INFLUENCE OF DIFFERENT ZINC SOURCES ON THE PRODUCTION PERFORMANCE OF *CIRRHINUS MRIGALA*

A Thesis

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In partial fulfilment of the requirements for the degree of

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In

AQUACULTURE

By

KHERWAL RAJ KISKU

B.F. Sc

DEPARTMENT OF AQUACULTURE
Faculty of Fishery Sciences
West Bengal University of Animal and Fishery Sciences
5 - Budherhat Road, Chakgaria, Kolkata - 700 094
WEST BENGAL, INDIA

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Dedicated to My
Parents
and
My Respected Guide

Department of Aquaculture
Faculty of Fishery Sciences
West Bengal University of Animal and Fishery Sciences
5, Budherhat Road, Chakgaria, P.O.- Panchasayar, Kolkata-94
(Main Campus: 68, Kshudiram Bose Sarani, Belgachia, Kolkata-700 037)

Dr. Surya Kanta Sau, *M.F.Sc., Ph.D.*
Assistant Professor

Date: 02/02/2023

Certificate

This is to certify that the work recorded in the thesis entitled "**Influence of different Zinc sources on the production performance of *Cirrhinus mrigala***" submitted by **Mr. Kherwal Raj Kisku** in partial fulfilment of requirements for the degree of Master of Fishery Sciences (Aquaculture) in the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, is the faithful and bonafide research work carried out under my supervision and guidance. The results of the investigation reported in this thesis have not so far been submitted for any other degree or diploma. The assistance and help received during the course of investigation have been duly acknowledged.

Dated: - 02/02/2023
Chakgaria Campus,
Kolkata -700094

(Dr. S. K. Sau)
Chairman
Advisory Committee

West Bengal University of Animal and Fishery Sciences

Faculty of Fishery Sciences
Department of Aquaculture
5, Budherhat Road, Chakgaria;
P.O. Panchasayar, Kolkata- 700094

Approval Sheet

APPROVAL OF EXAMINERS FOR AWARD OF THE DEGREE OF MASTER OF FISHERY SCIENCE (AQUACULTURE)

We, the undersigned, having satisfied with the performance of ***Mr. Kherwal Raj Kisku***, in the Viva– Voce Examination, conducted today, on 02/02/2023..... recommended that the thesis be accepted for the award of degree.

Name	Signature
1. **Dr. S. K. Sau** **Chairman, Advisory Committee**	02/02/23
2. Dr. R N. Mandal **External Examiner**	
3. **Prof. S. K. Das** **Member, Advisory Committee**	2/2/2023
4. **Prof. T. K. Ghosh** **Member, Advisory Committee**	2/2/23
5. **Dr. G. Dash** **Member, Advisory Committee**	
6. **Dr. B. Saha** **Member, Advisory Committee**	

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Place:Kolkata-700094

Kherwal Raj Kisku

(Kherwal Raj Kisku)

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Abbreviations

%	:	Percent
FAO	:	Food and Agriculture Organization
MT	:	Million tonnes
KG	:	Kilogram
USD	:	United States Dollar
Zn	:	Zinc
Fe	:	Iron
nm	:	Nano meter
NPs	:	Nanoparticles
ZnO	:	Zinc Oxide
Etc.	:	Etcetera
SiO_2	:	Silicon dioxide
TiO2	:	Titanium dioxide
nZnO	:	Nano Zinc Oxide
g	:	Gram
US	:	United states
$	:	Dollar
B.C	:	Before Christ
AD	:	Anno Domini
UN	:	United Nations
ICAR	:	Indian Council of Agricultural Research
FFDA	:	Fish Farmers' Development Agencies
t	:	Tonne
ha^{-1}	:	Per hectare
DADF	:	Department of Animal Husbandry & Dairying
PPP	:	Public Private Partnerships
yr^{-1}	:	Per year
USSR	:	Union of Soviet Socialist Republics
^oC	:	Degree Celsius
BW	:	Body weight
kJ	:	Kilojoule
CP	:	Crude protein
P:E	:	Protein : Energy
Kcal	:	Kilocalorie
Mg	:	Miligram
NRC	:	Nutrition Rehabilitation Centre
NSP	:	Non-starch polysaccharides
DNA	:	Deoxyribonucleic Acid
e.g	:	Exempli gratia
viz.	:	Videlicet
ROS	:	Reactive oxygen species
GPx	:	Glutathione peroxidase
Mn	:	Manganize
Cu	:	Copper

Se	:	Selenium
i.e	:	id est
Ag	:	Argentum/silver
µ	:	Micrometers or microns
l	:	Litre
$ZnCl_2$	:	zinc chloride
$ZnSO_4$	:	zinc sulphate
L	:	Lattitude
N	:	North
E	:	East
AM	:	Ante Meridiem
PM	:	Ante Meridiem
ml	:	Millilitre
B.P.	:	Boiling point
DM	:	Dry matter
h	:	Hour
APHA	:	American Public Health Association
@	:	At the rate
Mm	:	*millimeter*
dl	:	Decilitre
Rpm	:	Revolutions per minute
Dd	:	Double distilled
M	:	Moles
NaOH	:	Sodium hydroxide
SGR	:	Specific growth rate
FCR	:	Feed conversion ratio
Hb	:	Hemoglobin
RBC	:	Red blood cell
WBC	:	white blood cell
Hct	:	hematocrit test
MCH	:	Mean corpuscular hemoglobin
MCV	:	Mean corpuscular volume
MCHC	:	Mean corpuscular hemoglobin concentration
ALT	:	Alanine transaminase
AST	:	Aspartate aminotransferase
pH	:	Potential of Hydrogen
DO	:	Dissolved Oxygen
ABWG	:	Average body weight gain
DWG	:	Daily weight gain
Ltd.	:	Limited
UV	:	Ulta violet
SD	:	Standard deviation
U	:	Unit
ANOVA	:	Analysis of Variance
SPSS	:	Statistical Package for Social Sciences

ABSTRACT

Zinc as an essential trace element in fish diets that is required for growth, immunity and antioxidant defence mechanisms. The present investigation was performed to evaluate the efficiency of different sources of zinc as a fish feed additive in feeding *Cirrhinus mrigala*. Present study was conducted to compare the effect of different dietary inorganic sources of zinc i.e., Zinc oxide and zinc oxide nano particles on growth performance, some haematological indices and enzymatic activity of mrigal. This experiment was executed under laboratory condition for 56 days. Two hundred and twenty five mrigal advanced fry of uniform size were distributed into five experimental groups in triplicate manner and each tank stocked with fifteen number of fish. Each tank was filled with tap water of 120 litter with fully aeration system. One third water was exchanged alternative day by siphoning to control the water quality. Fish were fed with a basal diet(control) and treatment diet supplemented with zinc oxide at concentration of 30 mg/kg(T1) and zinc oxide nano particle at concentration of 30, 15 and 7.5 mg/kg (T2, T3, T4) respectively. The fish were fed daily two times at the rate of 3% of their body weight. The growth performance was recorded weekly basis and the haematology, enzymology parameters are tested after completion of the experiment. After 56 days of feeding trial, growth performance, haematological, enzymological changes of *Cirrhinus mrigala* showed significant ($P < 0.05$) differences between control and other treatments. The treatment group T1, T2, T3, T4 showed higher body weight gain, specific growth rate and low FCR. T3(15mg/kg) achieved highest growth than control and another treatment. Growth performance was impressive than basal diet. Haematological performance also assessed through blood parameter analysis like haemoglobin, RBC, WBC, PCV, MCV, MCH, MCHC counts. Hb, RBC, PCV and platelet counts showed increasing in numbers and WBC count was decreased in treatment as compare to control. Enzymatic activity recorded highest in T3 and lowest in control. The survivability was recorded with control group having 100% and T1, T2, T3 and T4 group having 98.61%, 98.26%, 99.31% and 91.67% survivability respectively. It may be concluded that 15mg nano ZnO/kg of supplemented feed showed better and significant ($p<0.005$) performance in mrigal which can substitute control diet for better production performance.

Key words: Mrigal, *Cirrhinus mrigala*, ZnO, nano ZnO, nanoparticles.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Aquaculture is an excellent option for dealing with, and even exceeding, the productivity of fishing activities while alleviating stress on natural resources (Freitas *et al.*, 2020). Aquaculture is regarded as the world's fastest growing food producing sector and now accounts for 52% of the global fish consumption (FAO, 2020). The nutritional benefits of fish have a positive effect on increased food security and decreased poverty rates in developing states. Intensive aquaculture has experienced tremendous growth in the last ten years (FAO, 2020). Global aquaculture production is expected to continue growing at a rapid pace, and comprise an increasing share of global protein production (Garlock *et al.*, 2020). Asia is found to have contributed 89 % of total world fed aquaculture production in terms of quantity and 69 % in terms of value. Fisheries and aquaculture are reported to have supplied almost 179 million tonnes (MT) of edible fish to the globe in 2018, yielding a per capita supply of 20.5 kg (live weight equivalent), with a total first sale value of USD 401 billion, with 82 MT originating from aquaculture output (FAO, 2020). Aquaculture is considered one of the most important food production systems both in terms of economic impact and food security, and the ongoing development of this industry is a key factor in the strategy to guarantee global nutritional safety. With increase in world population and rapid economic growth, the demands for protein are on rise. Due to positive health effects and important food features of composition, aquatic protein resources are highly appreciated. Therefore, global aquaculture has grown at impressive rate recently. Now a days, aquaculture is considered a fastest blooming global food industry, playing a crucial role in fulfilling the increased demand for animal protein requirements (Shah and Mraz, 2019).

Trace elements are dietary minerals required in minute quantities for normal physiological function. They are mostly structural components of enzymes or cofactors whose roles include the prevention of nutritional deficiencies, immune functions, regulation of gene expression, antioxidant defence, and prevention of chronic diseases. These essential micronutrients are absorbed from the gastro-intestinal tract and stored in the liver and these include: zinc, selenium, chromium, cobalt, copper, fluorine, iodine, iron, manganese and molybdenum (Strachan, 2010). The role of trace elements in biological systems has been described in several animals including fish. They are required for the normal life processes like skeletal formation, maintenance of colloidal systems, regulation of acid-base equilibrium and for biologically important compounds such as hormones and enzymes (Watanabe *et al.*, 1997; Ahmad *et al.*,

2013). Mineral deficiencies can cause biochemical, structural and functional pathologies, which depend on several factors including the duration and degree of mineral deprivation.

Zinc (Zn) is the second most abundant trace element in the animal body after iron (Fe). It cannot be stored in the body and can be consumed as regular dietary intake to meet the physiological need of the body (Zalewski *et al.,* 2005). It is required for growth, immune function, nutrition, metabolism, fertility, wound healing and maintenance of oxidative stress in animals (Kietzmann and Braun, 2006; Feng *et al.,* 2010; Liu *et al.,* 2011; Zhao *et al.,* 2014). Zinc is an essential trace element for finfish and plays a critical role in biological processes and physiological functions such as biosynthesis of hormones, enzymatic activity, and metabolism of proteins, carbohydrates and lipids (Muralisankar *et al.,* 2014). Zinc is an important trace element in fish nutrition as it is involved in various metabolic pathways. It is required for growth and metabolism of all vertebrates including fish. It is needed in more than 1000 structural, catalytic and regulatory proteins, which are important for growth, development and physiology of animals (Eide, 2006; Maret and Krezel, 2007). It is a specific cofactor of many enzymes, involved in different metabolic pathways and conformation of nucleoprotein filament (Eckerich *et al.,* 2001). Besides these, it is an integral part of about 20 different metalloenzymes, like alcohol dehydrogenase, alkaline phosphatase and carbonic anhydrase (Tan and Mai, 2001). The retardation of bone growth due to deficiency of Zn also proves its importance in the growth and mineralization of bone tissues (Liang *et al.,* 2012). Freshwater fish have the ability to take Zn from both food and water, nevertheless the diet is the predominant route for the absorption of this mineral (Spry *et al.,* 1988). However, under control condition of high water borne or low dietary Zn level, the gill showed their importance in the uptake of this mineral (Spry *et al.,* 1988). In freshwater fish the uptake of Zn from water occurs mainly through gills by calcium mediated pathway (Hogstrand *et al.,* 1998), while intestinal Zn uptake take place mainly by carrier mediated pathway (Glover and Hogstrand, 2002).

Nowadays, different types of nanotechnology based systems have been employed to increase production, efficiency and sustainability (Carlos *et al.,* 2022). Nanotechnology has enormous potential to provide innovative improvements to aquaculture systems to reduce costs, increase efficiency and to reduce our impact on the environment, as a necessity impacting our ability to feed the 7 billion plus inhabitants of the planet. China has been at the forefront of rapid development and deployment of nanotechnology in the agri-food sector. The applications of nano technology that would only be described as successful if the products satisfy the demands

of quality, reduced cost, environmental sustainability and low risk to human health (Chen and Yada, 2011).

Nanotechnology (from Latin "nanus" means dwarf) is a technology of materials and structures wherein the size is exhibited in nanometres. More specifically, the term nano signifies 10^{-9} or one billionth of a meter. The term nanomaterial refers to materials with dimensions ranging from 1 to 100 nm (Rai and Ingle, 2012). Nanotechnology is a very viable technology that has various scientific and technological applications. Rapid breakthroughs in nanoscience and nanotech in recent years have opened new vistas for so many consumer and industrial sectors, including agriculture and allied industries that have been viewed as the hotbed of a new revolution. Among the latest scientific advances, nanoparticles are quickly emerging as the future technology and science platform for the next era of agri-food system transformation and development as well as for improving the situations of the poor.

Nanoparticles (NP) have enormous potential in controlling pathogens, improving the immunity and growth functions in aquaculture (Brintha and Ajitha, 2015). Among the numerous metal oxide nanoparticles, zinc oxide plays a vital role in the nanotechnology field due to their specificity when compared to other metal oxide micro and nanoparticles. ZnO is a low-cost material that could be processed in many forms such as nanostructured thin films. Due to its easy processing in various forms, it is used in various applications from optoelectronics to energy conversion, photocatalysis, and sensors (Pislaru-Danescu *et al.*, 2018). ZnO NPs having exciting properties such as high stability, anti-corrosion, photocatalytic, antimicrobial, and UV absorption properties are used in various products including sunscreens, food packaging, drug delivery, cosmetics, paint, plastic, ceramics, and building materials (Osmond and McCall, 2010), textiles with self-cleaning fibers, rubber and papers (Pandurangan and Kim 2015, Saad *et al.*, 2016). It is also added to the diet and water as a micronutrient for the production of plankton and fish growth as a zinc source. Among different approaches, use of nanotechnology to produce nano sized Zn as ZnO called as nano ZnO (nZnO) is a potential alternative to both organic and inorganic Zn sources. The use of nZnO has shown to produce better results as compared with conventional Zn sources and less toxic (Sahoo *et al.*, 2014).

The zinc oxide nanoparticles (ZnO NPs) are the most commonly utilized nanomaterials (NMs). Globally, ZnO NPs have gained special attention because of their environmentally friendly characters with various excellent applications such as anticancer, antimicrobial, and photocatalysis, etc. (Jiang *et al.*, 2018).

In addition to organic and inorganic forms of minerals as feed supplements, nanotechnology has introduced a new atomic or molecular scale of Zn with better characteristics and availability. The nanoparticles of minerals have novel characteristics, including high specific surface area, activity, catalytic efficiency, stronger adsorbing ability and higher bioavailability (Sheikh *et al.,* 2016).

In aquaculture, nanotechnology involves the preparation and utilization of various nanoparticles such as iron, zinc, selenium, cobalt, etc is very helpful for many ways like, nutrient supplements, therapeutic agents and gene delivery, etc. (Dar *et al,* 2019). It has been reported with many advantages; for example, tissue-specific targeting, dose and toxicity reduction, as well as increased bioavailability, drug efficacy, and reduction of secondary adverse effects (Shah and Mraz, 2019).

Among metal nanoparticles (NP) annually produced, by volume, nano zinc oxide (nZnO) is the third highest globally produced nano metal after nano SiO_2 and nano TiO_2 (Piccinno *et al.,* 2012). The sudden rise in the demand in zinc oxide nanoparticles (ZnO NP) is mostly attributed to its better antibacterial properties than the conventional ZnO (Padmavathy and Vijayaraghavan, 2008). Zinc oxide nanoparticles are being used in the food industry as additives and during packaging due to their antimicrobial properties (Gerloff *et al.,* 2009; Jin et al., 2009). Studies have already proved the dose dependant effect of ZnO NP on growth performance in livestock and poultry (Hongfu, 2008; Yang and Sun, 2006; Lina *et al.,* 2009; Mishra *et al.,* 2014; Sahoo *et al.,* 2014) and also as antimicrobial and immune-modulatory agent by reducing the diarrhoea rate in piglets (Hongfu, 2008). Nanoparticles have enormous potential in controlling the pathogens in aquaculture. Different metal and metal oxide nanoparticles were screened for their antimicrobial activities against a wide range of bacterial and fungal agents including certain freshwater cyanobacteria (Swain *et al.,* 2014). Among different nanoparticles synthesized, copper oxide (CuO), zinc oxide (ZnO), silver (Ag) and silver doped titanium dioxide (Ag-TiO_2) showed broad spectrum antimicrobial activity (Swain *et al.,* 2014). Since CuO, ZnO and Ag nanoparticles showed higher antimicrobial activity, they may be explored for aquaculture use. Zinc oxide nanoparticles as one of metal oxides are versatile because they enter in a wide variety of applications ranging from sensing, catalysis, energy storage, electronic devices and biomedical applications. Chemically, zinc oxide (ZnO) and nano zinc oxide (nZnO) have the same chemical formula which suggests similar zinc to oxygen ratio, but at the nano scale are arranged with a wider energy level confinement than smaller size Zn, that could lead to more reactive as the surface is increased (Zhong, 2004)

Indian major carps, the prime cultivable freshwater fishes, are raised in India on the simple dietary combination of plant and animal feedstuffs. These fishes are fast growing, attaining a marketable size of 800-1000 g in less than a year and are generally propagated on extensive and or intensive scale of polyculture system (Jhingran and Pullin 1988). Among these carps, *Cirrhinus mrigala* is a detritus eater with narrow range in food variety. It is a bottom feeder fish and feeds mainly on decayed matters. It is fast growing fish and is used as a component of polyculture with other species of major carps. The Indian major carp mrigal (*Cirrhinus mrigala*) is of great commercial importance because it is the most common fish consumed by the largest population in India.

Although notable success has been achieved in the semi-intensive culture of Indian major carps through indigenous development and standardization of various artifacts of aquaculture, lack of proper feed development, requiring a precise knowledge of nutritional needs of concerned fish species, remains a major impediment towards more intensive culture of these fishes.

However, no study has been done to incorporate ZnO NP into fish feed based on growth performance, haematological responses of *Cirrhinus mrigala* in the agro-climatic condition of West Bengal, India. Very little information is available regarding the mineral requirements of Indian major carps, *Cirrhinus mrigala*, but the same assumes great importance in feed formulations. Therefore, in this study we evaluated the application of different zinc sources, including zinc nanoparticles (ZnO NP) as feed additives in diets for *Cirrhinus mrigala*.

Under this background, present research on "Influence of different Zinc sources on the production performance of *Cirrhinus mrigala*" intended to carry out with the objectives listed as follows:

1. Efficacy evaluation of different zinc sources on growth performance of *Cirrhinus mrigala*.
2. Determining the level of nano zinc to be incorporated in the diet of *Cirrhinus mrigala*.

CHAPTER 2

REVIEW OF LITERATURES

According to the Food and Agriculture Organization (FAO), aquaculture is understood to mean the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate ownership of the stock being cultivated. The reported output from global aquaculture operations in 2019 was over 120 million tonnes valued at US$274 billion.

Aquaculture (the farming of aquatic animals and plants) continues to dominate aquatic food production in Asia and globally. Over 91% of global aquaculture production currently being produced within the Asian region (102.9 million tonnes in 2017), and total global aquaculture production now exceeding global capture fisheries production by over 18.32 million tonnes. Moreover, in contrast to most terrestrial agricultural food production systems, over 95% of global aquaculture production is currently realized within developing countries, with production increasing within these countries at an average annual rate of 6.13% per year (FAO 2019).

2.1. History of aquaculture

Pisciculture devoted solely to fish which is as old as civilization itself. The hunting of fish in the prehistoric period was prevalent during the stone stage. Acceptance of fish culture as a lucrative commercial enterprise in the Indo-pacific region owes its inception to Chinese. Even today many countries follow the Chinese fish culture practices or are inspired by them.

However, the pictorial engraving on an Egyptian tomb shown tilapia being fished out if an artificial tank for about 2500 B.C. provides evidence that the people of Egypt were probably the first in the world to culture fish. But carp culture was wide spread in China in 2000 B.C. It had further developed in Chou Dynasty (1122-249 B.C). The earliest clear record on Chinese literature is said to be the “Classic of fish culture” believed to have been written by Fanli in 475 B.C. Who made a summary of the experiences in structure of ponds, method of propagation, rearing and growth of fry of common carp. It was further developed in Han Dynasty (206 B.C – 7 A.D.). But in Tang Dynasty (818-906 A.D.), there was a being change in fish culture in China and the people were prohibited for catching, selling and eating common carp because the common carp in Chinese is called Li, which is same as that of the surname of

the emperor. As a result the culture of carp which had developed for 1000 years suddenly came to a stop. This brought a new change in fisheries and people went for catching other group of fishes from natural water bodies. This resulted consequently the shifting of monoculture of common carp to polyculture of several species. In Sung Dynasty (906 – 1120 A.D.), the collection and transportation of fish fry were popular around rivers basins. It was further developed. Rearing of fry to adult fish were recorded in Ming Dynasty (1368 – 1644). In Ching Dynasty (1644 -1911), fry production season and biological basis of fry were found in the records. Commercial scale of fish Farming and transport of carp fry in bamboo baskets is recorded in the book "Wei-sn-chak-shik" written in 1243 A.D. In "A complete book of aquaculture" written in 1639 A.D., describes the collection of carp from the river and the methods of rearing them in pond. During 1619- 1904 A.D., only four types of fishes were cultured in China. After this period, another three species of fishes of fishes were brought under culture, making total seven types of cultivable fishes in China.

