Departmental Research Committee (DRC) in Zoology, and all the suggestions are taken care of and since been incorporated before the submission of the thesis.

16/8/24

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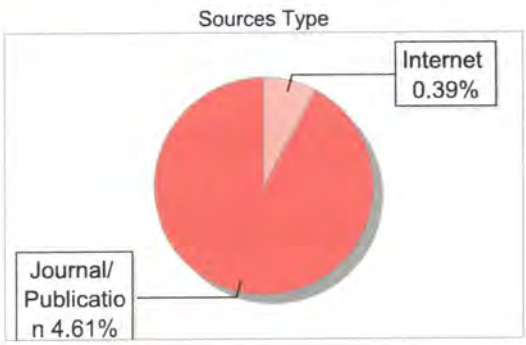
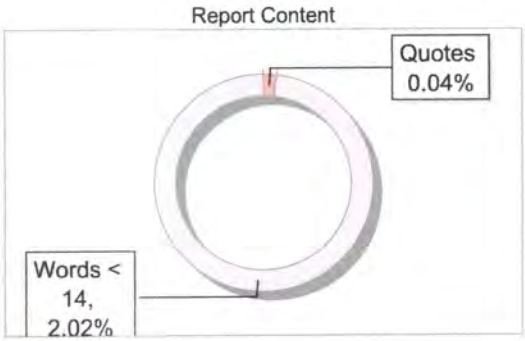
Submission Information

Author Name	Farha Yasmin
Title	Role of gut melatonin and dietary nutrients on the major digestive enzymes and management of oxidative stress in early and late juvenile female carp, Catla catla (Hamilton, 1822).
Paper/Submission ID	2187402
Submitted by	nbuplg@nbu.ac.in
Submission Date	2024-07-31 13:05:34
Total Pages, Total Words	122, 33579
Document type	Thesis

Result Information

Similarity **5 %**





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Database Selection

Language	English
Student Papers	Yes
Journals & publishers	Yes
Internet or Web	Yes
Institution Repository	Yes

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Acknowledgment

It has been a journey of almost six years of my life. There are many people without whom this accomplishment would not have been possible. I want to express my deepest gratitude and appreciation to all those who have supported and guided me throughout this process.

First and foremost, I would like to thank my supervisor, Dr. Sourav Mukherjee, Assistant Professor, Department of Zoology, University of North Bengal for his invaluable guidance, patience, and encouragement. His insightful feedback and unwavering support have been crucial in completing this thesis. I am deeply grateful for the countless hours he has dedicated to reviewing my work and providing constructive criticism.

I am grateful to the present and former Head of the Department of Zoology, University of North Bengal, for allowing me to use the central instrumentation facility during my research.

I would also like to extend my gratitude to all my teachers in the department: Prof. Min Bahadur, Prof. Soumen Bhattacharjee, Prof. Dhiraj Saha, Dr. Tilak Saha, Dr. Arpan Kr. Maiti for their expert advice, insightful comments, and continuous support.

I would also like to thank the non-teaching staff of the Department for their kind cooperation and support during my work.

I am extremely grateful to Prof. Koushik Ghosh, Department of Zoology, University of Burdwan, for allowing me to conduct the proximate analysis of diets used in the study in his laboratory.

I wish to express my gratitude to Prof. Asamanja Chattoraj, Department of Zoology, Kazi Nazrul University, for his encouragement, valuable concepts, insights, and precious suggestions regarding my research.

I am thankful to my school and college teachers, whose inspiration has always motivated

me to persevere. I want to acknowledge Dr. Dipanajana Majumdar, Assistant Professor at Siliguri College, whose guidance has inspired me a lot. Additionally, Mr. Sandeep Basu, my biology teacher from school, has consistently inspired and supported me in excelling in my career.

I am also grateful to my lab mates, Mr. Sona Sutradhar and Mr. Arun Roy, for their help and company during my research. I had the fortune to have outstanding seniors: Dr. Bappaditya Ghosh, Dr. Sutanuka Chattoraj, Dr. Uttara Dey Bhowmick, Dr. Subhasish Paul, Dr. Minu Bharati, Dr. Priyanka Rai, Mr. Abhishek Subba, Mr. Tanmoy Dutta, Mr. Debabrata Modak, Mrs. Trishita Majumdar, Mrs. Swati Singh Dey in the department. Dr. Bappaditya Ghosh has been a pillar of support throughout my Ph.D. journey, not only as a senior research scholar of the department but also as a mentor and an elder brother, especially during challenging times. I have learned many things from him. I want to extend my gratitude to all other fellow research scholars: Mr. Sudipta Kumar Roy, Mr. Sourav Sarkar, Miss Arpita Ray, Mr. Debojit Dutta, Miss Shaswati Ghosh, Mr. Rejuan Islam, Mr. Amlan Jyoti Ghosh, Mr. Sagar Sarkar, Mr. Manas Modak, Mr. Bhaskar Paul, Miss Prerna Bhujel, Miss Nilu Limboo, Miss Prapti Das, Mr. Supriyo Ghosh, Mr. Subhajit Das, Mr. Abhirup Saha, Mr. Arijit Deb.

