

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
(Farha Yasmin) 16/08/2024
Research Scholar
Department of Zoology

UNIVERSITY OF NORTH BENGAL

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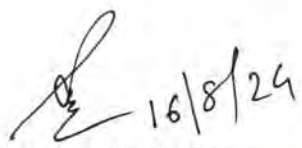
P.O. North Bengal University
Raja Rammohunpur, Dist. Darjeeling
West Bengal, India, PIN - 734013

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.

Date: 16th August, 2024


Dr. Sourav Mukherjee

(Supervisor)

Assistant Professor

Department of Zoology

University of North Bengal

Dist. – Darjeeling

West Bengal, INDIA

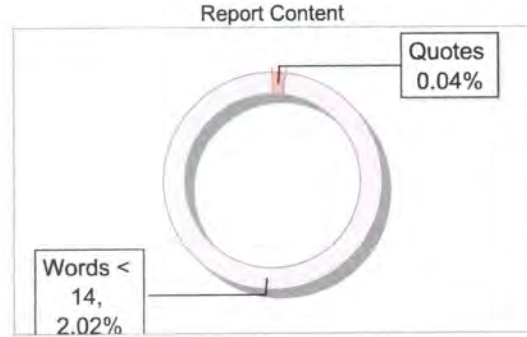
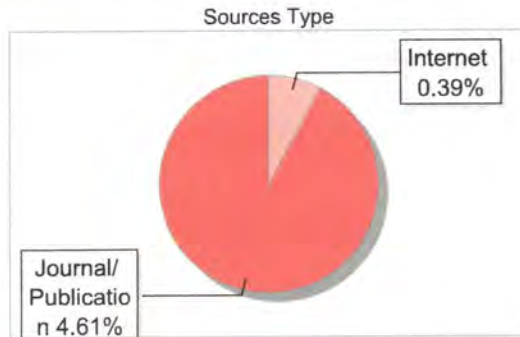
Dr. Sourav Mukherjee
Assistant Professor
Department of Zoology
University of North Bengal
Dist- Darjeeling, West Bengal
Pin- 734013

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Dr. Sourav Mukherjee
Assistant Professor
Department of Zoology
University of North Bengal
Dist- Darjeeling, West Bengal
Pin- 734013



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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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AANAT	Arylalkylamine- <i>N</i> -acetyltransferase
AMPK	5' AMP-activated protein kinase
ASMT	<i>N</i> -acetylserotonin <i>O</i> -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 ₂ O 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