Occurrence of fish in India dates back to three millennium BC. Fish remains and cut marks have been obtained from evacuations at Mohenjodero and Harappa of Indus Valley Civilization (2500 BC – 1500 BC) indicates utilization of fish as food. In India Kautilya, in his "Artha Shastra" written around 300 B.C. described how fish could be poisonous in tanks during war. King Someswara son of king Vikramaditya VI was the first to record the common sport fishes of India and group them into marine and freshwater forms in his book "Manasoltara" compiled in 1127 AD. During British rule in India, they developed sport fisheries through the introduction of trouts in the hill streams of Nilgris, Kashmir and Kulu valley.

2.2. World aquaculture

Realizing the challenge of feeding over 9 billion people in 2050, United Nations (UN) Member States adopted the 2030 Agenda for Sustainable Development, which offers a set of Sustainable Development Goals which include targets that can be aimed for by enhancing the contribution and conduct of fisheries and aquaculture towards food security and nutrition, especially in the use of natural resources. This unprecedented commitment was made in 2015, immediately after a milestone was reached in 2014 when the aquaculture sector's contribution to the supply of fish for human consumption overtook that of wild-caught fish for the first time. Aquaculture is still the fastest growing food producing sector in the world and it is expected to bridge the future global supply-demand gap for aquatic food. However, the challenge is to ensure that meeting this growing demand for fish as food will be met in conformity with the

2030 Agenda. Since capture fishery production became relatively static in the late 1980s, aquaculture has been responsible for the growth in the supply of fish for human consumption. Aquaculture provided only 7% of fish for human consumption in 1974. This share increased to 26% in 1994, 39% in 2004, and 53% in 2015.

Growth in the global supply of fish for human consumption has outpaced population growth in the past five decades, increasing at an average annual rate of 3.2% in the period 1961–2013, double that of population growth, resulting in increasing average per capita availability. World per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 14.4 kg in the 1990s and 19.7 kg in 2013, with preliminary estimates for 2015 pointing towards further growth beyond 20 kg (FAO, 2016).

2.3. Production and value

Aquaculture is a vibrant sector which produces high-protein, nutritious food, which can't be easily substituted by any other food commodity. Its contribution to human nutrition has been fully recognized (Chan *et al*., 2017), although nutrition-sensitive approaches must be promoted (Beveridge *et al*., 2013, Bogard *et al*., 2017) and the demand for aquatic food is expected to grow even higher in the coming decades. In 2015, aquaculture produced 76.6 million tonnes of aquatic animals, contributing 45% to the total global aquatic animal production and little over 53% to the total global fish consumption in the same year. Per capita food fish consumption has been estimated as 20.3 kg in 2015, compared to 19.7 kg in 2013 (FAO, 2017). In 2015, reported global aquaculture production was 106 million tonnes (both aquatic animals and plants). Asia dominated this production with a contribution of 89%. China, the world's largest aquaculture producer, contributed 47.6 million tonnes (69%) to the 2015 global production, while India and Indonesia maintained their second and third positions respectively. All other regions produced 11%, out of which 2 % by Africa, 4% by the America, 4% by Europe and 1% by the Near East. The value of the total global production of aquatic animals in 2015 has been estimated at US$158 billion. If aquatic plants are included, world aquaculture production in 2015 was 106 million tonnes, with an estimated value of US$163 billion. However, the actual total output value from the entire aquaculture sector value chain is significantly higher than this.

2.4. Indian aquaculture

Aquaculture is one of the fastest-growing industries in the World (Tacon, 2020) and has been playing an important role in the economic development front on account of its

contribution to food and nutritional security, national income, employment opportunities as well as generating livelihood options (Kumar and Shivani, 2014). It is the primary source of animal protein for billions of people Worldwide, where capture fishery and aquaculture serve the livelihoods of more than 10% of the global population. Fisheries and aquaculture supply not only dietary essentials for human consumption, but also provides excellent opportunities for employment and income generation, especially in the more economically backward rural areas (Jayasankar, 2018).

At the World Food Summit organised by the Food and Agriculture Organisation (FAO) in Rome in 1996, the participating countries committed to reduce the number of malnourished people in the world by half by the year 2015 (Haylor, 2004). It is a well-known fact that fish is rich in protein and essential amino acids. It is also a good source of calcium, vitamin A and B12 and omega-3 fatty acids. People irrespective of age who do not get sufficient nutrients from cereal-based diets, would be benefited from the inclusion of fish in the diet. Aquaculture not only supplies dietary essentials for human consumption, but provides excellent opportunities for employment and income generation, especially in the more economically backward rural areas. Sixty million people are directly engaged, part time or full time, in primary production of fish, either by fishing or in aquaculture, supporting the livelihoods of 10-12% of world population (FAO, 2016). Aquaculture currently accounts for over 50% of the global food fish consumption (Subasinghe et al., 2009). While in India, the culture system is based on 3-6 species combination, Chinese have 10 or more species in a single pond thus maximizing productivity.

The three IMCs, namely catla (*C. catla*), rohu (*L. rohita*) and mrigal (*C. mrigala*) contribute the bulk of production to the extent of 70 to 75% of the total freshwater fish production, followed by exotic carps comprising silver carp, grass carp and common carp forming the second important group contributing to the balance 25 to 30% (FAO, 2017). Indian aquaculture has demonstrated a six and half fold growth over the last two decades, with freshwater aquaculture contributing over 95% of the total aquaculture production. India is bestowed with 3.15 million ha of reservoirs, 2.36 million ha of ponds and tanks as well as 0.19 million ha of rivers and canals. Freshwater aquaculture with a share of 34% in inland fisheries in mid 1980s has increased to about 80% in recent years (DADF, 2017). The technologies of induced carp breeding and polyculture in static ponds and tanks have brought about remarkable upward trend in aquaculture productivity and turned the sector into a fastest growing industry. The research and development programs of the Indian Council of Agricultural Research

(ICAR), as well as the development support provided by the India Government through a network of Fish Farmers' Development Agencies (FFDA) have been the principal vehicles for this development. Additional support has been provided by several other organisations, state departments and financial institutions. So far, about 0.65 million ha of water area has been brought under fish farming covering 1.1 million beneficiaries. Currently the average annual yield is around 3 t ha^{-1}. At the same time, training has been imparted to about 0.8 million fishers (DADF, 2017).

Despite the importance of freshwater aquaculture in Indian food sector, no extensive reviews have been made on the sector. However, preliminary review on recent advances in freshwater aquaculture (Jayasankar, 2014), documentation on freshwater aquaculture technologies for bringing about blue revolution (Jayasankar, 2017), promotion of PPPs (Public Private Partnerships) for technology adoption (Jayasankar and Barik, 2015) and vertical expansion strategy for increasing aquaculture production (Jayasankar and Das, 2015) are available. The present paper forms a comprehensive attempt to review the current status of freshwater aquaculture in India.

2.4.1. Freshwater aquaculture production trends in India

Aquaculture production in India has been increasing steadily over the years and during 2015-16 the figure was about 5.77 million t. Bulk of the carp production in the country is contributed by the three Indian major carps (IMCs), namely catla, rohu and mrigal. Exotic species, namely silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*) form the second important group. National freshwater fish productivity has registered marked rise from 0.6 t ha^{-1} yr^{-1} (1974) to 3 t ha^{-1} yr^{-1} at present. Many farmers have demonstrated productivity levels as high as 8-12 t ha^{-1} yr^{-1} (Jayasankar, 2014). Further, freshwater aquaculture production has begun to diversify, incorporating medium and minor carps, catfishes and murrels. The only species from the freshwater sector that goes to the export market has been the giant freshwater prawn (*Macrobrachium rosenbergii*), while carps and other finfishes are grown for the domestic market. Recently, production of *M. rosenbergii* has plummeted, while that of white legged shrimp *Penaeus vannamei* enhanced considerably.

With a national per capita consumption of 11 kg, fish is recognised as one of the chief components in the domestic food security in India (Jayasankar and Barik, 2015). Freshwater aquaculture is a homestead activity in several parts of the country. Besides contributing to the

nutritional security, it also helps in bringing additional income to the poor rural households. Aquaculture brings about socio-economic development in terms of income and employment through the use of unutilized and underutilized resources in many parts of the country.

2.4.2. Major players in freshwater aquaculture in India

Andhra Pradesh, West Bengal, Bihar and Chhattisgarh are among the top producers of freshwater fish through aquaculture. Andhra Pradesh producing around 15 lakh t of fish of which 92% is supplied to other states and West Bengal with a current production of around 13 lakh t of fish and still sourcing fish mainly from Andhra Pradesh are the two top producers of freshwater fish in the country (Jayasankar, 2014). Bihar, Chhattisgarh, Assam and Jharkhand are also enhancing their stakes in freshwater aquaculture production. Bihar and Chhattisgarh together produce around 3 lakh t; while the former state has negligible dependence on fish from other states with about 20% marketed to outside states; the latter gets about 25% from Andhra Pradesh for internal market. Assam produces around 2 lakh t, though still sources around 30% of their fish requirement from other states considering high domestic demand. Jharkhand produces about 1 lakh tonne freshwater fish, but still procures 20% of its need for internal market from outside states. Jharkhand is all poised to boost its freshwater fish production significantly through cage culture of pangas in reservoirs (Jayasankar, 2014).

2.4.3. Present production of carp culture

India is the second largest producer of fish next to China and Indonesia ranks third in aquaculture production (FAO, 2016). In India, the major carps: Catla, Rohu and Mrigal are the mainstay of freshwater aquaculture. The major carps are the most preferred farm fishes because of their fast growth and higher acceptability to consumers (Saini *et al*., 2014) Indian major carps are the most cultivable fish species in India contributing about 87% of the total freshwater aquaculture production of the country (Ayyappan and Jena, 2003). India is one of the major fish producing countries in the world employing over seven million persons in fishing and allied industries and contributing 60 crores annual1y to national income. World freshwater aquaculture production reached 47.9 million tons in 2016, and 59.7% is destined to carps). India is the third largest aquaculture producer in the world with 4.2 million tons of carps, which is about 73.7% of the total India aquaculture production in 2016 (FAO, 2018).

2.5. Studies on *Cirrhinus mrigala*

Mrigal (*Cirrhinus mrigala*), a carp endemic to Indo-Gangetic riverine systems, is one of the three Indian major carp species cultivated widely in Southeast Asian countries. This species has long been important in polyculture with other native species, mainly in India. The traditional culture of the species was restricted to eastern parts of India until the 1950s. The technology of artificial propagation, which assured seed supply in the 1960s, led to the foundation of scientific carp culture.

The initially higher growth rate of mrigal, coupled with its compatibility with other carps, has helped in establishing this species as one of the principal component species in pond culture. The species was transplanted in the peninsular riverine systems of India, where it has established itself. Subsequently it has spread over whole of India. In addition, mrigal has become an important component in the fish culture systems of Bangladesh, Pakistan, Myanmar, the Lao People's Democratic Republic, Thailand and Nepal. Mrigal has also been introduced into Sri Lanka, Vietnam, China, Mauritius, Japan, Malaysia, Philippines and the former USSR (FAO, 2009). The common Indian species is *Cirrhinus mrigala*, but other species like *C. cirrhosa, C. latia, C. reba* and *C. fulungee* are also found in India.

It breeds during monsoon months. It is most suited for induced breeding and now available throughout India. More than five inter-generic hybrid fries are available for culture. The fingerlings and adult feed more on animal protein. Both male and female mature at the age of two years. It is said that the induced breed fish mature only at the age of one year. C. mrigala breeds during monsoon. The fingerlings are available from natural grounds from July to November. The fish breeds naturally in rivers or induced riverine conditions due to the effect of pituitary hormone or other synthetic hormones. Mrigal is popular as a food fish and an important aquaculture freshwater species throughout South Asia. It is widely farmed as a component of a polyculture system of three Indian major carps. The introduction to aquaculture across India started in the early 1940s and in the 1950s and in the 1960s to other Asian countries. The mrigal carp fails to breed naturally in ponds, thus induced breeding is done (Rema Devi et al., 2011).

2.5.1. Biological features of *Cirrhinus mrigala*

Body of mrigal is bilaterally symmetrical and streamlined, its depth about equal to length of head. Its body is with cycloid scales, head without scales; snout blunt, often with pores; mouth broad, transverse; upper lip entire. It has single pair of short rostral barbels;

pharyngeal teeth in three rows, 5.4.2/2.4.5 pattern; lower jaw with a small post-symphysial knob or tubercle. The origin of dorsal fin nearer to end of snout than base of caudal. The dorsal fin as high as body with 12 or 13 branched rays; last unbranched ray of dorsal fin non-osseous and non-serrated. The pectoral fins are shorter than head; caudal fin deeply forked; anal fin not extending to caudal fin. The lateral line with 40-45 scales; usually dark grey above. The dorsal fin greyish, pectoral, pelvic and anal fins are orange-tipped (especially during breeding season).

2.5.2. Habitat and biology

Hatchlings of mrigal normally remain in the surface or sub-surface waters, while fry and fingerling tend to move to deeper water. Adults are bottom dwellers. Mrigal feed on detritus and decayed vegetation form its principal food components, while phytoplankton and zooplankton comprise the rest. Mrigal is eurythermal, appearing to tolerate a minimum temperature of 14 ºC. In culture, the species normally attains 600-700 g in the first year, depending on stocking density and management practices. Among the three Indian major carps, mrigal normally grows more slowly than catla and rohu. The rearing period is usually confined to a maximum of two years, as growth rate reduces thereafter. However, mrigal is reported to survive as long as 12 years in natural waters. Maturity is attained in two years in captivity. As mrigal needs a fluviatile environment for breeding it does not breed in ponds. However, captive breeding in hatcheries has been made possible through induced breeding by hypophysation and the use of synthetic hormones. Mrigal is a highly fecund fish. Fecundity increases with age, and normally ranges from 100000-150000 eggs/kg BW. The spawning season depends upon the onset and duration of the south-west monsoon, which in India, Bangladesh and Pakistan extends from May to September. Mrigal usually breeds at 24-31ºC (FAO, 2009).

2.5.3. Reproduction

They sexually mature within two years. During the monsoon or from May-July, they lay eggs in the aquatic vegetation in the shallow areas of the flooded river. During the breeding season, a mature female fish lays about one lakh to eight lakh eggs.

2.5.4. Growth

Temperature is an important environmental factor that play important role in the growth and metabolism in fish. The increase of water temperature in the consequence of global warming is alarming for aquaculture. Increased temperature affects physiological processes causing a decrease in fish abundance and even the extinction of certain species (Ashaf-Ud-Doulah *et al.*,

2019). It has been reported that the survival, distribution, reproduction and normal metabolism of fish depend on aquatic environmental temperature (Shahjahan *et al.*, 2017). Being coldblooded animal, fish is affected by the temperature of the surrounding water which influences the body temperature, growth rate, food consumption, feed conversion and other body functions (Britz *et al.*,1997). The effects of temperature changes on fish species may be predict through physiological studies (Somero, 2010). Almost all biochemical and physiological activity is greatly affected by rising water temperature that causes stress and alteration of blood chemistry standards because of fish being aquatic poikilothermic animal. Chatterjee *et al.* (2004) stated that high temperature increases the chemical reactions in fish body and greatly affect the physiological process when exceed the level of tolerance. The rise in environmental temperature reduced the dissolved oxygen content in the water which in turn increase the fish metabolism, and the fish adjust the adverse environmental condition by raising total hemoglobin level (Brix *et al.*, 2004). It is an ideal species for carp polyculture system and can be stocked with other carps like catla and rohu. By improving feed efficiency of fish maintained at higher temperature might be the increased feed intake of the fish with increase in water temperature, which resulted in better growth of the fish, leading to better feed conversion ratio.

The preferred temperature is considered to coincide with the optimum temperature for growth (Brett, 1971). The growth of fish at all stages is largely governed by the kind of food, ration, feeding frequency, food intake and its ability to absorb the nutrients. Among these, feeding frequency is an important aspect for the survival and growth of fish at the early stage (Mollah and Tan, 1982). Optimum feeding frequency seems to be dependent on fish size and higher frequency of feeding was found to be advantageous for higher growth and survival in younger age groups (Murai and Andrews, 1976). The fishes should have the access to feed up to satiation for their optimum growth. However, over-feeding leads not only to reduction in feed conversion efficiency and increase in input cost, but also accumulation of wastes that adversely affects the water quality. Plankton being the most preferred food for carp at early stages, pond fertilization is carried out intermittently for its sustained supply during seed rearing.

2.5.5. Nutritional requirements

The nutrient requirements of most Indian major carps are incompletely documented. This is because most cyprinids are cultured either extensively or semi-intensively, and are rarely fed formulated commercial feeds. Most of the investigations on the nutrient requirements of Indian major carps including mrigal, have been carried out on fry and fingerlings and there is considerable variation in the results. Some of the variation can be attributed to biological differences such as fish size, age, temperature, while the remainder are a consequence of different experimental procedures and protocols. The optimum dietary protein requirement for maximum growth is dependent on protein quality and fish size and ranges from 40 to 45 percent for mrigal fry and 30 to 45% for fingerlings (Mohanty *et al.,* 1990). Kalla *et al.* (2004) suggested that mrigal fry and fingerlings be fed on supplementary diets containing about 40 percent protein, preferably of plant origin. Hassan *et al.* (1995) also recorded optimal growth and feed conversion of mrigal fry and fingerlings with a 40 percent CP diet, P:E ratio of around 8.9 kcal/g and an energy content of 15.09 kJ/g, although maximum protein utilization and conversion occurred at a similar energy content but a lower protein content (30 percent CP) in the diet (P:E ratio of 12.1 kcal/g). Mrigal requires the same ten essential amino acids as other finfish under farming conditions. Indian major carps obtain their vitamin requirements mainly from natural food in ponds. Based on weight gain, mortality, behavioural and morphological criteria the optimum vitamin C requirement for newly hatched mrigal is between 650 and 700 mg/kg diet.

4.6. Additives in aquaculture

The rapid growth of modern aquaculture is driven by a variety of factors, which include the increasing use of formulated aquafeeds and intensification of the culture systems (Dawood *et al.,* 2014). Feed additives have been defined as non-nutritive ingredients or non-nutritive components of ingredients that are included in formulations to either influence physical or chemical properties of the diet or affect aquatic animals' performance or quality of resulting products (Barrows, 2000). The chemical nature of these feed additives is quite diverse, and their use in commercial diet formulations for aquatic species varies considerably. Additives that influence feed quality in commercial formulations include pellet binders, preservatives (such as antimicrobial compounds and antioxidants) and feeding stimulants (NRC, 2011). Such additives are commonly included to achieve and maintain optimal physical and chemical characteristics. Other additives that may directly affect fish performance or product quality

including probiotics, prebiotics, acidifiers and plant- or animal-derived extracts, are also commercially available for aquatic animals (Ng and Koh, 2016).

The range of feed additives used in aquatic feeds is very diverse. Certain feed additives target the feed quality, including pellet binders, antioxidants, and feed preservatives (antimold and antimicrobial compounds). Enzymes are used to improve the availability of certain nutrients (proteases, amylases) or to eliminate the presence of certain antinutrients (phytase, nonstarch polysaccharides (NSP) enzymes. In case of aquaculture, intensive fish farming has promoted the growth of several bacterial diseases, which has led to an increase in the use of antimicrobials (Defoirdt *et al.,* 2011). Current levels of antimicrobial use in aquaculture worldwide are not easy to determine because different countries have different distribution and registration systems. Antibiotics are used in aquafeeds as growth promoters for improving health of aquatic animal species. Other growth-promoting agents and feed additives comprise of probiotics, prebiotics, synbiotics, organic acids, medicinal herbs, antioxidants and nucleotides which have been used widely to promote aquatic animal species health and production (NRC, 2011).

These are given at sub-therapeutic dosage for stabilization of the microflora of intestine and for improving the performance in general along with prevention of certain specific pathological conditions of the intestine (Romero *et al.,* 2012). The over-use of antibiotics can create antibiotic-resistant bacteria. Antibiotic-resistant bacteria can spontaneously arise when selective pressure to survive results in changes to the DNA sequence of a bacteria allowing that bacteria to survive antibiotic treatments (Anderson *et al.,* 2003). A promising alternative to promote growth performance and to increase immune resistance in fish is the use of functional feed additives (Dawood *et al.,* 2015). They can be used in addition to chemotherapeutic agents and vaccines (Iwashita *et al.,* 2015), as probiotic is a live, dead or component of a microbial cell, which is administered via the feed or to the rearing water, benefiting the host by improving disease resistance, health status, growth performance, feed utilization, stress response or general vigour, which is achieved via improving microbial balance of the host ambient environment (Dawood *et al.,* 2016). Inactivated probiotic preparations appeared as an alternative to live probiotics, which could potentially cause safety problems in open aquatic environments (Salinas *et al.,* 2006).