True friends are rare gems in everyone's life, and I am incredibly fortunate to have such friends. Mr. Sudipta Kumar Roy has stood by my side since college, supporting me through every personal, academic, and research-related challenge. Mrs. Aishwaria Banik, Mrs. Ankita Ghosh, and Miss Esha Roy hold a special place in my heart. I have shared my joys and frustrations with them. The countless good times we have spent together have been like a balm, providing comfort and happiness. I would also like to acknowledge Mr. Russel Sarkar for his unconditional help and support during last phase of my Ph.D.

I also want to express my gratitude to those who misjudged or mistreated me, as their

actions gave me strength and resilience. I extend my heartfelt good wishes to them.

I would like to express my heartfelt gratitude to Mr. Ayan Mahanty and Ms. Soumita Bhattacharjee for their invaluable assistance whenever I needed it.

I wish to express my deep respect and regard for my parents, Mr. Mir Murshed Ali and Mrs. Benajir Ahmed, whose unwavering encouragement and endless blessings have made it possible for me to complete this work.

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

The Council of Scientific and Industrial Research (CSIR), Govt. of India, New Delhi (37(1737)/19/EMR-II), and North Bengal University (1950/R-2019 and 2309/R-2022) are thankfully acknowledged.

This thesis would not have been possible without the contributions and support of all these wonderful individuals. Thank you all from the bottom of my heart.

Farha Yasmin
16/08/2024

PREFACE

The research presented in this thesis, titled **'Role of gut melatonin and dietary nutrients on major digestive enzymes and management of oxidative stress in early and late juvenile female carp, *Catla catla* (Hamilton, 1822),'** was initiated by me in 2019 under the supervision of Dr. Sourav Mukherjee in the 'Fish Biology and Endocrinology Laboratory,' Department of Zoology, University of North Bengal, Darjeeling.

Melatonin (5-methoxy-*N*-acetyltryptamine) is an indole amine hormone that is synthesized in the pineal, retina, gut, and many other tissues in vertebrates. Tryptophan is the precursor molecule that is converted into melatonin through a series of four consecutive steps involving four enzymes (5-tryptophan hydroxylase, 5-hydroxy tryptophan decarboxylase, arylalkylamine-*N*-acetyltransferase, hydroxy Indole-O-methyltransferase/ N-acetylserotonin O-methyltransferase. The environmental synchronizer of melatonin within the gut tissue is different from that in the pineal organ/gland of a vertebrate. Unlike melatonin synthesis in the pineal organ/gland, gut melatonin synthesis is not dependent on the light-dark cycle. However, factors such as the availability, timing, and frequency of daily feedings, as well as the quality of the food, may influence melatonin levels in the gut, at least in fish species. During the current decade, there has been increasing interest in research on gut melatonin. Apart from its endocrine functions, this molecule is emerging as a significant autocrine, paracrine, luminal effector, or detoxifying modulator. It plays a crucial role in the regular activities of digesting food particles for better nutritional assimilation by the animal.

In natural environments, fish species often exhibit changes in feeding preferences during different growth phases of the juvenile stage. These changes are attributed to their varying feeding and digestive capabilities. These feeding variations may also coincide with the annual periodic abundance of natural foods in the environment, which correlates

with changes in gut mass parallel to body mass fluctuations. Until ingested food is processed for absorption through digestion, it can be viewed as a foreign particle by the body's physiology. The gut wall serves as the initial barrier, activating various mechanisms, among which the antioxidative defense mechanism plays a crucial role in safeguarding against potential harm. A significant relationship between gut melatonin, detoxifying enzymes, and vital digestive enzymes in fish has been noted during the past decades.

Until now, research has explored the role of L-tryptophan (Trp) as a nutraceutical component in dietary modulation of gut melatonin synthesis across various animals. These studies suggest that Trp availability in food could be a key factor influencing melatonin synthesis in the digestive tract. However, the response may vary depending on the amount of Trp administered. While studies have investigated the role of Trp content in the diet, no research has yet explored the influence of other major nutritional components, such as protein, carbohydrates, and lipids, on the regulation of gut melatonin synthesis in carp. Furthermore, the role of diet-modulated gut melatonin in gut histo-architecture and ovarian physiology remains an unexplored field for investigation. Hence, the study has been designed.

This thesis is structured into four chapters. **Chapter 1** provides a brief **'introduction'**. **Chapter 2** covers the **'review of literature and objectives of the study.'** **Chapter 3** details the **'materials and methods'** used. **Chapter 4** entails the **'results'** of the study. **Chapter 5** contains a detailed **'discussion'** of the study's findings. Lastly, **Chapter 6** describes **'summary and conclusion.'** The list of research articles and the papers presented at different conferences, along with the bibliography, have been added in the last section of the thesis.