Moreover, probiotics can be used in various aquatic environments: freshwater (Rahiman *et al.,* 2010), brackish water and sea water (Vijayan *et al.,* 2006). Generally,

probiotics which are administered to improve the rearing water quality, to enhance the physiological and immune responses of aquatic animals and to reduce the use of chemicals and antibiotics in aquaculture. It can be used as a suitable alternative to the prophylactic use of antibiotics and chemicals. They can compete for chemicals, nutrition/energy or even oxygen, enhance health and immune systems, elevate growth and survival rates as well as feed utility, and improve water quality. Several review articles have investigated the different aspects of prebiotic application (e.g. inulin, oligofructose, xylo oligosaccharide, fructo oligosaccharide, mannan oligosaccharide, galacto oligosaccharide, b-glucan) in aquatic animal species, and the results revealed that prebiotics are promising and have beneficial effects on growth performance, gut microbiota, immunity and disease resistance. Prebiotics have the potential to enhance many aquatic animal biological responses and reduce the mortality caused by invasion of pathogens (Dawood and Koshio, 2016). They also modify the microbial community within the GI tract to boost non-specific immune responses (Song *et al*., 2014).

Synbiotics contain both probiotics and prebiotics (Akhter *et al*., 2015). The synbiotic concept may give the benefit of both prebiotics and probiotics on growth of aquatic animals mainly due to the synergistic effect (Dawood *et al*., 2015).It could significantly enhance growth performance, digestive enzyme activity which leads to digestive system efficiency and the immune-haematological response in fish (Ringo and Song, 2016).The application of natural, safe and cost-effective additives that can stimulate the immunological response of aquatic animal species has received heightened attention among the feed manufacturers. Consequently, research has aimed to identify promising safe, natural and cost-effective immunostimulating agents. Inactivated natural microbes or microbial products such as b-glucans, lipopolysaccharides, chitin, fucoidan and peptidoglycans can stimulate the immune system (NRC, 2011). Chitin is a polymer of glucosamine and is found in shells or cell walls of invertebrates, fungi and yeasts and this compound is the main component of crustacean exoskeletons and consisting of calcium oxide and protein units. Chitosan, an amino polysaccharide, is prepared from shellfish chitin by treatment with alkali. Both chitin and chitosan exerted immunostimulatory effects on fish (Yan *et al*., 2017). By using the natural immunostimulants in aquafeeds for the activation of the immune response is promising to increase disease resistance (Traifalgar *et al*., 2013). The alternative herbal bio-medicinal products in the aqua cultural operations, which have the characteristics of growth-promoting ability and tonic to improve the immune system, act as appetite stimulators (Adel *et al*., 2016). They increase consumption, induce maturation and have antimicrobial capability and also

antistress characteristics that will be of immense use in the culture of shrimps and other fin fishes without any environmental and hazardous problems (Van Hai, 2015). Plants can be used as promising antibiotics that after challenging with pathogens, the survival rates of infected fish priority fed various immunostimulants, vaccines and probiotics, increased (Brunt *et al*., 2007). In aquaculture, medicinal plants are also used as chemotherapeutics and feed additives (Chang, 2000). They have the properties of growth promoting ability, a tonic to improve the immune system, antimicrobial capability, and stimulating appetite and anti-stress characteristics (Citarasu, 2010). Herbs contain many types of active components, such as polysaccharides, alkaloids or flavonoids (Ardo *et al.,* 2008). The herbal-compound extracts act as immunostimulants to enhance the immune response of fish (Thompson *et al.,* 1993) viz. lysozyme, complement, antiprotease, meloperoxidase, reactive oxygen species (ROS), reactive nitrogen species, phagocytosis, respiratory burst activity, nitric oxide, total haemocytes, glutathione peroxidase (GPx) and phenoloxidase, against bacterial, fungal, viral and parasitic diseases (Harikrishnan *et al.,* 2011). Using of natural vitamin sources, which are required for normal cell function, growth and development of aquatic organisms, leads to the belief that the absence of vitamins leads to characteristic deficiency diseases in aquatic species (Chen *et al.,* 2015). They are unable to be synthesized by the host in sufficient amounts to meet the physiological needs of the animal and therefore must be obtained from the diet. Vitamin C is one of the important nutrients because it is a powerful antioxidant and immunomodulator for fish/ shrimps. The fish and shrimp body need vitamin C to remain proper health condition (Dawood and Koshio 2016). Vitamin C has been known to be an important micronutrient correlating with enhanced aquatic animal performances (Shahkar *et al.,* 2015). It may play a role in reproduction of cultured fish species. Reduced reproductive performance has been reported in female tilapia (*Oreochromis mossambicus*) and rainbow trout (*Salmo gairdneri*) fed vitamin C deficient diets (Sandnes *et al.,* 1984). Furthermore, supplementation of vitamin C in egg, larval development and broodstock showed enhanced immune function and overall health (Shahkar *et al.,* 2015). The large variability observed in sensitivity to vitamin E deficiency in cultured aquatic species (Lovell *et al.,* 1984). Gatlin *et al.* (1986) examined the hypothesis that marginal vitamin C status would increase sensitivity of channel catfish to vitamin E deficiency. Channel catfish fed diets without vitamin C had reduced weight gain and feed efficiency regardless of vitamin E supplementation.

Although the signs of vitamin E deficiency were not observed in fish fed the vitamin E–deficient diet with supplemental vitamin C. *Gatlin et al.* (1986) suggested that vitamin E–

deficient diets supplemented with high or adequate levels of vitamin C still caused elevated lipid peroxidation, as the lack of a vitamin C sparing effect on vitamin E nutrition of channel catfish. Dietary bioactive food components that interact with the immune response have considerable potential to reduce susceptibility to infectious diseases. A functional immune system is essential for the survival and performance of shrimp/fish in aquaculture. Every trace mineral components are having their specific role in immunity of cultured animals, but the crucial trace metals that have been associated with an improvement in immunity or function that support immunity are Zn, Mn, Cu and Se (El Basuini *et al.*, 2016). The immune system uses several methods to detoxify these foreign agents or antigens. The trace elements that have been combined with an improvement in immunity, or function that support immunity. The microelements have especially been strengthened by the importance of their roles in immune defence and antioxidative protection. variety of additives have always played a role, either alone or as a combination, in compounded aquafeed. A better understanding of the mechanisms whereby feed additives influence aquatic animal's growth performance and health condition will lead to the development of alternative feed additives while minimizing the use of antibiotics.

4.7. Nanoparticles in aquaculture

Nanotechnology, as a new emerging technology, has lots of potential for the development and transformation of agri-food industry including aquaculture (Kumari and Yadav, 2014; Rodrigues *et al.*, 2017). Its application has already grounded in textiles, electronics, engineering and medicine industry (Smith *et al.*, 2007). It enables scientist to measure, manipulate and manufacture products at the nanometer scale i.e. one-billionth of a meter which impart unique physical and chemical properties of the materials. It has been reported that aquaculture industries can be benefited by the use of recent techniques developed through nanotechnology. Nanotechnological formulations are suitable for multiple applications in aquaculture like administration of vaccines, antibiotics, pharmaceuticals and nutraceuticals (Rather *et al.*, 2011). Nanotechnology based intervention can also minimize the problem associated with various hydrobiological issues in the aquaculture system.

The sustainable growth of nanotechnology calls for a better structure to comprehend the influence of the nano sized materials on aquaculture sector, it also causes pollution and adverse effects due to inadequate handling and disposal, leading to adverse effects to biological system and the environment (Samrot *et al.*, 2019). To achieve this goal, significant research on

nanotoxicology is undergone in many ways in-vitro and in-vivo models (Gornati *et al.*, 2009). Nanomaterials are corner stones in the field of nanotechnology. They are defined as materials possessing dimension between 1 and 100 nm in diameter. A nanometer is one millionth of a millimeter 100,000 times smaller than diameter of a human. Nanomaterials has gained a huge popularity because they exhibit unique optical, magnetic, electrical and other properties at nanoscale (De Crozals *et al.*, 2016).

There consist of two major approaches to synthesis of nanomaterials – top-down approach and bottom-up approach. Nanomaterials can be metals or polymers or non-polymers, polymers that may be derived from biological or non-biological sources, either be incorporated to metals or not. Minerals are essential for normal physiological functioning in animals including fish, although mineral requirements could be different depending on forms, interactions with other elements, and the fish species itself. Zinc (Zn) is the second most abundant trace element in the animal body after iron (Fe). Regular dietary intake of Zn is indispensable as it cannot be stored in the body (Zalewski *et al.*, 2005), and is required for growth, immune function, nutrition, metabolism, fertility, wound healing and maintenance of oxidative stress in animals (Zhao *et al.*, 2014). Nanotoxicity studies helps in understanding the properties of nanoparticles which induces negative impact on environment (Buzea *et al.*, 2007) where most nanoparticles cause toxicity through reactive oxidative species (ROS) and leading to damage of biological macromolecules (Peter *et al.*, 2014). Moreover, the Zn content in the raw feed ingredients of the diet or natural feed sources is too low to accomplish the need of the animals (Zhao *et al.*, 2014). Nanotechnology is already being applied in the food industry (Chaudhry *et al.*, 2008). The evidence suggests that public perception is generally supportive of nanotechnology, and this is in part due to efforts to ensure that information about NMs were in the public domain at an early stage (Anderson *et al.*, 2005).

For traditional chemicals, target organs are often identified by measuring the contaminant of interest in the tissues. This is problematic for NMs because reproducible, reliable methods for detecting NMs in tissues are still underdevelopment. For metal-based NPs, it may be possible to measure total metal concentrations in tissues e.g. tissue Ti(Titanium) levels for rainbow trout *Oncorhynchus mykiss* exposed to TiO2 NPs (Federici *et al.*, 2007), but methodology may require extensive modifications. Man-made NMs, sometimes called engineered NMs or manufactured NMs, are novel materials with nanoscale dimensions. Water quality is, of course, a critical factor in fish health. The standard concerns include ensuring water quality for the immediate needs of the species (e.g. dissolved oxygen levels, temperature,

salinity), removal of nitrogen wastes as well as the interactions of these parameters (Handy and Poxton, 1993). This might involve electron microscopic studies to confirm primary particle size and shape, dynamic light scattering measurements to confirm particle size distributions in liquid samples, measurements of the zeta potential to estimate particle charge as well as mass concentration measurements. Despite multiple advantages, use of nanoparticles also brings some concern to the scientific communities especially on the toxicity of certain nanoparticles to organisms and environment. In some of the scientific reports, it has been said that the potential toxicity and environmental implication of nanomaterials on aquatic organisms must be evaluated as water resources are particularly vulnerable to direct and indirect contamination of nanoparticles (Wang *et al*., 2008). Due to uncertainty on the dose and concentration of NPs, there is a possibility of negative implication on fish and its environment. There are reports on accumulation of Se-NPs in liver cells of Medaka, *Oryzias latipes* (Li *et al.,* 2008). Ag-NPs, when administered to *Cyprinus carpio* at a concentration of 200µg/ L resulted in significant reduction of enzyme activity in the brain (Lee *et al.,* 2012). Some reports have also been published on the negative effect of nanoparticles during the developmental process of fish embryo (Zhu *et al.,* 2008) observed the changes in survival, development and hatching rate of both embryo and larvae of zebra fish when ZnO-NPs were introduced. Iron oxide NPs (10 mg/L) were also shown to develop toxicity in the embryos of Danio rerio, causing mortality, delayed hatching and malformation (Zhu *et al.*, 2012). Toxic accumulation of Cu-NPs was also found in *Oncorhynchus mykiss* (Al-Bairuty *et al.*, 2013).

4.8. Different zinc forms and importance in aquaculture

Zinc (Zn) is the second most abundant trace element in the animal body after iron (Fe). Regular dietary intake of Zn is indispensable as it cannot be stored in the body (Zalewski *et al.,* 2005) and is required for growth, immune function, nutrition, metabolism, fertility, wound healing and maintenance of oxidative stress in animals. Zn absorption in animals is very less and differs with the sites of gastrointestinal tract and age of the animal (Swain *et al.,* 2016). Moreover, the Zn content in the raw feed ingredients of the diet or natural feed sources is too low to accomplish the need of the animals (Zhao *et al.,* 2014). Thus, Zn has extensively been incorporated as a component in the mineral and vitamin premix used in formulation of diets for the farmed animals including fish. Freshwater fish have the ability to take Zn from both food and water, nevertheless the diet is the predominant route for the absorption of this mineral (Willis and Sunda, 1984; Spry *et al.,* 1988). However, under control condition of high water borne or low dietary Zn level, the gill showed their importance in the uptake of this mineral

(Spry *et al.,* 1988). In freshwater fish the uptake of Zn from water occurs mainly through gills by calcium mediated pathway (Hogstrand *et al.,*1998), while intestinal Zn uptake take place mainly by carrier mediated pathway (Glover and Hogstrand, 2002). It is well documented that normal Zn levels in freshwater (Spry *et al.,* 1988) and seawater (Willis and Sunda, 1984) are insufficient to meet the requirement of growing aquatic species. Therefore, Zn is considered as an essential nutrient in finfish feed (Wei *et al.,* 1999). Dietary Zn requirements have been established for a number of different fish species by using zinc sulfate (ZnSO4) as a dietary source and found to be between 15–30 mg kg^{-1}diet for common carp (Ogino and Yang, 1979) and rainbow trout (Oncorhynchus mykiss) (Ogino and Yang, 1978), 20 mg kg^{-1} diet for channel catfish (*Ictalurus punctatus*) (NRC, 1993), 35 mg kg^{-1}diet for juvenile abalone (*Haliotis discus hannai*) (Tan and Mai, 2001), between 20 – 25 mg kg^{-1} diet for red drum (Gatlin *et al.,* 1991).

Zinc is essential for growth, metabolism, immune function and inhibiting the action of reactive oxygen species (ROS) or free oxygen radicals in fish (NRC, 2011). The importance of zinc in the proteins structure is known, and also a large number of enzymes have been identified that need this element to modify their activity (Muralisankar *et al.,* 2015). The normal levels of zinc in freshwater (Spry *et al.,* 1988) has been reported not to be enough to meet the growth requirement. Therefore, this element is considered as an essential nutrient in fish feeding and can be added to the diet in order to meet the nutritional requirements of fish (Tan and Mai, 2001).

On the other hand, excessive zinc in the diet could be toxic to fish and should be avoided (Chupani *et al.,* 2018). Zn may be included in the diets either as inorganic salts, viz. Zn oxide (ZnO) and Zn sulphate (ZnSO4), or as organic chelates, for example Zn propionate, Zn gluconate and Zn acetate. Even if, bioavailability of Zn in organic chelates is higher than the inorganic Zn salts, the use of organic Zn sources in animal diets is limited due to its higher cost (Zhao *et al.,* 2014). There are several Zn sources, including Zn carbonate, chloride, oxide, and sulfate. However, the primary mineral element content is higher in the Zn oxide form than other sources (NRC, 2007). Thus, it necessitates reduction of the supplemental dose of Zn to the fish feed so as to keep aquatic environment pollution free and maintain good health of the fish. Considering Zn sources with apparently better bioavailability, nowadays nanoparticulate Zn (nano Zn) appeared as an alternative Zn source for prospective utilization (Swain *et al.,* 2016).

Different sources of zinc have different functions (Lin *et al.,* 2013). The inorganic sources of zinc include a variety of chemical salts containing this element such as zinc oxide (ZnO), zinc chloride ($ZnCl_2$) and zinc sulphate ($ZnSO_4$), which all of them can be used in the production of aquatic animal diets (Reilly, 2004). It is also added to the diet and water as a micronutrient for the production of plankton and fish growth as a zinc source. Zinc is an essential trace element for finfish and plays a critical role in biological processes and physiological functions such as biosynthesis of hormones, enzymatic activity, and metabolism of proteins carbohydrates, and lipids (Muralisankar *et al.,* 2014). Various organic sources of zinc have also been investigated in many studies as additives to aquatic animal diets, which include a mixture of amino acid chelate or complex with zinc, zinc methionine, zinc lysine, zinc gluconate, zinc acetate, zinc picolinate, zinc propionate and zinc proteinase (Kucukbay *et al.,* 2006). Various studies have shown that organic and inorganic sources have different effects on production performance (Zhao *et al.,* 2014). Since usability of inorganic zinc is low, added concentration is 20 –30 times higher than the natural requirement of animals in order to meet the animals' needs (Bratz *et al.,* 2013).

In addition to common forms of inorganic and organic, nano forms have recently been considered as a new form of minerals and have recently been used in fish diets because of high bioavailability and low toxicity (Dekani *et al.,* 2019). It was evident that ZnO-NPs improved growth performance and feed utilization in pigs and broilers (Zhao *et al.,* 2014).

4.8.1. Effect of Zinc Oxide Nanoparticles on fish

Feed additives in nano forms have been reported to enhance growth and immunity through antioxidant effects at lower concentrations when compared to other forms of additives (Awad *et al.,* 2019, Mohd Yusof *et al.* 2019). Dietary supplementation of zinc oxide-nanoparticles (ZnO-NPs) plays an important role in regulating growth, immunity and enzymatic profiles in many aquatic species including Nile tilapia, common carp, freshwater prawn and rohu (Awad *et al.,* 2019). Nanoparticles have enormous potential in controlling pathogens, improving the immunity and growth functions in aquaculture (Brintha and Ajitha, 2015). Among the numerous metal oxide nanoparticles, zinc oxide nanoparticles play a vital role in the nanotechnology field due to their specificity when compared to other metal oxide micro and nanoparticles. ZnO is a low-cost material that could be processed in many forms such as nanostructured thin films. Due to its easy processing in various forms, it is used in various

applications from optoelectronics to energy conversion, photocatalysis, and sensors (Pislaru-Danescu *et al.,* 2018).

Previous studies have shown that dietary supplementation of ZnO in nano form is more effective than the organic form in enhancing growth performance in African catfish (Onuegbu *et al.,* 2018), and in the immunological response and disease resistance of Nile tilapia (Awad *et al.,* 2019). In addition, ZnO-NPs exhibit strong antimicrobial properties against various pathogens, including those affecting fish such as the gram-negative bacteria *Aeromonas hydrophila, A. salmonicida, Edwardsiellaictaluri, E. tarda, Francisellaorientalis* and *Yersinia ruckeri* and the fungus *Aphanomyces invadans* (Shaalan *et al.,* 2017, Mohd Yusof *et al.,* 2019, Swain *et al.,* 2019). *Pseudomonas aeruginosa* is a gram-negative bacterium composed of 2 cell membranes; the outer membrane and a plasma membrane with a thin layer of peptidoglycan (Fu *et al.,* 200; Diggle and Whiteley 2020). The mechanisms by which ZnO-NPs exert antibacterial activity are not fully understood. Sirelkhatim *et al.* (2015) reviewed mechanisms of their antibacterial activity, including direct contact of ZnO-NPs with cell walls, resulting in loss of bacterial cell wall integrity, liberation of antimicrobial ions, mainly Zn^{++} ions, and stimulation of reactive oxygen species production.

Growth rates of the fishes in both control and treatment diets were within the normal range for *L. rohita* raised under pond culture, and supplemental Zn and Se had higher growth rates in treated groups (SGR) as compared to control group. During 120 days of experimental period (Swain *et al.,* 2018). Feed consumption and feed conversion efficiency of mrigal were higher in feed containing 15 mg of zinc oxide nanoparticles. (Onuegbu *et al.,* 2018) reported an increase in the concentration of Zinc Oxide nanoparticles with feed consumption and feed conversion. Fish fed diet containing Se-NP, Zn-NP, and Se/Zn-NP showed higher final body weight, weight gain, weight gain rate, specific growth rate, and lower feed conversion ratio in Nile Tilapia (Ghazi *et al.,* 2021). In his study he also reported that 10 mg/kg diet ZnO-NP shows higher final body weight, weight gain, weight gain rate, specific growth rate, and lower feed conversion ratio in Nile Tilapia than control diet. Faiz *et al.* (2015) reported that the feed conversion ratio was higher in ZnO nanoparticles incorporated feed of juvenile grass carp. The effects of nano zinc oxide (nZnO) on the growth and hematological parameters of grass carp, have been investigated against ZnO and ZnSO4, as dietary zinc supplements in basal feed. Two levels of supplementation level 1 (30mg/kg) and level2 (60mg/kg) were considered for each treatment. Percent weight gain (%WG), specific growth rate (SGR), and feed conversion ratio (FCR) were reported to be significantly ($p < 0.05$) higher in fish fed level 1, followed by level

2 of nZnO supplemented diets. Nano ZnO level 2 significantly decreased hematological parameters such as red blood cells (RBCs) and white blood cells (WBCs). This suggests 30 mg/Kg feed to be the optimal dietary supplementation level of nZnO for *C. idella.* Thangapandiyan and Monika (2019) reported that the fish fed with 10 mg/kg ZnONP-supplemented diet shows that the growth performance was highly increased followed by 7.5 mg/kg and 5 mg/kg ZnONPs when compared with the control. The biochemical, hematological, and digestive enzyme activities were also significantly increased with different concentrations of ZnONPs. The effects of zinc oxide nanoparticles show the higher improvement of growth and metabolic functions in rohu. The hematological analysis acts as a rapid and economical method for assessing metal oxide toxicity on fishes. Shah and Altindag (2005) reported that the hematological parameters such as hematocrit, Hb, RBC, and WBC are used to assess the functional status of the oxygen-carrying capacity of the bloodstream and have been used as an indicator of metal pollution in the aquatic environment. Fırat (2007) reported that the decrease in WBC count could be associated with the cortisol hormones which play an important role in the prevention and healing of inflammation on fish by the introduction of toxicants. Akbary *et al.* (2018) reported that the Hb, Hct, and RBC counts are decreased and WBC count significantly increased compared to the control in grey mullet fish exposed to sublethal concentration of copper oxide nanoparticles. Ali *et al.* (2015) reported the increase of blood parameters compared to control with a high concentration of selenium nanoparticles supplemented feed to African catfish, *Clarius gariepinus*. Aasma Noureen *et al.*, (2018) reported significant variation in blood parameters such as MCH, MCV, MCHC, Hct, and RBC levels of *C. carpio* exposed to both bulk and CuO NPs when compared to the control group after 14 days of fish exposure. Latifeh Chupani *et al.* (2018) reported that when common carp (Cyprinus carpio L.) exposed to diet-born ZnO nanoparticles had no effects on hematology. Fish fed with Se/Zn-NP showed higher hemoglobin, red blood cells, and globulin in Nile Tilapia (Ghazi *et al.*, 2010). Mondal *et al.* (2020) reported that nano ZnO of 20mg/kg concentration shows highest weight gain, specific growth rate and low FCR than 10mg/kg, 30mg/kg concentration nano ZnO. 10, 30 mg/kg diet shows retarded growth in Rohu. Significantly ($p < .05$), higher activities of the digestive and metabolic enzymes were recorded in the rohu, fed with ZnO-NP containing diets as compared to the diets containing inorganic Zn or control diet (Mondal *et al.* (2020). Amylase activity did not differ too much time in 10mg/kg nano ZnO diet. Maximum amylase activities were noticed in 20mg/kg nZnO diet. The maximium lipase activity was noticed in 30mg/kg nZnO diet.

Tawfik *et al.* (2017) reported a study on *Oreochromis niloticus* where fishes were fed for 120 days on Zinc oxide conventional bulk scale (ZnO) and nanoscale (nZnO) supplemented feed in different concentrations (15, 30, 45 and 60 mg/kg of the feed) in addition to the control which was fed on ZnO free feed. nZnO (15mg/kg) achieved specific growth rates like the higher concentrations of bulk ZnO (60mg/kg). The 60mg/kg nZnO gave the highest rates of Specific growth rates (4 folds than control). growth hormone was higher in serum of fish fed on nZnO supplemented feed than the bulk form.

Study on Nile tilapia results displayed that the best growth and digestive enzyme activity ($P < 0.05$) were noticed in fish fed 60 mg kg^{-1} Nano-ZnO. Moreover, significant ($P < 0.05$) improvement in intestinal topography was observed in 60 mg kg^{-1} Nano-ZnO group versus other treatments. Furthermore, fish fed 30 mg kg^{-1} Nano-ZnO recorded the best values of hematological indices ($P < 0.05$). The alanine and aspartate aminotransferase (ALT and AST) values were lower, while total serum protein, albumin, and globulin contents were clearly higher in fish fed diet that contained 30 mg kg^{-1} Nano-ZnO (Ibrahim *et al.,* 2021). Large-scale production and huge use of zinc oxide leads to the direct and indirect release into an aquatic ecosystem which ultimately affects the aquatic biota. ZnO is one of the most harmful products present in the aquatic environment, due to its toxic and biochemical altering properties in aquatic organisms (Kahru and Dubourguier, 2010). Fish, occupying high trophic levels in the aquatic ecosystem and being an important food source, are regarded as indicators of ZnO contamination in the aquatic environment (Agah *et al.,* 2008). The Survival Rate of *Cirrhinus mrigala* was 100% in feed containing 0, 5, and 20 mg of zinc oxide nanoparticles, and 80, 90, and 90% were observed in 10, 15, and 25 mg (Rajan and Rohini, 2020). In Nile tilapia the highest survival rate was recorded in the groups that supplemented with 30mg/kg diet nZnO (90%) and in that supplemented with 60 mg/kg diet conventional ZnO (86.6%) (Awad *et al.,* 2019). The survival rate of rohu in the concentrations of ZnONPs (mg/kg) 5, 7.5, 10 is reported 94%, 95% and 96% respectively by Thangapandiyan and Monika (2019).

CHAPTER 3

MATERIALS AND METHODS

3.1. Experimental site

The present experiment was carried out with the aim to find out the **influence of different quantity of zinc sources on the production performances of *Cirrhinus mrigala*.** The experiments were conducted in the laboratory conditions of the Department of Aquaculture, Faculty of Fishery Sciences, West Bengal University of Animal and Fisheries Sciences, Chakgaria, Panchasayar, Kolkata-700094 (Lat. 22º 82'N; Long. 88º 20'E).

The glass tanks of size 120 × 45 ×45 cm (capacity - 243L) were used for the initial acclimatization of experimental fish.

3.2. Preparation of experimental tanks

The growth experiment was carried out with 15 tanks of 243 litre capacity. The tanks were cleaned thoroughly using scrub and then sun dried for three days. Tanks are placed in triplicate manner and then they were filled with tap water up to 162 litre maintaining 30cm height and provide sufficient aeration throughout the experiment.

3.3. Stocking of experimental fish

Healthy and uniform mrigal (*Cirrhinus mrigala*) fingerlings of irrespective sex were used for all the experiments. Three hundred (300) fishes were collected from Naihati fish seed hatchery, Kolkata, West Bengal. Fish were brought to the laboratory with proper oxygen packaging and on reaching the laboratory, the experimental fishes were disinfected by immersion in 0.02 ppm potassium permanganate ($KMnO_4$) solution for 10 min, then released into the glass tanks. Keeping them unfed for 24 hours, then they are fed with sinking palleted control feed (28.37% protein) for 7 days @ 3% body weight with proper aeration facilities where they got acclimatized fully before experimentation. Chlorine-free aerated tap water was used throughout the experiment. Faecal matters were siphoned out and one third of water exchange was done with fresh water on every alternative day. During the acclimatization period, the fish showed no abnormalities such as lethargy, opercular flaring, abrupt swimming, etc.

3.4. Experimental design

Two hundred twenty five (225) fish with average body weight (3.63±0.01 g) were randomly distributed into five experimental groups. Each group is sub-divided into three replicates where each replicate comprises of fifteen (15) fish selected in random. Body weight of individual fish was recorded and during the time of allocation the mean body weight was kept identical among the experimental groups.

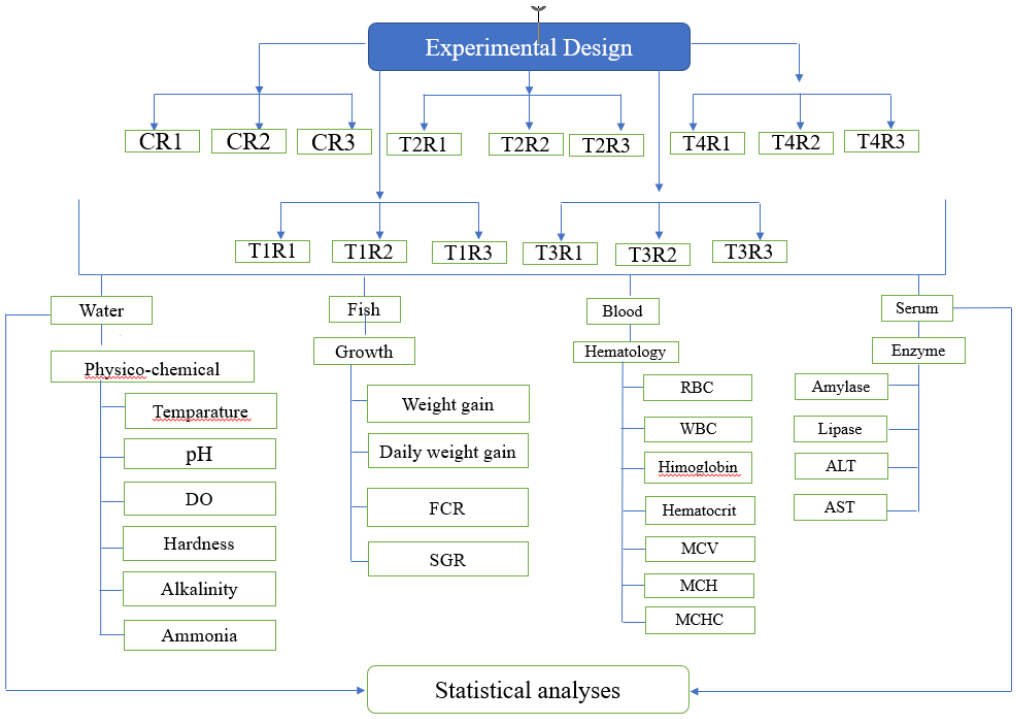


Fig.-1: The experimental design

3.5. Fish and Rearing Conditions

After acclimatization of 225 fishes with average body weight of (3.63±0.01 g) were stocked in 15 aquaria tank at a density of 15 fishes per tank in triplicates. Body weight of individual fish was recorded and during the time of allocation the mean body weight was kept identical ($P>0.05$) among the experimental groups. The tanks were covered with nylon netting to prevent the jumping out of fishes as well as to avoid insects and dust introduction. Feeding was done twice a day (9 AM and 4 PM) with at 3% body weight. The excess feed and faecal matters were siphoned daily before and after feeding. The physicochemical characteristics of the water were measured periodically to maintain the optimal level throughout the experiment.

3.6. Feed preparation

The raw materials are selected based on the ability to supply nutrients. Soyabean meal and mustard cake were used as a protein source; wheat flour was used as carbohydrate source; fish oil and sunflower oil were used as fat sources. The different supplements were also added to make the feed balanced. The components used for feed preparation were dried, powdered, and sieved through a 425-micron sieve. After knowing the protein content of major ingredients by the Micro-Kjeldahl method, the ingredients were weighed and mixed thoroughly with 130 - 150 ml of distilled water. The mixed feedstuff was put in the autoclave for 15 minutes at 100°C and later on cooled.

After cooling, fish oil, sunflower oil, lecithin, vitamin mixtures and binders and different concentration zinc oxide nanoparticles and one treatment feed for normal zinc oxide were mixed with the ingredients. The control feed was prepared without any zinc source. The composition of different feed is given in table 1. The treatment feeds were prepared with normal zinc of 30mg/kg (T1), nano zinc 30mg/kg (T2), nano zinc 15mg/kg (T3) and nano zinc 7.5mg/kg (T4). With the help of hand pelletizer machine, the pellets are prepared and they were dried at room temperature. The formulated feed was kept in an airtight container at -20°C until used to prevent contamination.

Table 1: Composition of different feeds (Dry matter basis)

Ingredients (g)	Control	T1	T2	T3	T4
Wheat flour	37	37	37	37	37
Mustard cake	25	25	25	25	25
Soyabean meal	26	26	26	26	26
Dry fish	2.5	2.5	2.5	2.5	2.5
Shrimp dust	2	2	2	2	2
Mineral Mix	2	2	2	2	2
sunflower oil	1	1	1	1	1

Fish oil	1	1	1	1	1
Lecithin	2	2	2	2	2
Vitamin Mixtures	1	1	1	1	1
Binder	0.5	0.5	0.5	0.5	0.5
Total (g)	**100**	**100**	**100**	**100**	**100**
ZnO (mg)	0.0	3.0	0.0	0.0	0.0
nZnO (mg)	0.0	0.0	3.0	1.5	0.75

3.6.1. Proximate composition of feed ingredients

All the ingredients used in feed preparation were analyzed for their proximate composition such as moisture, crude protein, crude fat, and ash prior to the formulation of test diets.

3.6.2. Crude protein (%)

Crude protein content of sample was determined by the standard Micro-Kjeldahl method. One gram of feed sample was digested with 25 ml concentrated sulfuric acid and 2.5 g digestion mixture (copper sulfate: sodium sulfate in 1: 9 ratio) until it became clear. Volume was made to 250 ml with distilled water by transferring the content of digestion flask to volumetric flask with several rinsing with distilled water. 5 ml aliquot of digested feed samples was distilled in a Micro-Kjeldahl assembly by adding 5 ml of 40% sodium hydroxide solution. Gaseous ammonia thus released was trapped in 10 ml boric acid containing Toshiro's indicator {boric acid 20 g, methyl red (1%) 12 ml, bromocresol green (1%) 6 ml, dehydrated alcohol 200 ml and distilled water 782 ml}. The nitrogen trapped in boric acid was estimated by titrating it against N/100 sulfuric acid. A blank was also run, the value of which was subtracted from sample's readings. The normality of acid was checked by titrating against sodium carbonate using methyl orange as indicator. The nitrogen content was determined by the formula:

$$\text{Nitrogen (\%)} = \frac{\textit{Volume of } N/10\ H2SO4\ X\ 0.0014\ X\ \textit{aliquot taken}}{\textit{Weight of dry sample}} \times \mathbf{100}$$

Crude protein (%) = Percent nitrogen × 6.25

3.6.3. Crude fat (%)

A weighed amount of the ground sample (about 2-3 g) was placed in a cotton-pumped thimble before being extracted in a Soxhlet apparatus for 8–10 hours with petroleum ether (B.P. 60–80 ˚C). At 100 ˚C, the extracted oil in the oil flask was dried to a fixed weight. The amount of ether extract was determined by the difference between the weight of the oil flask before and after extraction and was calculated on a DM basis using the following formula:

Weight of dry sample = (Weight of thimble + Oven dried sample) - Weight of thimble

Weight of crude fat = (Weight of thimble + Oven dried sample) – (Weight of thimble + Oven dried sample after extraction)

$$\text{Crude fat}(\%) = \frac{\text{Weight of ether extract}}{\text{Weight of dry sample}} \times 100$$

3.6.4. Total ash (%)

Approximately 3-4 g of oven dried sample (exactly weighed) was taken in a pre-weighed silica crucible and charred on heater to make smoke free. The crucible along with the sample was transferred to a muffle furnace and kept at 600 ˚C for 3hr. Remove the crucible from the furnace, cool it in a desiccator and weigh. The previously recorded empty crucible weight is now subtracted and the weight of ash is, thus determined. Total ash was expressed on DM basis by the formula:

$$\text{Ash } (\%) = \frac{\text{Weight of ash}}{\text{Weight of dry sample}} \times 100$$

3.6.5. Moisture (%)

Moisture was determined by oven drying at 100°C for 12 to 14 h till constant weight is achieved.

$$Moisture = \frac{\text{Initial weight of sample} - \text{Final weight of sample}}{\text{Initial weight of sample}} \times 100$$

3.7. Physico-chemical analysis of water sample

Water quality parameter test was done for monitoring important physico-chemical parameters of water taking all the necessary precautions not to entrap any air bubbles. The important physicochemical parameters of water such as temperature, pH, dissolved oxygen, total hardness, total alkalinity, salinity and ammonia were analyzed. The method for different analysis and the different instruments were used.

Table-2: Methods used for analyses of physico-chemical parameters of water sample

Water quality parameters	Methods/instruments used	Reference
Temperature (˚C)	Mercury thermometer	APHA,2005
pH	Digital pH meter (Waterproof Multi-Parameter PCSTestr™ 35, Oakton	APHA, 2005
Dissolved oxygen (DO) (mg/l)	Winkler's method	APHA, 2005
Total hardness (mg/l)	Titration method	APHA, 2005
Total alkalinity (mg/l)	Titration method	APHA 2005
Ammonia(mg/l)	Titration method	APHA 2005

3.7.1. Temperature

Water temperature (˚C) of all aquariums was measured by centigrade thermometer (Range 0 ˚C to 50 ˚C) at 10:30 am on 1st day, 28th day and 56th day. (APHA, 2005).

3.7.2. pH

The pH of were estimated by digital pH meter (APHA, 2005)

3.7.3. Dissolved Oxygen (DO)

Dissolved oxygen of water sample was estimated following the Winkler's method (APHA, 2005). water sample collected in a 300 ml sampling bottle avoiding any air bubbles then 1 ml of Winkler's A (Manganous Sulphate Solutions) followed by 1 ml of Winkler's B (potassium Iodide) were added. When precipitated settled sufficiently to a level, 1 ml of concentrate Sulfuric acid (H_2SO_4) was added and colour turned to golden yellow. Sample of 50 ml was taken in a conical flask and few drops of starch indicator were added to it. Then the sample was titrated against N/40 $Na_2S_2O_3$(Sodium thiosulphate) till the samples became colourless.

The volume of the titrant was recorded and the result were expressed as mg/l.

Dissolved oxygen was calculated as follows:

$$D.O\ (mg/l) = 8 \times 100 \times N/V \times v$$

Where:

V = Volume of sample taken (ml)

v = Volume of used titrant (ml)

N = Normality of titrant

8 is the constant since 1ml of 0.025N Sodium thiosulphate solution is equivalent to 0.2mg oxygen.

3.7.4. Total hardness

The total hardness of water samples was measured by titrating the 50 ml of sample against EDTA (Ethylene di-amine tetra acetic acid) after adding ammonia buffer and erichrome black T (APHA, 2005) as indicated. The value was expressed in mg/l.

$$\text{Total Hardness (mg/l)} = \frac{\text{volume of EDTA used (ml)}}{\text{volume of samples taken (ml)}} \times 100$$

3.7.5. Total alkalinity

Total alkalinity was calculated through titration method (APHA, 2005). Water samples (50 ml) was taken in a conical flask, and then 2-3 drops of phenolphthalein indicator were added to it,

if pink colour developed, sample was titrated against 0.02 N H_2SO_4 till the colour disappear then the burette reading was noted down and after that 2 drops of methyl orange indicator was added and the solution turned into orange colour.

The titration was continued with 0.02 (N)H_2SO_4 added and the solution turned into pink. If pink colour did not appear with phenolphthalein indicator, The total burette reading was noted down and the result was expressed as mg/l.

$$\text{Total Alkalinity(mg/l)} = \frac{\text{volume of H2SO4(ml)}}{\text{volume of samople taken(ml)}} \times 100$$

3.7.6. Total Ammonia

After proper filtration, phenol solution (11.1%), sodium nitroprusside solution and oxidizing solution and oxidizing solution (alkaline citrate and sodium hypochlorite @ 4:1) were added to the sample. The samples were then wrapped with paper and kept at room temperature (22-27ºC) in subdued light for at least 1 hour. A blue colour appeared due to the formation of indophenol, which was stable for 24 hrs. The ammonia concentration of the samples were directly estimated through a double beam UV-VIS-Spectrophotometer (CECIL CE-4002) at 640 nm wavelength.

3.8. Growth performance analysis

3.8.1. Growth study

The growth performance of mrigal fingerlings in freshwater studied for. Fish sampling was done weekly basis and record their growth in terms of weight increment. Individual fish were caught using scoop net from each replicate tank during every sampling and blotted on absorbent paper to record their total weight (to the nearest g) using electronic balance. Average weight of fishes of each replicate was then calculated separately for each tank.

The growth indices were calculated using the following formula-

Table-3: Indices used to evaluate growth performance

Index	Formula
1. Average Body Weight gain (ABWG)	(Final weight – Initial weight)
2. Daily weight gain (DWG)	(Final length – Initial length)
3. Specific Growth Rate (SGR)	$\text{SGR} = \frac{(\text{LnW2} - \text{LnW1})}{\text{Days of experiment}} \times 100$ Where, W1 =Initial Weight of fish, and W2 = Final Weight of fish
4. Feed Conversion Ratio (FCR)	$\text{FCR} = \frac{\text{Feed intake}}{\text{Weight gain}}$

3.8.2. Survivability

Observations on survivability were recorded daily. Cumulative survivability rate were calculated as follows (Thangapandiyan and Monika, 2020)

$$\text{Survival rate (\%)} = \frac{\text{Number of fish survived}}{\text{Initial number of fish}} \times 100$$

3.9. Estimation of hematological parameters

The changes in haematological parameters like total erythrocyte count, total leukocyte counts, thrombocyte counts, haematocrit values, haemoglobin levels, white blood cell, red blood cell indices, i.e., mean corpuscular volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), differential leukocyte counts and serum biomarkers like aspartate aminotransferase (AST), alanine aminotransferase (ALT) and blood

cells morphology were monitored after experiment. The blood samples were collected from all the tanks.

3.9.1. Collection of blood

Three fish from each tank of the relevant groups were sampled from the experimental tanks with minimum handling stress and transferred to the plastic buckets containing water of the same temperature and instantly anaesthetized with clove oil (40 µl/l). The blood from the experimental fish was drawn using a 2 ml sterile syringe through a caudal vein puncture (Roberts, 2012). The use of a plastic syringe is a necessary precaution with fish blood because contact with glass results in shortened coagulation times (Smith *et al*., 1987). The anticoagulant used was 3% ethylenediamine tetra acetic acid (EDTA) and blood samples were collected in 1.5 ml Eppendorf tubes rinsed with the anticoagulant to prevent coagulation and further used for the quantification of haematological parameters.

3.9.2. Blood smear preparation, fixation, and staining

Blood smear preparation

A drop of blood was placed on one end of a chemically cleaned slide. A second slide was held at an angle of 45º at about the centre of the first slide. The slide was brought back against the blood until it spread by capillarity along with the interface between the slides. The slide was then moved in the reverse direction, creating a thin, uniform film of blood on the slide, which was then allowed to air dry.

Fixation

When the smear was dry, it was placed into a Coplin jar filled with 95% methanol for at least 5 min for fixation of the cells on the slide and stored in a dust-free condition until staining.

Staining

The methanol-fixed slides were then stained by the May Grunwald Giemsa method. The undiluted Giemsa stain was poured on the slide and allowed to stain for 5 min. Then the slides were washed in phosphate buffer and allowed to dry.