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	(SOD), (B) catalase (CAT), (C) glutathione peroxidase (GPx), (D) glutathione reductase (GRd), (E) glutathione S-transferase (GST), (F) reduced glutathione (GSH) in the ovary of the late juvenile (LJv) carps, which were supplied with SD, or PRD1, PRD2, or TrpRD1, or TrpRD2, or CRD, or ORD daily at ~10:00 h for 30 days. Different letters on the mean values indicate significant ($p < 0.05$) differences among the experimental groups.	

List of tables

List of abbreviation

AANAT	Arylalkylamine-*N*-acetyltransferase
AMPK	5' AMP-activated protein kinase
ASMT	*N*-acetylserotonin *O*-methyltransferase
BHA	Butylated hydroxyanisole
BL	Body length
BSA	Bovine serum albumin
BW	Body weight
Ca^{2+}	Calcium cations
cAMP	Cyclic adenosine monophosphate
Carb.	Carbohydrate
CAT	Catalase
CCD	Charge-coupled device
CCK	Cholecystokinin
CDNB	1-chloro-2, 4-dinitrobenzene
CF	Coniferyl ferulate
cGMP	Cyclic guanosine monophosphate
CMC	Carboxymethylcellulose
CRD	Carbohydrate-rich diet
D	Dimension
˚C	Degree centigrade
DD	Constant dark
DNSA	3,5-dinitro salicylic acid
DTNB	5'-dithiobis (2-nitrobenzoate)
EDTA	Ethylene diamine tetra acetic acid
EJv	Early juvenile
ELISA	Enzyme-linked immunosorbent assay
FAA	Food anticipatory activity
FI	Feeding intensity
FO	Fish oil
FRTC	Fisheries Research and Training Centre
FTT	Food transmission time

G	Gram
GaSI	Gastro-somatic index
GH	Growth hormone
GH–IGF	Growth hormone-insulin-like growth factor
GHR	Growth hormone receptor
GIT	Gastrointestinal tract
GPx	Glutathione peroxidase
GRd	Glutathione reductase
GSH	Reduced glutathione
GSI	Gonado-somatic index
GSSG	Glutathione oxidized
GST	Glutathione S-transferase
GVBD	Germinal vesicle breakdown
H	Height
H	Hour
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
5-HT	5-hydroxytryptamine
5-HTP	5-hydroxy-tryptophan
Hiomt	Hydroxy Indole-O-methyltransferase
HIOMT	Hydroxyindole-O-methyltransferase
IGF1	Insulin-like growth factor 1
IGF1R	Insulin-like growth factor 1 receptor
IGF2	Insulin-like growth factor 2
Kg	Kilogram
L: D	Light: Dark
LC-PUFA	long-chain polyunsaturated fatty acids
LH	Luteinizing hormone
LJv	Late juvenile
LP	Long photoperiod
LV	Length of villi
M	Mucosal
MDA	Malondialdehyde
mg	Milligram

µm	Micrometer
$MgCl_2$	Magnesium chloride
MIH	Maturation-inducing hormone
Min	Minute
mL	Milliter
Mm	Millimeter
MPF	Maturation-promoting factor
MU	Muscularis
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
NBT	Nitro blue tetrazolium
NBU	North Bengal University
nm	Nanometer
NP	Natural photoperiod
OPD	o-phenylenediamine
ORD	Oil-rich diet
PBS	Phosphate buffer saline
%	Percentage
PMS	5-methylphenazinium methyl sulfate
PMSF	Phenyl methyl sulphonyl fluoride
PNPP	p-nitrophenyl phosphate
PRD1	Protein-rich diet 1
PRD2	Protein-rich diet 2
Pro.	Protein
RGC	Residual gut content
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RT	Room temperature
SCN	Suprachiasmatic nuclei
SD	Standard diet
SE	Standard error
SGR	Specific growth rate
SM	Submucosa

SO	Serosa
SOD	Superoxide dismutase
SP	Short photoperiod
TBA	2-thiobarbituric acid
TCA	Trichloroacetic acid
TOR	target of Rapamycin
TPH1	Tryptophan hydroxylase 1
Trp	L-Tryptophan
TrpRD1	Tryptophan rich diet 1
TrpRD2	Tryptophan rich diet 2
TRPV1	Transient receptor potential vanilloid family 1
TRPV2	Transient receptor potential vanilloid family 2
UV Vis	Ultraviolet visible
UV	Ultraviolet
V	Villi
VO	Vegetable oil
WG	Weight gain
WT	Water temperature
WV-B	Width of villi at the base
WV-T	Width of villi at the tip