3.9.3. Erythrocytes Count

The erythrocytes were counted by using a haemocytometer. Cell counts were performed by using a Neubauer's counting chamber (Behera *et al*., 2014). The total number of erythrocytes was then calculated using the following formula;

$$\text{No. Of RBCs} = \frac{\text{No.of erythrocytes} \times \text{dilution factor}}{\text{Are counted} \times \text{depth of fluid}}$$

(million /cu mm of blood)

$$\text{Dilution} = 200 \text{ area counted} = 5 \times 0.04 = 0.2 \text{ mm}^2$$

3.9.4. Estimation of Haemoglobin (Hb)

The haemoglobin level of blood was estimated by the cyano-methemoglobin method using Drabkin's fluid (Drabkin, 1950). The haemoglobin concentration was then calculated using the following formula:

$$\text{Haemoglobin (g/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

3.9.5. Haematocrit (Packed Cell Volume)

Haematocrit was determined by micro-haematocrit (capillary) technique described by Dacie and Lewis (Dacie and Lewis ,1991) using RM 12 °C micro-centrifuge and a micro-haematocrit reader.

3.9.6. Mean Cell Volume

The mean cell volume (MCV) is the average volume of red blood cells and is calculated from the haematocrit (HCT) and the red blood cell count (RBC) (Stoskopf,1993).

$$\text{MCV (\%)} = \frac{\text{HCT(\%)}}{\text{RBC count in millions/mm} \times 1000} \times 10$$

3.9.7. Mean Cell Haemoglobin

The mean cell hemoglobin (MCH) is the content (weight) of haemoglobin of the average red cell. It is calculated from the haemoglobin concentration and the red blood cell count (Drabkin ,1950).

$$\text{MCH (pg)} = \frac{\text{hb}(\frac{\text{g}}{\text{dL}})}{\text{RBC count in million/mm}\times\times 1000} \times 10$$

3.9.8. Mean Cell Haemoglobin Concentration

The mean cell haemoglobin concentration (MCHC) is the average concentration of haemoglobin in a given volume of packed red blood cells. It is calculated from the haemoglobin concentration and the haematocrit (Stoskopf, 1993).

$$\text{MCHC (g/dl)} = \frac{Hb(\frac{g}{dL})}{HCT(\%)} \times 100$$

3.9.9. Leucocyte Count

Leucocytes were counted by using the haemocytometer. Cell counts were performed by using a Neubauer's counting chamber (Behera *et al.*,2014). The total number of leucocytes was then calculated using the following formula:

$$\text{No. of WBCs} = \frac{\text{Number of leucocyts} \times \text{dilution factor}}{\text{Area counted} \times \text{depth of fluid}}$$

(1000/cu mm of blood) Dilution = 20,

Area counted = 4× 1 = 4 mm^2

and Depth of fluid = 0.1 mm

3.10. Enzymological analyses

Collection of serum and analysis

For serum, collect blood without anticoagulant and allowed to clot at about 30°C by keeping the syringe in a slanting position for 2 hour and then kept at 4°C overnight. The serum samples were collected by centrifugation at 2500 rpm for 15 min, transferred to Eppendorf tubes with a micropipette, and stored at -20°C for further analysis of biomarkers (Kishawy *et al.*,2020).

3.10.1. Estimation of digestive enzymes

3.10.1.1. Amylase estimation (Bernfield,1988)

Apparatus - Test tube, micro pipette with tips, incubator, spectrophotometer.

Chemicals

A) Starch solution - 1.0% starch solution was prepared fresh by dissolving1.0 gm of soluble starch in 100 ml of 0.02M sodium phosphate buffer (pH - 6.9). Bring it to a gentle boil to dissolve. Incubate at 25^0c for 5 minutes prior assay.

B) DNS reagent - 1gm 3,5 dinitrosalicylic acid was dissolved at room temperature in 50 ml of ddH_20 then added 20 ml of 2M NaOH solution and 28.2 gm Rochelle salt. Finally made upto 100 ml by distilled water. Gentle heat the solution not boiled. The reagent was stored at room temperature.

Method

Incubate 0.1ml of crude enzyme (homogenate) for 5 minutes at 35^0 C with 0.1 ml of substrate solution. Then add 1 ml of DNS reagent for interrupt the enzymatic reaction. The tube containing the mixture was heated for 5 minutes then boil in water bath and cooled in running tap water. After that 10 ml of distilled water was added in the test tube. The production of reducing sugar (maltose) from starch because of amylovtic activity. Amount of maltose was measured at 540 nm. The blank was prepared in the same manner using 1ml sterile distilled water in the place of crude enzyme.

$$\text{Ezyme(Units /ml)} = \frac{\text{mg of maltose released}}{\text{volume of enzyme / 5 min}}$$

3.10.1.2. Lipase estimation (Ogunbivi and Okon, 1976)

Apparatus -Test tube, micro pipette with tips, incubator, titration apparatus.

Chemicals

A) Olive oil emulsion: It is prepared by dissolving 200mg sodium benzoate in 100 ml distilled water. Then 7 gm gum Arabic was added to the solution. Mix well in blender until it gets dissolved. Mix 25 ml of olive oil and mix for 10 minutes. Keep the emulsion in refrigerator and shake well before use.

B) Phenolphthalein indicator
C) 95% ethanol
D) Phosphate buffer (0.1M)
E) 0.05 M NaOH.

Method

The reaction mixture consisted of 1ml of crude enzyme, 0.5ml phosphate buffer and 1 ml of olive oil emulsion. Shake the solution. Then heat it at 100^0 C for 5 minutes. Then incubate test and blank at 37^0C for 1 hr. Add 3 ml of 95 % ethanol and two drops of phenolphthalein to each test tube including the blank. Then titrate the reaction mixture against 0.05 M NaOH to a similar pink colour.

$$\text{Enzyme(Units/ml)} = \frac{[(\text{consumed volume of NaOH}) \times (\text{molarity of NaOH}) \times (1000) \times (2)]}{\text{volume of enzyme}}$$

3.10.2. Serum alanine aminotransferase (ALT) and Aspartate aminotransferase (AST)

The serum ALT and AST were determined by using the ALT and AST test kits (Erba diagnostics Ltd., Mannheim, Germany) following the modified UV (IFCC), and kinetic assay methods. The serum ALT and AST were measured at room temperature by following the kit procedure using a Photometer (Model: 5010 v5+, Robert Riele KG, Berlin)**.** The absorbance was measured at 340 nm and calculated the ALT and AST concentrations (Wolf *et al.,* 1972).

3.11. Statistical analyses

The data were collected and presented in thesis as mean ± standard deviation. The data on survivability, biomass, haematological parameters, blood cell morphometric parameters and serum biomarkers were analysed by one-way ANOVA test and confirmed the significance of difference among the treatments and dosing periods by Tukey post-hoc test for comparison of means. All the statistical analyses were done using Statistical Package tools for Social Sciences (IBM-SPSS), version: 22.0, considering the probability level of $P<0.05$.

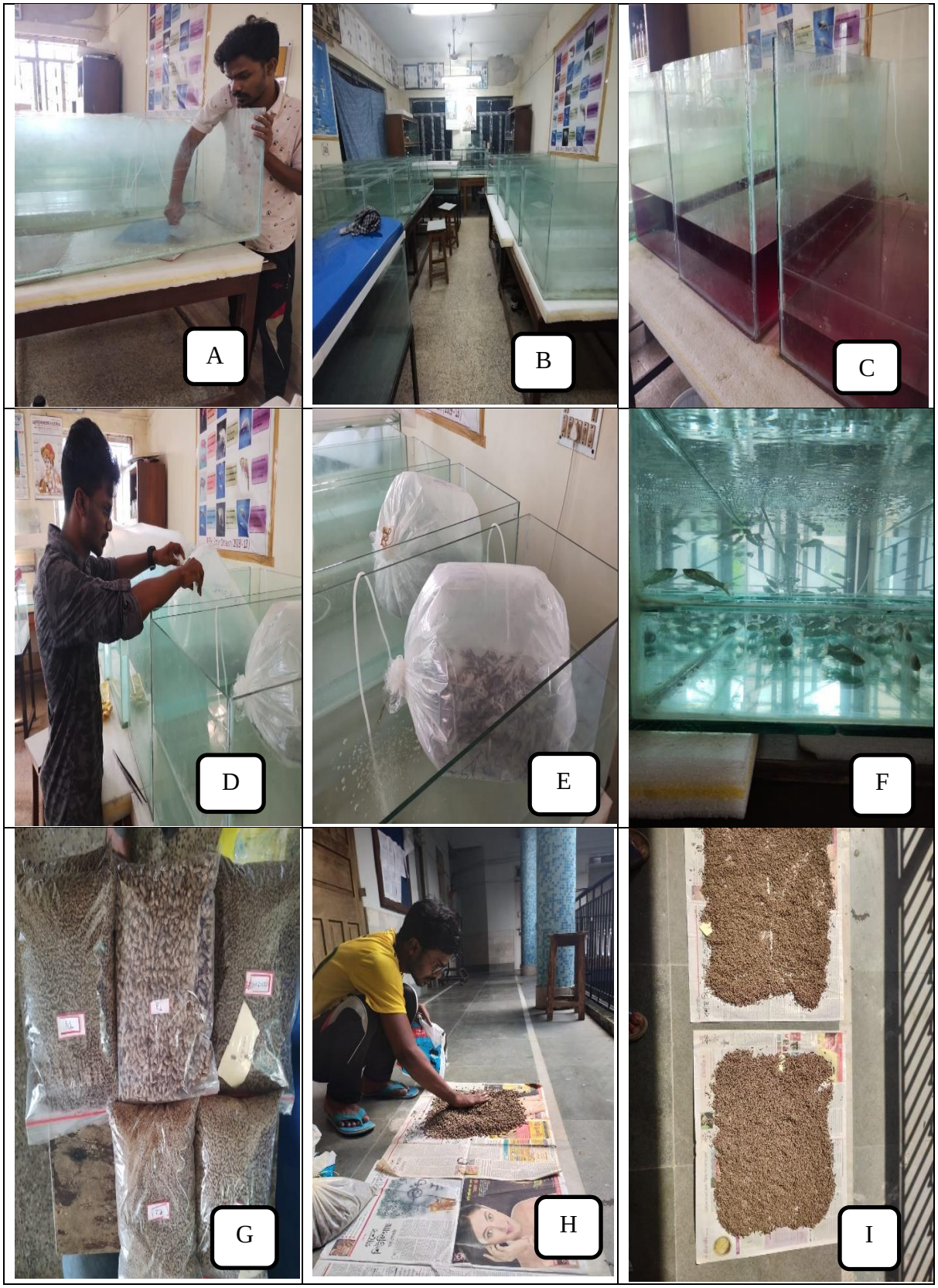


Plate I: A. Tank Cleaning B. Tank Setup C. Tank Disinfection using $KMnO_4$ D. Fish Acclimatization E. Acclimatization F. fish released in tank G. Feed H. Feed drying I. Feed Sun drying

Plate II: A. Feeding B. Tank covered with net C. Siphoning D. Weighing of fish E. Blood sample for serum F. Blood sample for hematology G. DO estimation H. feed ash proximate analysis I. Feed Fat proximate analysis

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Physico-chemical parameters of water

Water quality management in aquaculture is vital, fish are highly sensitive to changes in parameters such as temperature, pH, dissolved oxygen, alkalinity, total hardness, total ammonia etc. The water quality must therefore be consistently monitored and controlled for the fish to maintain optimum health, productivity and quality. During the experiment water quality parameters were monitored and recorded.

4.1.1. Temperature

The temperature of experimental tanks water did not differ so much among the treatments during the periods of investigation. The temperature ranges between 26.6 °C to 26.85 °C. The highest temperature value was recorded 26.85 °C in 56th day in T4 tank and lowest temperature value was recorded 26.6 °C in T1 tank on 1st day of the experiment. The temperature value in control tank initially increased on 28th day and decreased gradually. T1 tank temperature value initially increased up to 28th day than gradually decreased up to 58th day. T2 tank temperature value decreased from 1st day to 58th day of the experiment. Control and T3 tanks temperature value increased up to 28th day and slightly decreased after that up to 58th day. The temperature changes in T4 tank was not significantly changed up to 28th day but it increased at 56th day. Temperature variation of experimental tanks at 1st, 28th and 56th day was presented in figure-2.

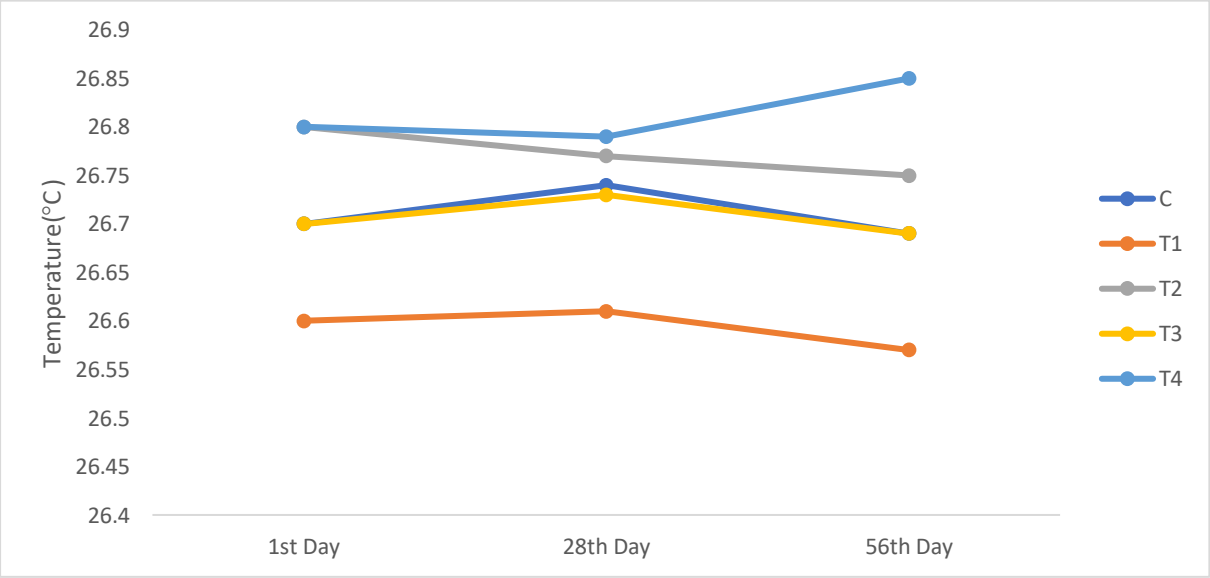


Fig.-2: Temperature variation of experimental tanks at 1st, 28th and 56th day

Temperature plays an important role in regulating the metabolism of animals. It has a direct effect on important factors influencing growth including food consumption and food conversion efficiency. Hence, an optimum range of temperature is required for the optimum metabolic activity, which in return gives maximum yield. Santhosh and Singh (2007) suggested that favourable water temperature for freshwater carp culture is between 24 to 30°C. Singh *et al*. (2013) reported that temperature between 25 - 30°C is favourable for magur and below this range will affect the physiological response. In this present experiment, the average water temperature recorded was 26.71±0.03.

4.1.2. pH

Water pH remains within normal range in all the experiments tanks employed in the present study. The highest pH value was 7.65 and lowest pH value was 7.51. There were no significant changes in the pH value between the treatments during the experiments. Experiment tanks shows slight changes in the pH value. But values are not changed significantly ($p>0.05$). The pH variation of experimental tanks at 1st, 28th and 56th day was presented in figure-3.

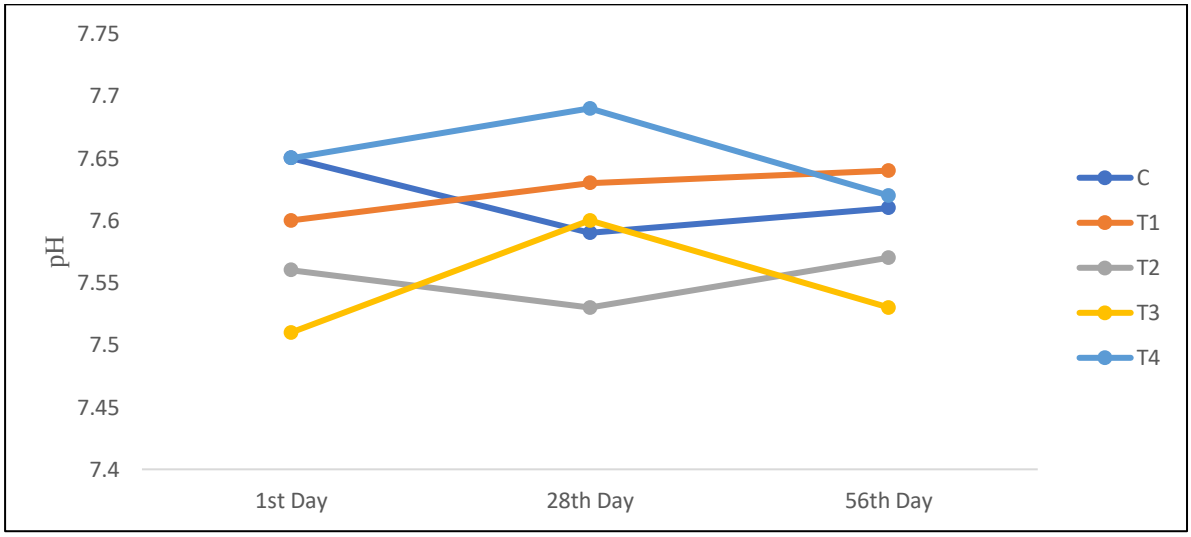


Fig.-3: pH variation of experimental tanks at 1st, 28th and 56th day

The pH is hydrogen ion concentration in water and refers to how acidic or basic the water is. It is an important parameter to consider because it affects the metabolism and other physiological processes of finfish and shellfish. According to Santhosh and Singh (2007) the suitable pH range for fish culture is between 6.7 to 9.5 and ideal pH level is between 7.5 to 8.5 and above and below this is stressful to the fishes. The average pH in all the experimental tanks throughout the experimental period was recorded 7.6±0.03.

4.1.3. Dissolved Oxygen

The temporal trend in the values of dissolve oxygen, in general exhibited a more or less steady state condition under all the treatments. However, in T2 and T4, it intended to decline during 1st to 28th day. T2 declined up to 5.66 mg/l at 28th day and then increased to 5.72 mg/l in 56th day, and the DO of T4 treatment increased after 28th day. But in control tanks, DO was increasing up to 5.74 mg/l at 28th and then 5.75 mg/l at 56th day. There was slight variation of DO level observed in T1 treatment which was 5.7 mg/l and in T3, and it increased up to 28th day and then it declined up to 5.7 mg/l in 56th day. The dissolved oxygen variation of experimental tanks at 1st, 28th and 56th day was presented in figure-4.

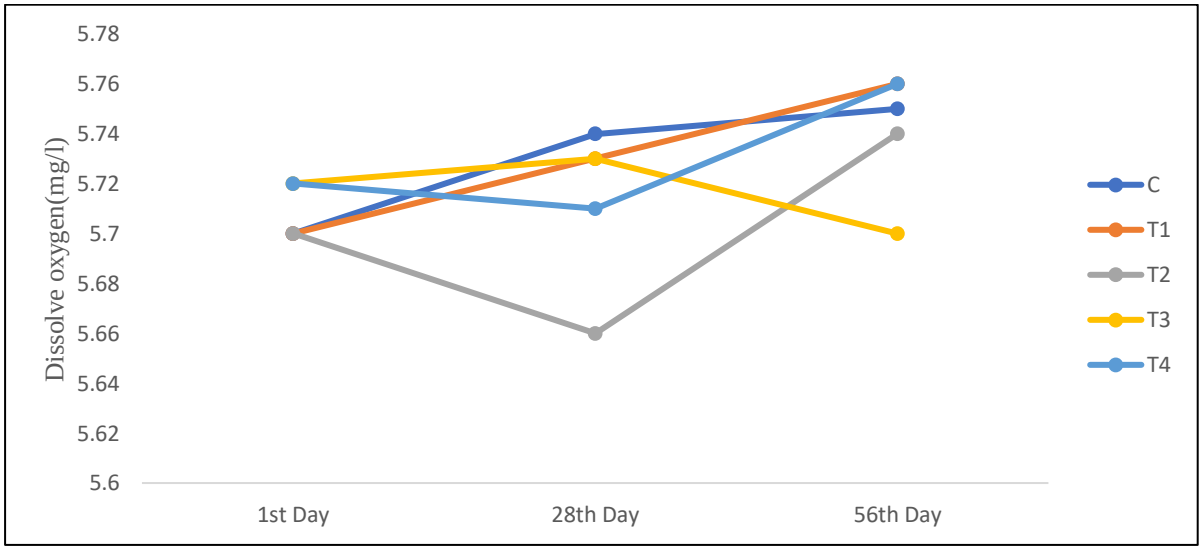


Fig.-4: Dissolve oxygen variation of experimental tanks at 1st, 28th and 56th day

Dissolved oxygen is one of the important chemical parameters in water body and should be maintained within optimum range. The level of dissolved oxygen in water always varies with a large number of factors such as water temperature, metabolic rate, biomass density etc. Bhatnagar and Singh (2010) and Bhatnagar *et al*. (2004) suggested the optimum DO level >5 mg/l is essential to support good fish production. In this experiment dissolved oxygen level in the experimental tanks recorded 5.72±0.02. Such an optimum DO level in the experimental tanks may be attributed to the continuous aeration of the culture water.

4.1.4. Hardness

The temporal variation in the concentration of hardness was observed and those were not significant differences ($p>0.05$). The hardness value ranged in between 630 mg/l to 660 mg/l. In control hardness decreased slightly in the 28th day and 56th day. During experiment observed lowest value was 634 mg/l and highest was 658 mg/l. Control groups hardness value decreased from 1st to 28th day,1st day value was 658 mg/l then 28th day value was 651 mg/l and then decreased to 647 mg/l in 56th day. T1 increased in between 1st and 28th day from 645 mg/l to 647 mg/l and then decreased to 644 mg/l during 28th day and 56th day. In T2 treatment value decreased in between 28th day and then increased slightly to 655 mg/l. Value in T3 decreased during 1st interval and then increased during 28th and 56th day. T4 value increased and then decreased in between experimental period. The variation of hardness of experimental tanks was shown in figure 5.

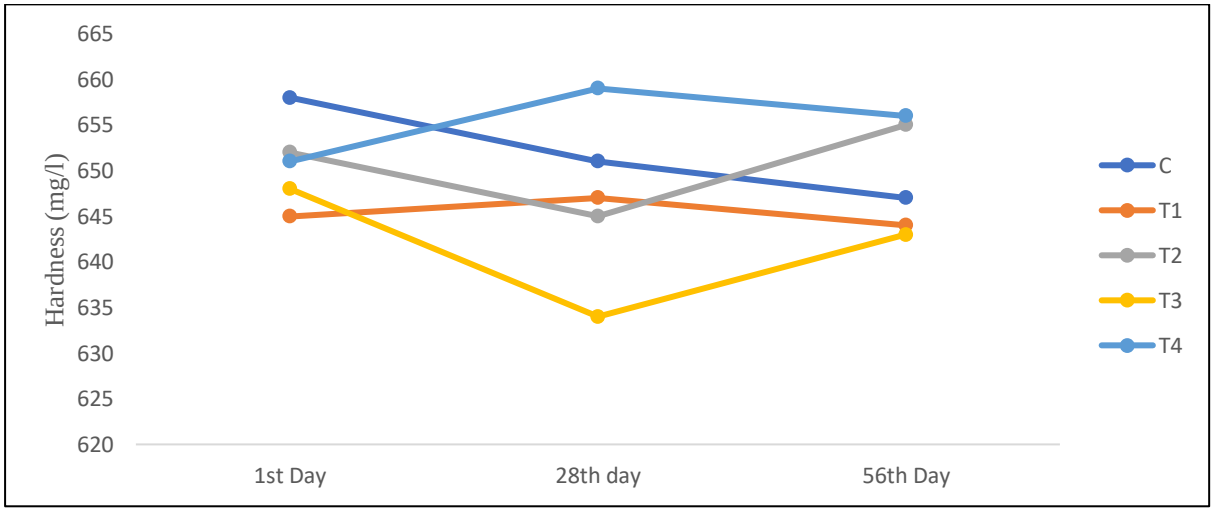


Fig.-5: Hardness variation of experimental tanks at 1st, 28th and 56th day

Hardness of water refers to the total concentration of divalent metallic cations like calcium and magnesium dissolve in water. Hardness of water is due to presence of soluble salts of calcium, magnesium and other heavier metals in water which is expressed in terms of equivalent calcium carbonate. Bhatnagar *et al*. (2004) suggested hardness values less than 20 mg/l causes stress, 75-150 mg/l is optimum for fish. The recommended range of hardness for fish culture is at least 20 mg/l (Swann, 1997) and the optimum range is 30-180 mg/l (Santhosh and Singh, 2007).

4.1.5. Total alkalinity

Total alkalinity of water intended to increase over time in all the treatments groups at 28th day. Then the total alkalinity level was decreased at 56th day. Only T4 treatment value decreased in 1st interval and then increased in 2nd interval of the experiment period. The overall mean value of alkalinity was highest at T3 (137.1 mg/l), and lowest at T4 (135 mg/l). There were not shown significant difference of total alkalinity within control, T1, T2, T3 and T4 ((p>0.05). The total alkalinity variation of experimental tanks at 1st, 28th and 56th day was noted in figure-6.

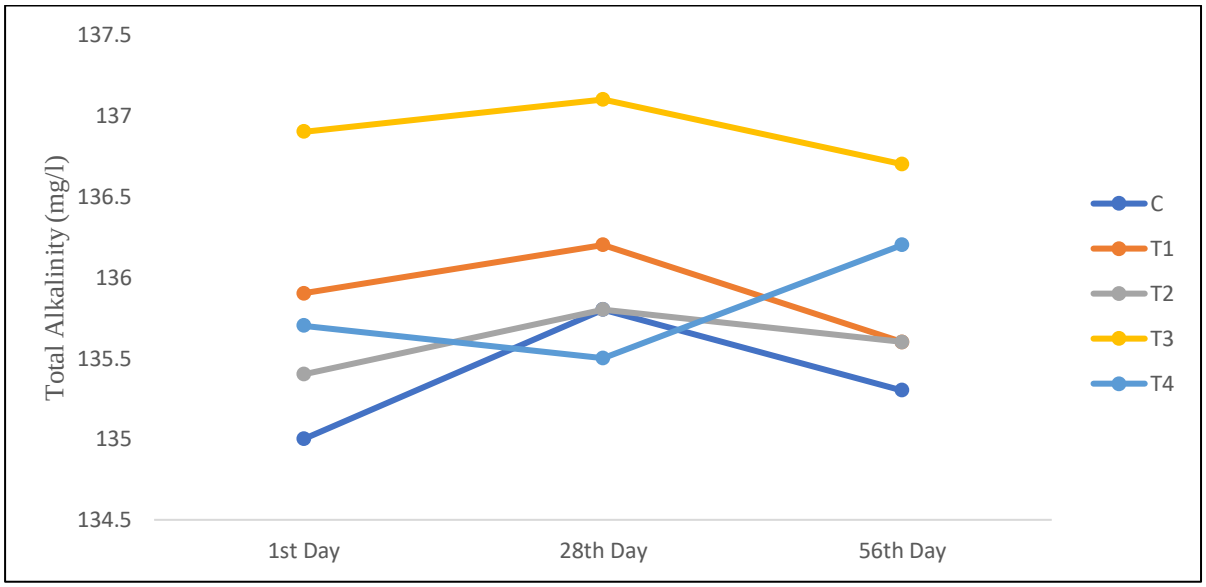


Fig.-6: Total alkalinity variation of experimental tanks at 1st, 28th and 56th day

Alkalinity is one of the important chemical parameter that is wholly depend on the presence of calcium salts in the forms of bicarbonates and carbonates. Alkalinity is also responsible for stabilizing the pond pH at an optimum range. Wurts and Durborow (1992) suggested that the ideal range of alkalinity for freshwater fish lie between 75 to 200 mg/l, but not less than 20 mg/l. According to Santhosh and Singh (2007) the ideal alkalinity value for fish culture is 50-300 mg/l. In this experiment alkalinity level in the experimental tanks recorded 136.05±0.02

4.1.6. Total ammonia

Total ammonia value was non-significant, it ranges between 0.01 to 0.04 mg/l. The highest value observed was 0.04 mg/l at T2 on 28th day and the lowest was 0.01 mg/l at control on 28th day; T2 and T3 at 1st day. There was decreasing trend of total ammonia at control and

T4 from 1st to 28th days and then increased in 29th to 56th day. The T2 and T3 groups ammonia increased in 1st interval and then decreased in 2nd interval of the experiment of 56 day. The variation of total ammonia of experimental tanks at 1st, 28th and 56th day was shown in figure-7.

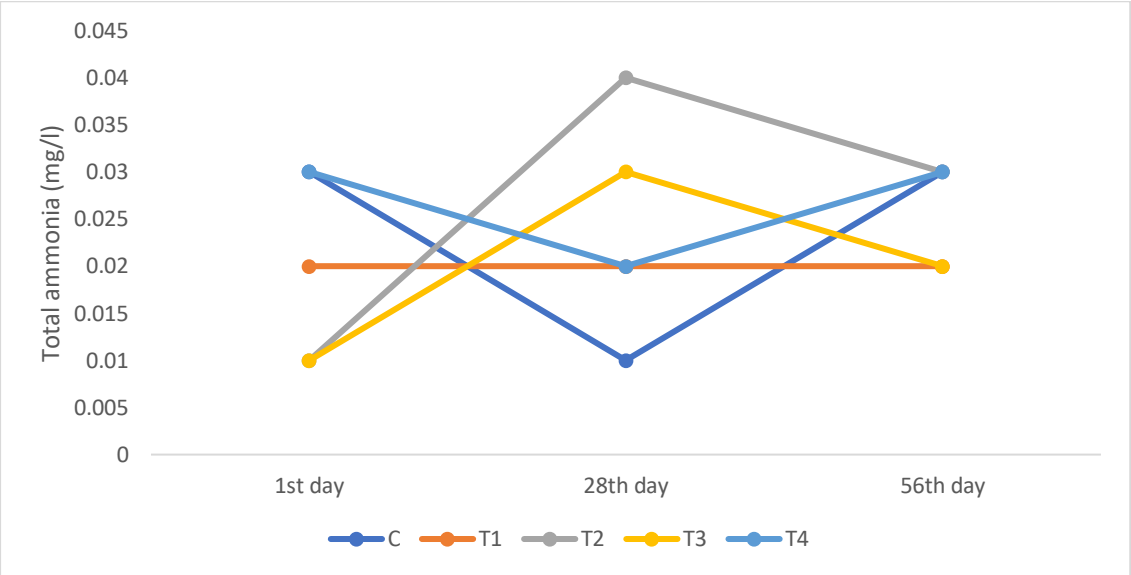


Fig.-7: Total ammonia variation of experimental tanks at 1st, 28th and 56th day

Ammonia nitrogen in a culture tank refers to the decomposition of organic matter such as uneaten feed or excreted ammonia through their gills and in their feces that indicates toxic residues which is composed of un-ionized and ionized ammonia so its need to be strictly monitored well within the tolerable limit of the culture animals. Bhatnagar and Singh (2010) recommended the level of ammonia nitrogen less than 0.02 mg/l suitable for pond fishery. Some researchers also suggested that maximum limit of ammonia concentration for aquatic organisms is 0.01 mg/l (Meade, 1985; Santhosh and Singh, 2007).

4.2. Proximate composition of experimental diets

The proximate composition of different experimental diets on dry weight basis are presented in Table -4. The moisture (%) content of all the experimental diets varied from 8.5 to 8.8. Highest moisture content was found in T1 and T2 feed valued 8.8 % and lowest moisture value was recorded in control feed valued 8.5 %. The crude protein (%) content ranged from 28.37 to 28.42. Highest crude protein percentage recorded 28.42 in T3 feed and lowest crude protein percentage was recorded in control feed. The fat value ranged from 8.5 to 8.98 %. Highest fat content was recorded 8.98 % in T3 feed and lowest fat content was recorded 8.5 % in T1 feed.

The ash (%) content ranged from 8.16 to 8.49 %. Highest ash value was recorded 8.49 % in control feed and lowest ash value was recorded 8.16 % in T3 feed.

Table-4: Proximate composition of experimental diets (on wet weight basis)

Sl. No.	Experimental Diet	Moisture	Crude protein	Fat	Ash
1	C	8.5	28.37	8.8	8.49
2	T_1	8.8	28.4	8.5	8.44
3	T_2	8.8	28.38	8.7	8.28
4	T_3	8.6	28.42	8.98	8.16
5	T_4	8.7	28.39	8.66	8.39

4.3. Growth performance

The growth performance and feed efficiency parameters of the experimental fish (*Cirrhinus mrigala*) such as, weekly body weight, average body weight gain (ABWG), daily weight gain (DWG), specific growth rate (SGR), food conversion ratio (FCR), and survivability have been calculated using standard protocol.

4.3.1. Body weight

Temporal trend of body weight of fish tended to increase over time as expected, however the pattern of growth differed in the test fish in different treatment tanks. The growth rate differed in different weeks of the experiment period. The highest average body weight was 21.62g at T3 and the lowest average weight was 15.33g at control groups. From ANOVA analysis, there was significant difference shown ($p<0.05$). From the table-5, it was clear that the weekly growth obtained of *Cirrhinus mrigala* showed significantly for all the treatments including control ($p<0.05$). But there were variations of growth obtained within the treatments. At 1st week, there was not significant change of growth among T1, T2 and T4. But T3 showed significant change from others groups. Same types of relation among the treatments and control group were found at 5th, 6th, 7th and 8th week also. The control group showed retarded growth compared to treatments during all weeks.

The Relationship of body weight (g) week-wise of different treatment groups of *Cirrhinus mrigala* (C, T1, T2, T3, T4) was presented in figure 9.

Table-5: The weekly growth (g) of *Cirrhinus mrigala* treatments wise

Weeks	C	T1	T2	T3	T4
Initial	3.61 ± 0.06^{A}	3.63 ± 0.06^{A}	3.63 ± 0.08^{A}	3.64 ± 0.03^{A}	3.62 ± 0.05^{A}
1st	4.83 ± 0.07^{aB}	5.22 ± 0.12^{bB}	5.29 ± 0.05^{bB}	5.71 ± 0.19^{cB}	5.14 ± 0.04^{bB}
2nd	6.18 ± 0.07^{aC}	6.77 ± 0.09^{bC}	6.95 ± 0.05^{cC}	7.67 ± 0.16^{dC}	6.60 ± 0.07^{bC}
3rd	7.67 ± 0.04^{aD}	8.44 ± 0.08^{cD}	8.68 ± 0.05^{dD}	9.71 ± 0.25^{eD}	8.21 ± 0.08^{bD}
4th	9.22 ± 0.04^{aE}	10.17 ± 0.12^{cE}	10.41 ± 0.07^{dE}	11.87 ± 0.18^{eE}	9.85 ± 0.11^{bE}
5th	10.74 ± 0.05^{aF}	11.90 ± 0.09^{bF}	12.16 ± 0.08^{bF}	14.66 ± 0.19^{cF}	11.59 ± 0.08^{bF}
6th	12.26 ± 0.20^{aG}	13.67 ± 0.14^{bG}	13.94 ± 0.13^{bG}	16.77 ± 1.10^{cG}	13.39 ± 0.07^{bG}
7th	13.79 ± 0.40^{aH}	15.43 ± 0.16^{bH}	15.78 ± 0.10^{bH}	18.97 ± 1.27^{cH}	15.11 ± 0.09^{bH}
8th	15.33 ± 0.63^{aI}	17.41 ± 0.38^{bI}	17.95 ± 0.47^{bI}	21.62 ± 1.22^{cI}	16.84 ± 0.18^{bI}

*Data presented in mean ±SD. Capital superscripts showing significance level column-wise ($p<0.05$), small superscripts showing significant level row-wise ($p<0.05$).

There were significant differences of growth obtained among all groups at 3rd and 4th week. But there was no significant difference of growth obtained within T1 and T4 at 2nd week. Temporal variation of average body weight of fishes in the system was shown in figure- 8.

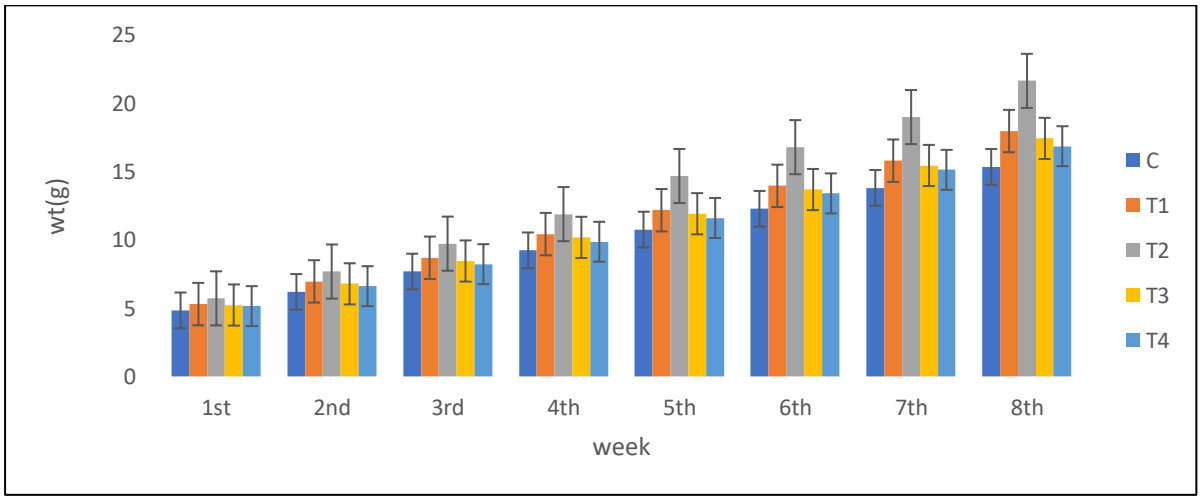


Fig.-8: Weekly variation of average body weight of *Cirrhinus mrigala*

Same as present study, in grass carp Faiz *et al*., (2015) reported that end of the experiment a significant difference in body weight was observed in Zn supplemented diet as compared to basal diet. Mondal *et al*., (2020) also experienced that in rohu fish ZnO nano particle supplemented feed can achieve higher body weight than fish basal diet and 20 mg/kg nZnO supplemented diet achieve highest body weight among another treatments. Present study showed highest body weight in 15 mg/kg nZnO supplemented feed. Mahboub *et al*., (2020) observed that in African catfish nZno 20 mg/kg showed highest final body weight as compare to ZnO 20 mg/kg, nZnO 30 mg/kg supplemented diets. Swain *et al*., (2018) reported that body weight increased higher in nano zinc and nano selenium as compared to basal diet.

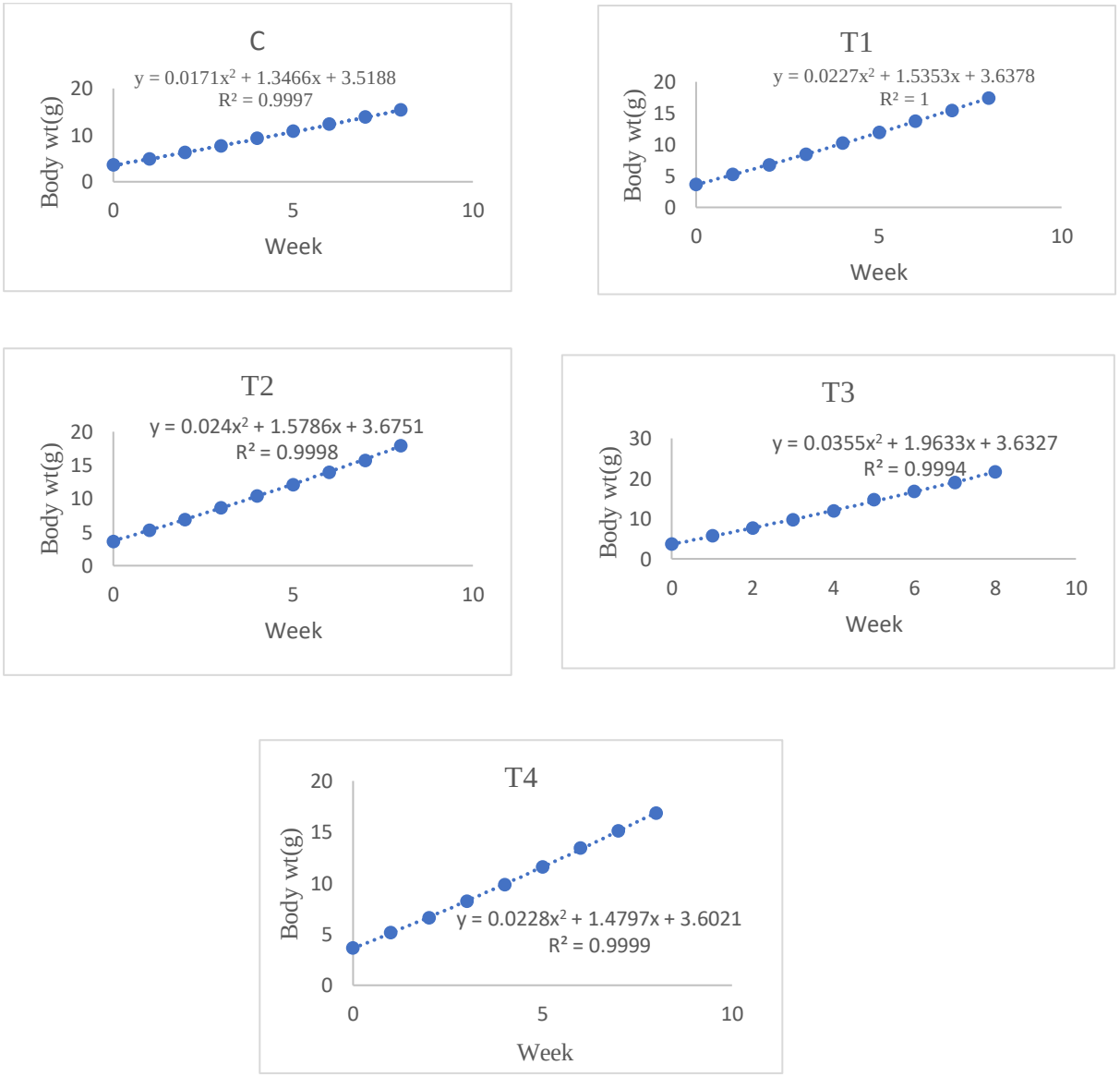


Fig.-9: The relationship of body weight with week-wise of *Cirrhinus mrigala* under control and different treatments.

4.3.2. Average body weight gain (%)

In the present study, weight gain was increased with the advancement of the study and average body weight gain (ABWG) attained by the experimental fish recorded in weekly basis. Based on that we can see optimal ABWG was attained by the fishes except T3 treatment fishes. The highest ABWG found was 17.93±1.20(g) in T3 treatment and the lowest ABWG found was 11.65±0.65g in control tank. T3 tank showed highest peak in between 4th and 5th week and then decreased and again increased during 6th and 8th week. The ABWG did not show significant differences within T1 and T4 but, there was significant differences among other groups ($P<0.05$) (Table-6).

Table-6: Average body weight gain (ABWG), daily weight gain (DWG), feed conversion ratio (FCR) and specific growth rate (SGR) of *Cirrhinus mrigala.*

Treatment	ABWG	DWG	SGR	FCR
C	11.65±0.65^{a}	0.208±0.01^{a}	2.58±0.003^{a}	2.06±0.01^{d}
T1	13.76±0.33^{b}	0.245±0.01^{c}	2.8±0.00^{b}	1.72±0.01^{b}
T2	14.26±0.57^{c}	0.254±0.01^{c}	2.85±0.01^{b}	1.60±0.02^{b}
T3	17.93±1.20^{d}	0.320±0.02^{d}	3.18±0.01^{c}	1.54±0.01^{a}
T4	13.24±0.05^{b}	0.236±0.01^{b}	2.74±0.02^{b}	1.96±0.02^{c}

*Data presented in mean ±SD. Small superscripts showing significance level column-wise ($p<0.05$).

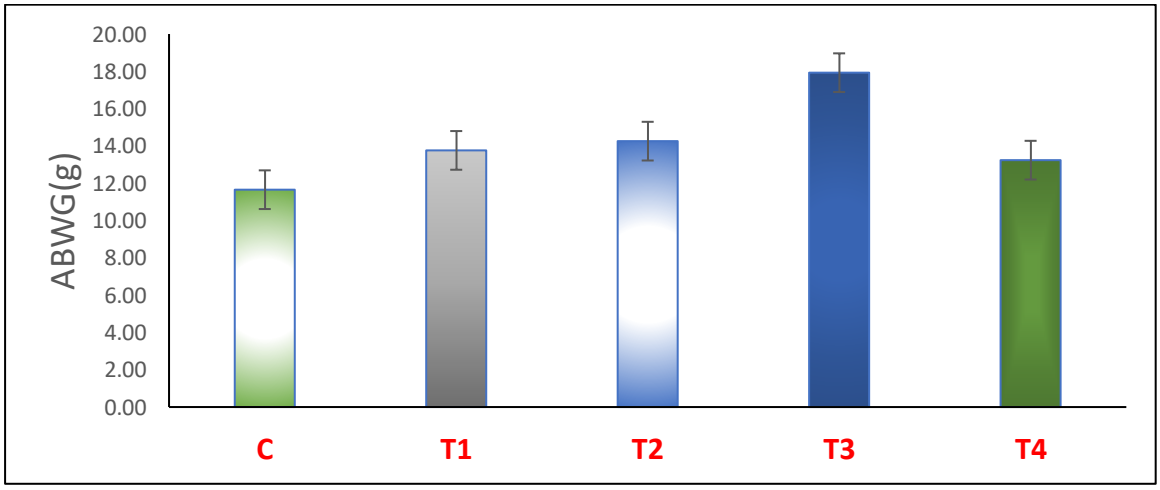


Fig.-10: Variation of total average body weight gain of *Cirrhinus mrigala*

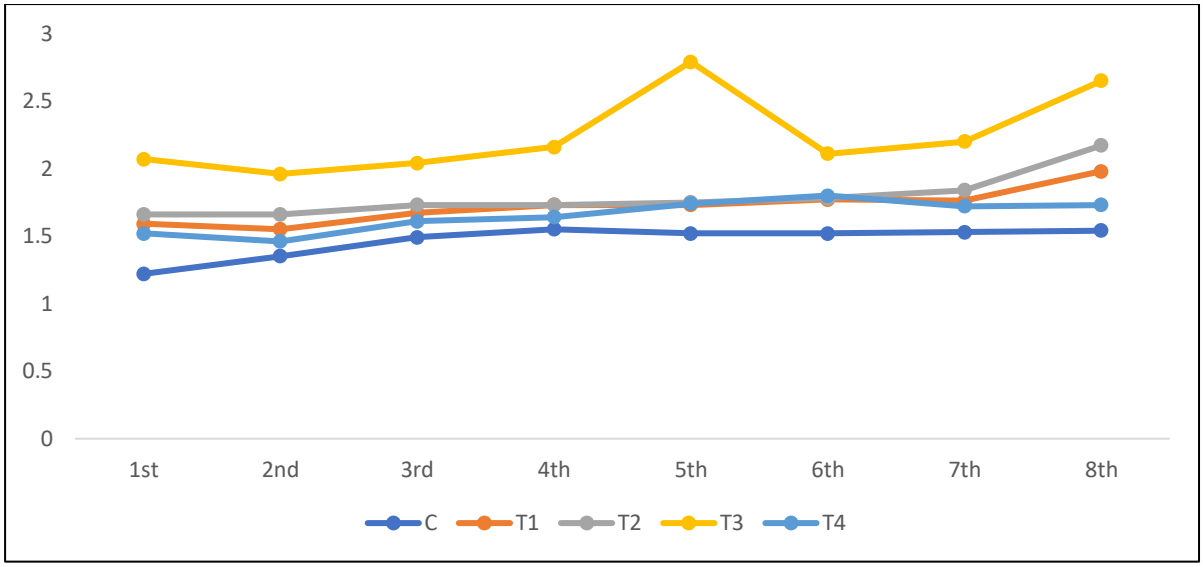


Fig.-11: Temporal variation of average body weight gain of *Cirrhinus mrigala*

The weekly pattern of average body weight gain was in slight increasing trend but more increased trend of weekly weight gain was observed in 5th week for T3 group (figure-11). Current study on mrigal showed that higher weight gain is achieved in 15mg/kg of nZnO as compare to other diet of ZnO 30 mg/kg concentration and nZn concentration of 7.5 and 30 mg/kg diet. Rajan and Rohini (2020) reported a same study where they recorded mrigal achieved better weight gain in 15 mg/kg diet of nZnO than any other 5,10,20,25 gm/kg concentration of nZnO, which is in the favour of my experiment. According to Ghazi *et al.* (2021) 10 mg/kg diet ZnO-NP shows higher weight gain in tilapia than control diet. Based on the study on grey mullet Shukry *et al.*, (2021) observed that the weight gain was significantly higher in fish treated ZnO nano particle than the control and that fed diet had 20-40 mg/kg nano ZnO. But present study showed higher weight gain in 15 mg/kg ZnO nano particle diet. Onuegbu *et al.*, (2018) experienced ZnO 30 mg/kg nano particle diet shows significantly($p<0.05$) higher weight gain in catfish. Tawfik *et al.*, (2017) reported that the weight gain of tilapia gradually enhanced by supplementation of nano zinc oxide than the conventional zinc oxide. Kishawy *et al.*, (2020) reported that nano zinc significantly improved the weight gain than organic zinc.

4.3.3. Daily weight gain (DWG)

Onuegbu *et al.* (2017) reported that nano particle supplemented feed showed higher daily weight gain in catfish. In this study daily weight gain was observed in 56 days experiment period, and it is documented treatment wise. Present study revealed that T3 treatment shows the highest DWG and control tank shows lowest DWG. Highest DWG is 0.32g and lowest

DWG is 0.2g. For T1, T2 and T4 tank DWG was 0.25g, 0.26g and 0.24g respectively. Present study showed that there was no significant($p>0.05$) differences among the control and treatments groups. Daily weight gain represented in figure-12.

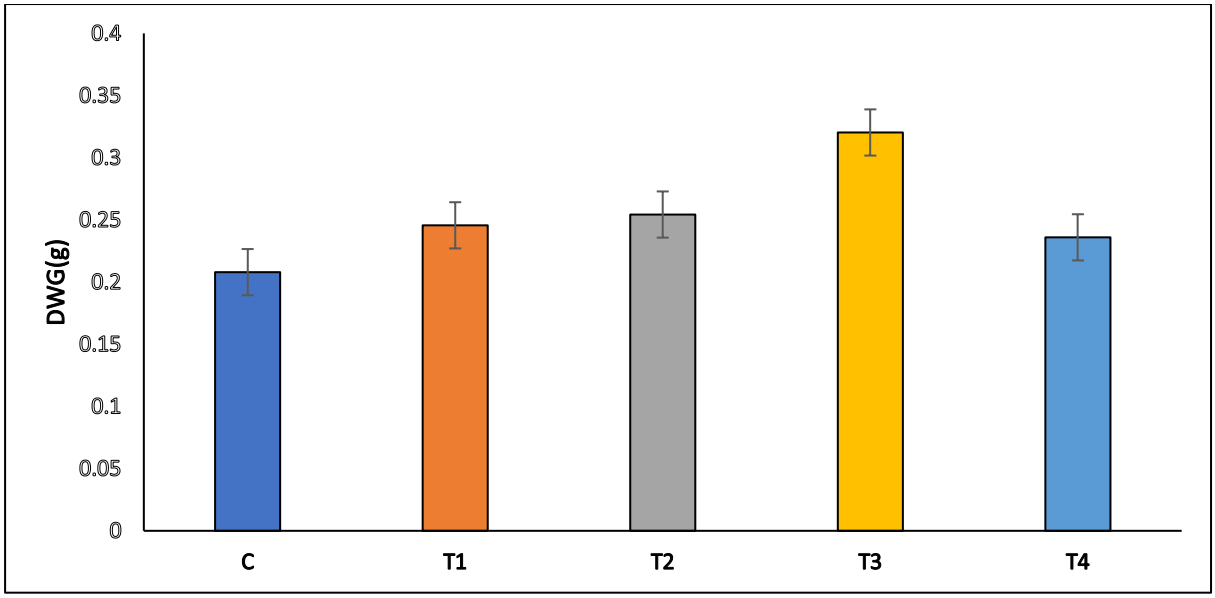


Fig.-12: Treatment wise variation of DWG of *Cirrhinus mrigala*

4.3.4. Specific growth rate (SGR)

The SGR value of different treatment groups are shown in Table-6. The result obtained in specific growth rate of the experimental fish showed a significant($P<0.005$) difference among different treatment groups. The highest SGR value was recorded in T3 (3.181g) which was significantly different from another treatment. The lowest value was founded in Control group (2.582g). There were no significant differences ($p>0.05$) among treatment groups of T1, T2 and T4 (figure-13). There was significant difference of T3 with other three treatments and control group. Present study showed that T3 containing nZnO 15 mg/kg concentration achieve higher SGR in Mrigal. Previous study on *Oreochromis niloticus* by Tawfik *et al*. (2017) reported that fishes were fed for 120 days on Zinc oxide conventional bulk scale (ZnO) and nanoscale (nZnO) supplemented feed in different concentrations (15, 30, 45 and 60 mg/kg of the feed).

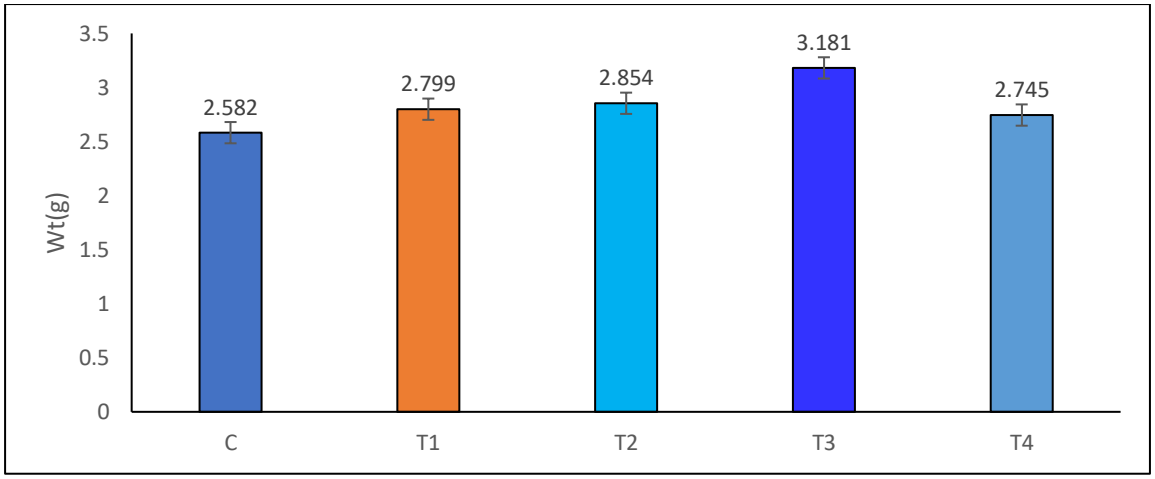


Fig.-13: Treatment wise variation of SGR of *Cirrhinus mrigala*

nZnO (15mg/kg) achieved higher specific growth rates like the higher concentrations of bulk ZnO (60mg/kg). Growth rates of the fishes in both control and treatment diets were within the normal range for *L. rohita* raised under pond culture, and supplemental Zn and Se had higher growth rates in treated groups (SGR) as compared to control group during 120 days of experimental period (Swain *et al*., 2018).

4.3.5. Feed conversion ratio (FCR)

The FCR of the different treatment groups are given in Table-6. Better FCR was recorded in T3 (1.54±0.01) which was significantly differed from other groups ($p<0.05$). The highest FCR was found in control group (2.06±0.01). There was no significant difference between T1 (1.72±0.01) and T2 (1.60±0.02). The significant difference was found in T4(1.96±0.01) with other treatments.

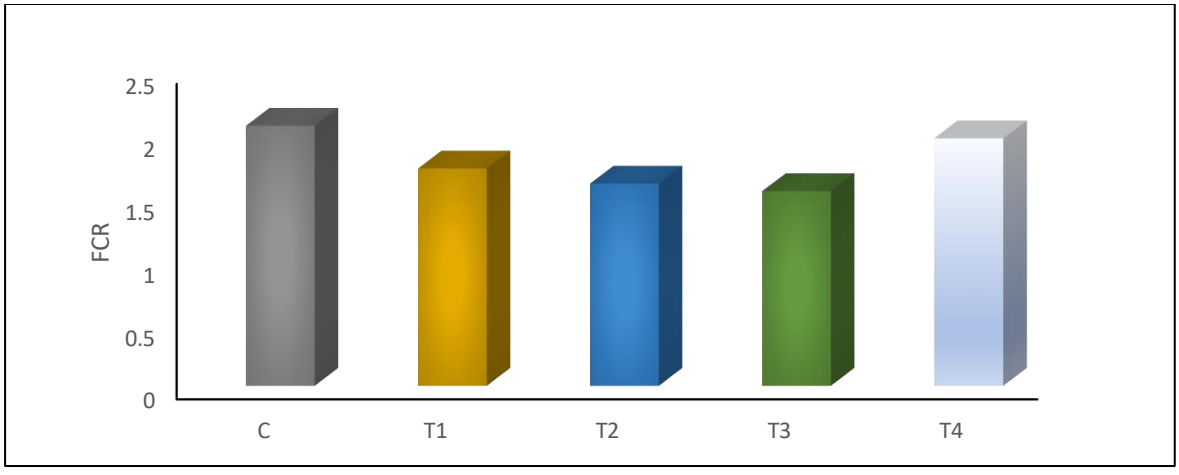


Fig.-14: Variation of feed conversion ratio of *Cirrhinus mrigala*

Present study showed T3 treatment containing nZno 15mg/kg concentration diet achieve low FCR (figure-14). In similar study Mondal *et al*. (2020) observed that in 20 mg/kg concentration of nZnO showed low FCR in rohu. Faiz *et al*., (2015) showed that the feed conversion ratio was higher in ZnO nanoparticles incorporated feed of juvenile grass carp. Feed consumption and feed conversion efficiency of Mrigal were higher in feed containing 15 mg of zinc oxide nanoparticles. Onuegbu *et al*. (2018) reported an increase in the concentration of Zinc Oxide nanoparticles with feed consumption decreased feed conversion ratio. Relationship between body weight and FCR in mrigal in control and different treatments shown in figure 15. It was found that there was very strong relationship between FCR and body weight at different treatment gropus along with control as R^2 is nearer to 1.

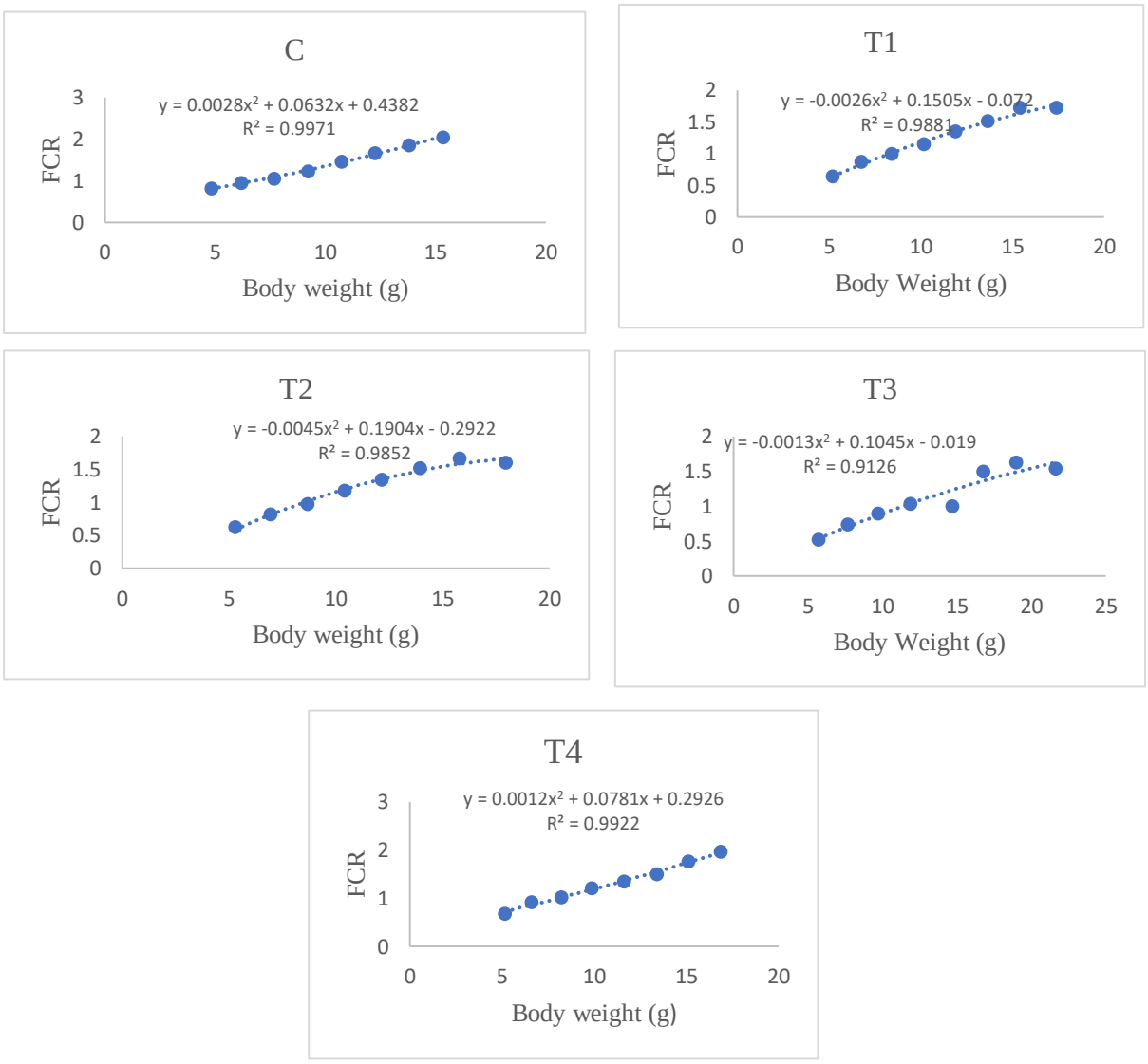


Fig.–15: Relationship between body weight(g) and FCR in mrigal in control and different treatments.

4.3.6. Survivability

It is defined as the ratio of the fish survived with initial number of fish described in percentage**.** In the present study, survivability was observed on weekly basis upto 8th week of experimental period. Control group having 100% survivability and T1, T2, T3 and T4 group having 98.61%, 98.26%, 99.31% and 91.67% survivability respectively. T3 treatment having highest survivability of 99.31% and T4 treatment having lowest survivability of 91.67% (figure-16).

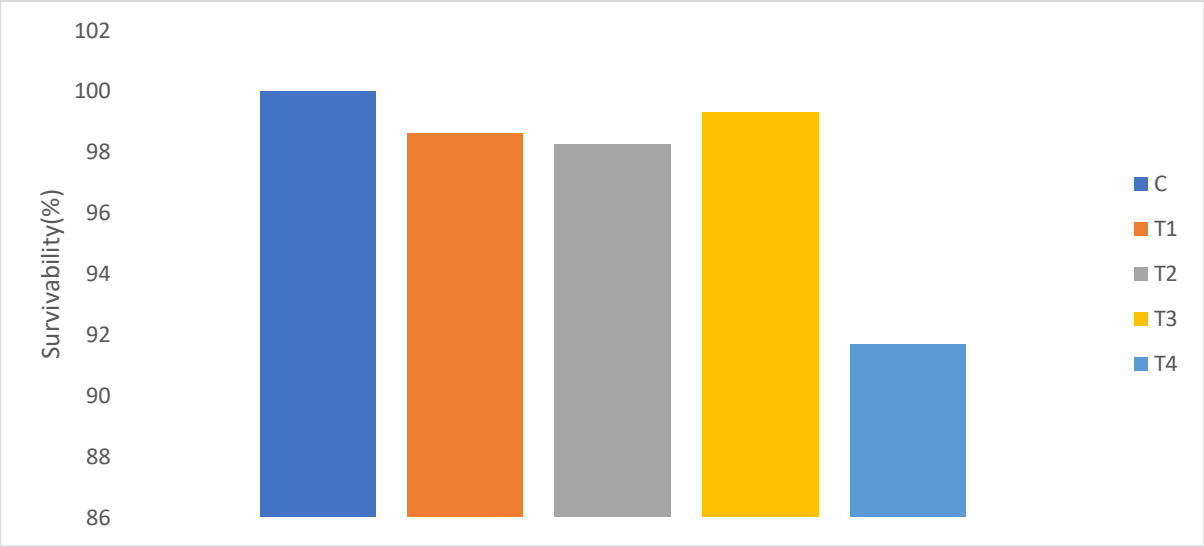


Fig.-16: Survivability (%) variation of *Cirrhinus mrigala*

Rajan and Rohini(2020) experinced that *Cirrhinus mrigala* having survival rate of 100% in feed containing 05 and 20 mg of nZnO, and 80, 90 and 90% observed in 10, 15 and 25 mg. Awad *et al*. (2019) reported that 30 mg/kg diet of nZnO showed 90% survivability and 60 mg/kg diet of nZnO showed 86.6% survivability in Nile tilapia. The survival rate of rohu in the concentrations of ZnONPs (mg/kg) 5, 7.5 ,10 was 94%, 95% and 96% respectively (Thangapandiyan and Monika, 2019).

4.4. Haematological parameters

The haematological analysis acts as a rapid and economical method for assessing metal oxide toxicity on fish (Rajan and Rohini, 2021). Shah and Altindag (2005) reported that the haematological parameters such as haematocrit, haemoglobin, red blood cell, white blood cell are used to assess the functional status of the oxygen-carrying capacity of the bloodstream and have been used as an indicator of health condition of fish. This analysis are very helpful in judgement of health conditions of fish and now commonly used as an effective index for monitoring the physiological and pathological changes in fish (kori-siakpere *et al*., 2008).

Table-7: Different haematological parameters treatment-wise of *Cirrhinus mrigala*

	C	T1	T2	T3	T4
Haemoglobin (g/dl)	6.3 ± 0.37^{a}	6.7 ± 0.20^{b}	6.8 ± 0.14^{b}	8.0 ± 0.11^{d}	7.2 ± 0.12^{c}
RBC (Million/Cu-mm)	2.1 ± 0.01^{a}	2.2 ± 0.03^{a}	2.2 ± 0.06^{a}	2.6 ± 0.04^{b}	2.4 ± 0.02^{b}
WBC (no/Cu-mm)	3900 ± 25^{d}	3600 ± 23^{d}	1300 ± 24^{a}	3200 ± 25^{c}	1900 ± 28^{b}
Neutrophil	52±0.20	57±0.10	51±0.20	51±0.10	48±0.10
Lymphocyte	45±0.20	40±0.10	46±0.20	44±0.20	49±0.10
Monocyte	1±0.10	1±0.10	1±0.10	2±0.20	1±0.20
Eusinophill	2±0.02	2±0.01	2±0.02	3±0.01	2±0.02
Platelets count ($x10^5$)	0.64±0.02	0.45±0.01	0.6±0.02	0.9±0.03	0.8±0.01
ESR (mm in 1st Hour)	14±0.10	16±0.20	29±0.10	21±0.20	10±0.10
P.C.V (%)	18.9±0.11	20.1±0.12	20.4±0.14	24±0.14	21.6±0.13
M.C.V (FL)	90±0.02	91.3±0.03	92.7±0.06	92.3±0.05	90±0.02
M.C.H (Pg)	30±0.01	30.4±0.02	30.9±0.05	30.7±0.01	30±0.03
M.C.H.C (g/dl)	33.3±0.15	33.3±0.20	33.3±0.30	33.3±0.40	33.3±0.22

Different superscripts showing significance level row-wise ($p<0.05$).

Present study showed that blood parameters are increased and decreased value in effect of nZnO against control. Haemoglobin, RBC, platelets count and PCV showed increased value in treatment than control and these parameters showed highest value in T3 (8.0±0.11g/dl). But WBC was decreased in treatment tank than control tank (table 7). Some of the parameters were remain minor changes like monocytes, eusinophil, MCV, MCH, MCHC counts. The relationship of body weight (g) with RBC, haemoglobin and platelets were presented in figure-18. There is strong relationship of RBC and haemoglobin content with body weight as R^2 is nearer to 1. But there is moderate relationship of platelets count with body weight (R^2 =0.6404).

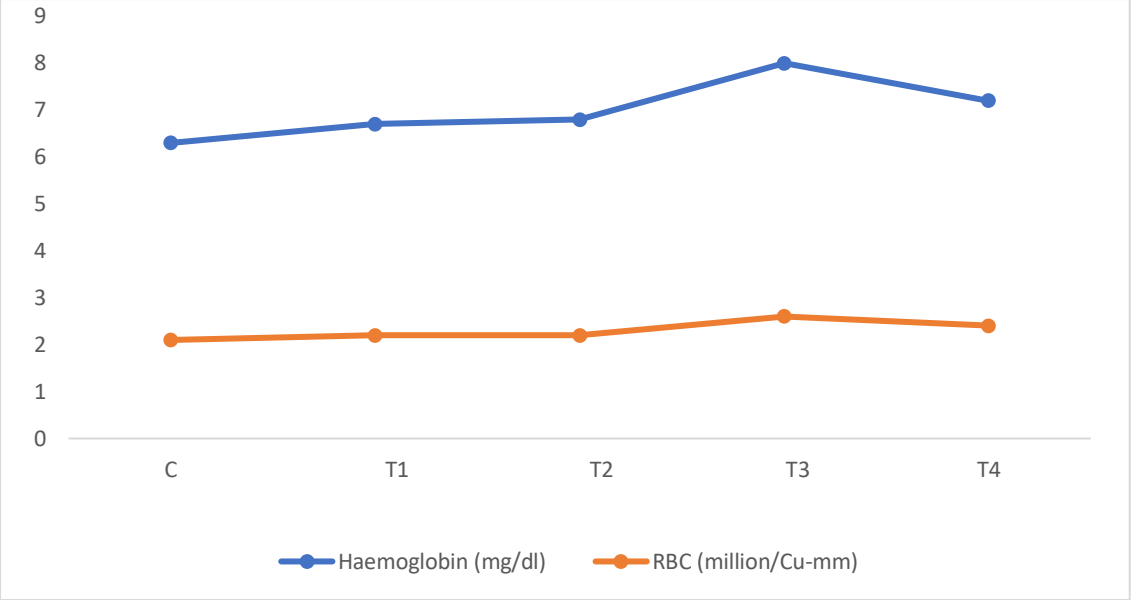
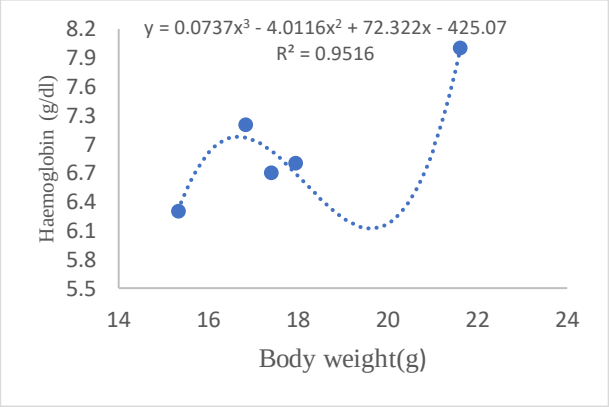
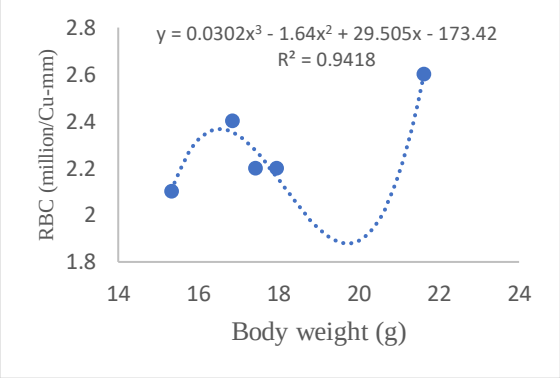


Fig.-17: Line diagram showing haemoglobin (mg/l) and RBC (million/Cu-mm) treatment-wise





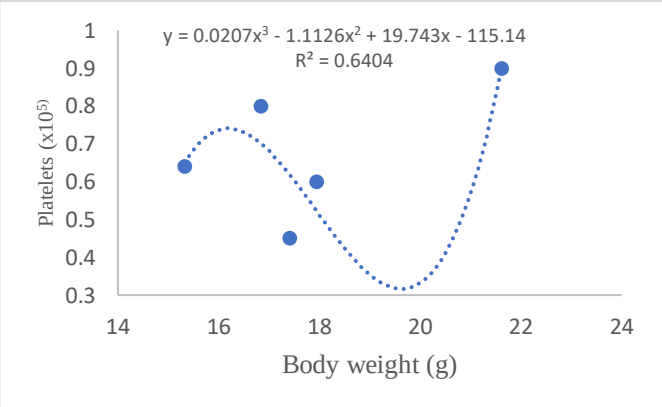


Fig.-18: Relationship of Haemoglobin, RBC and platelets with body weight(g).

T3 treatment showed progressively better changes than any other treatments and control tank. Rajan and Rohini (2020) reported that the haematology performance was higher in nZnO 15mg/kg diet of mrigal than 5,10,20 and 25 mg per kg diet of nZnO. Haemoglobin, RBC, Haematocrit, MCV, MCH, MCHC of mrigal progressively increased and WBC and platelets decreased with an increase in the quantity of Zinc Oxide nanoparticles. As similar to Present study, (Firat, 2007) experienced that the decrease in WBC count could be associated with the cortisol hormone which play an important role in prevention and healing of inflammation on fish. Similar to present study Faiz *et al*. (2015) reported that in grass carp higher concentration of nZnO (60mg/kg) significantly decreased RBC, WBC counts than concentration of 30mg/kg of nZnO diet. The fish fed with 10 mg/kg ZnONP-supplemented diet shows progressively increased haematological changes (Thangapandiyan and Monika, 2019). Akbary *et al*. (2018) observed that the Hb, Hct, and RBC counts are decreased and WBC count significantly increased compared to the control in grey mullet fish exposed to sublethal concentration of copper oxide nanoparticles, which is totally against my findings. Chupani *et al*. (2018) reported that when common carp (*Cyprinus carpio*) exposed to diet-born ZnO nanoparticles had no effects on haematology, but present study showed that nZnO effects the hematology. There were changes in haemoglobin, RBC, WBC, neutrophil, platelets count, ESR, PCV, MCV shown in Table-4.

4.5. Enzymological performance

Present study showed that amylase activity is highest in T3 treatment containing nano ZnO concentration of 15 mg/kg. After blood serum analysis highest amylase activity was recorded 51.00±0.22u/l in T3 tank and lowest amylase activity was recorded 32.00±0.15u/l in control tank (table 8). There was no significant difference within T3 and T4 but these two treatments are significantly differed from other groups. The highest lipase activity was recorded in T3 (76.50±0.18u/l) and lowest lipase activity was recorded in control (47.00±0.15u/l). Like amylase, there was no significant difference within T3 and T4 but these two treatments are significantly differed from other groups. ALT and AST increased with increase of Zn concentration and control diet reveal lowest AST and ALT value. Lowest AST value was recorded 14.00±0.53 in control tank and highest AST value was recorded 29.00±0.35 in T2 (30 mg/kg) diet. Lowest ALT value was recorded 28.00±0.51 in control diet and highest ALT value was recorded 41.00±0.75 in T2 tank.

Relationship of body weight (g) with amylase and lipase enzyme was presented in figure 21. There is very strong relation of body weight change with amylase level (R^2=0.8099) and with lipase (R^2=0.834). Relationship of ALT and AST with body weight(g) was shown in figure 22. There is medium relation of body weight with ALT (R^2=0.6844) and with AST (R^2=0.5145).

Table -8: Different enzymological performance of *Cirrhinus mrigala* at different treatments.

Treatment	Amylase(u/l)	Lipase(u/l)	AST/SGOT(u/l)	ALT/SGPT(u/l)
C	32.00±0.15^a	47.00±0.15^a	14.00±0.53^a	28.00±0.51^a
T1	37.00±0.25^b	55.00±0.22^b	16.00±0.41^a	30.00±0.47^a
T2	42.00±0.21^c	61.00±0.20^c	29.00±0.35^c	41.00±0.75^c
T3	51.00±0.22^d	76.50±0.18^d	23.00±0.51^b	34.00±0.60^b
T4	47.00±0.23^d	70.50±0.20^d	21.00±0.51^b	33.00±0.32^b

Different superscripts showing significance level column-wise ($p<0.05$).

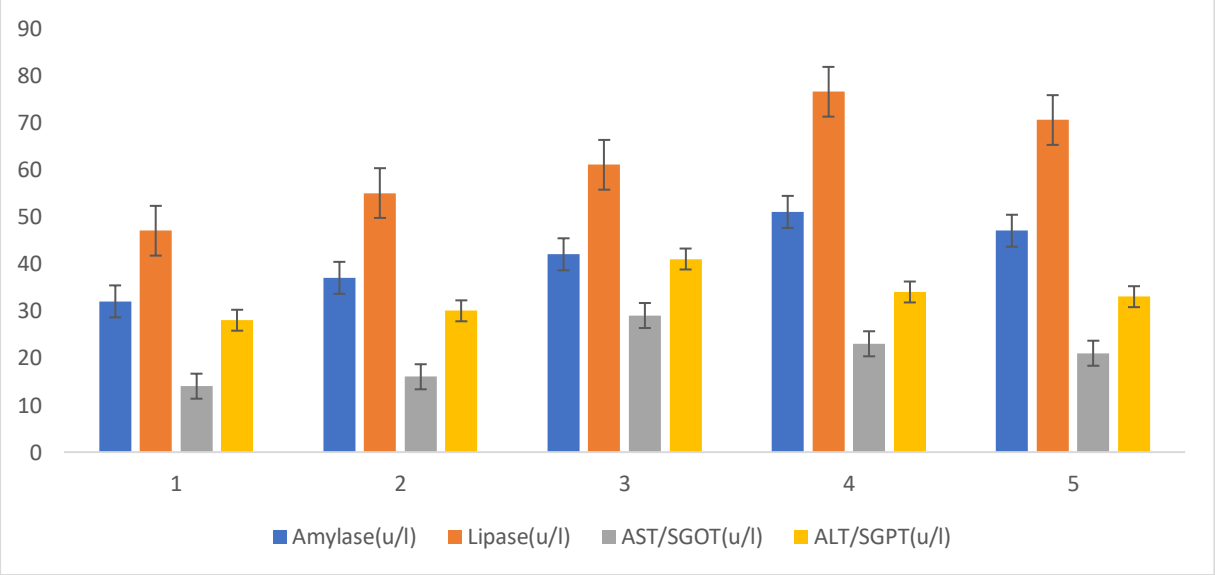


Fig.-19: Enzymological variations treatment-wise of *Cirrhinus mrigala*

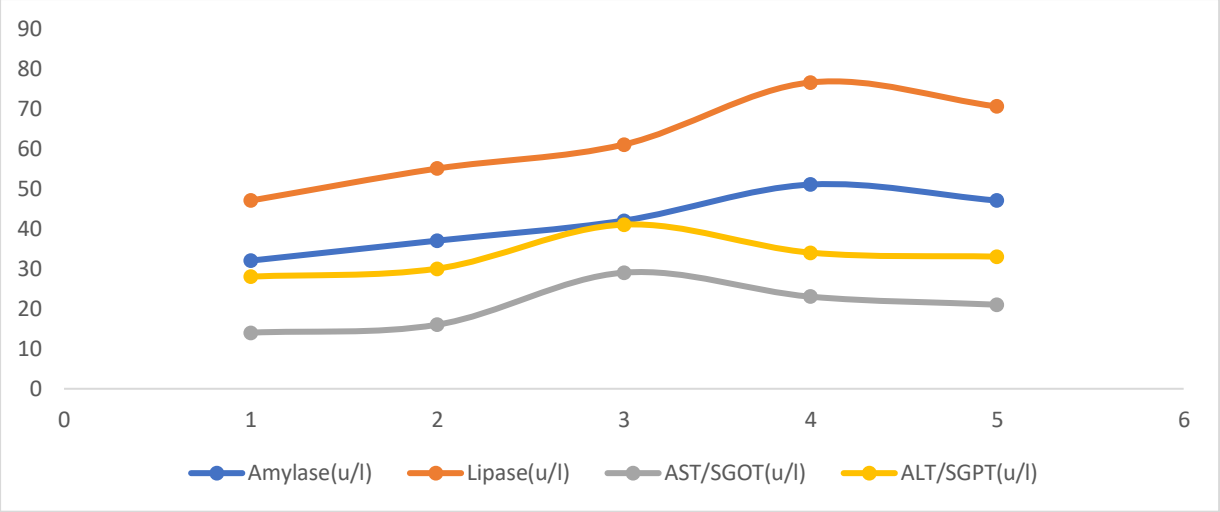
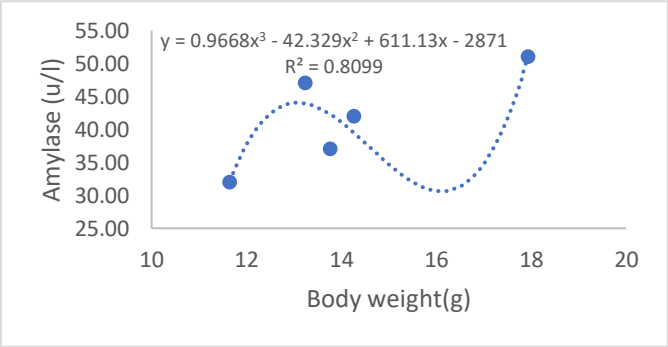


Fig.-20: Enzymological variations treatment-wise of *Cirrhinus mrigala*.



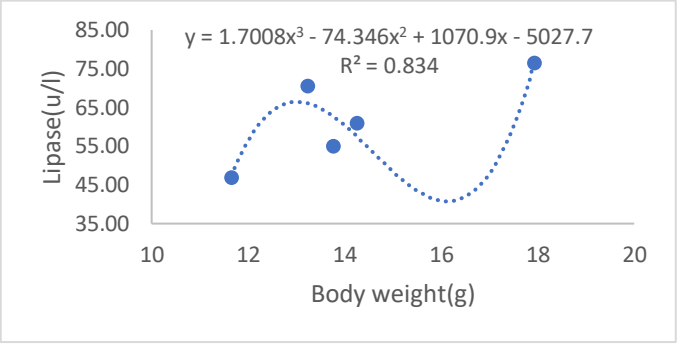
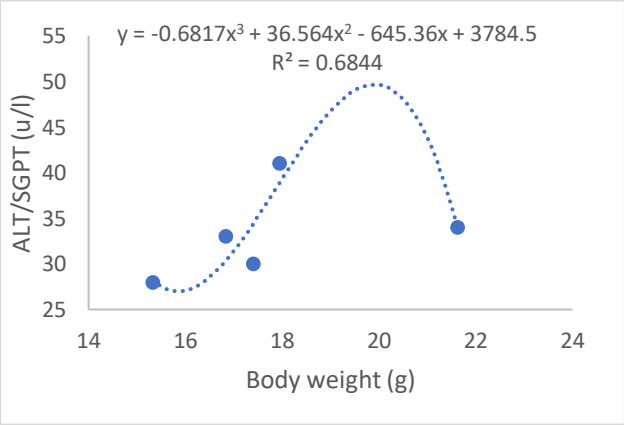


Fig.-21: Relationship of amylase and lipase enzyme with body weight (g).



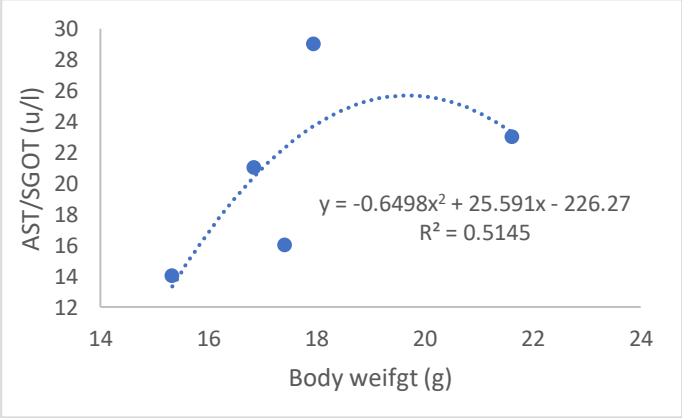


Fig.-22: Relationship of ALT/SGPT and AST/SGOT with body weight(g).

The activities of ALT and AST are pointers of healthy liver or liver dysfunction. Mondal *et al*. (2020) reported that digestive enzymes activities were significantly ($p<0.05$) higher in the diet containing nano ZnO in rohu. Amylase activity was higher in nano ZnO 20mg/kg concentration and lipase activity was higher in nZnO concentration of 30mg/kg. They also observed that ALT and AST increased with increasing in nano ZnO concentration. Present study also revealed that nano zinc supplemented diet increased digestive enzyme activity in blood and ALT and AST increased with increasing in nano ZnO concentration. Similar to the study Ibrahim *et al*. (2021) observed that the ALT and AST values were lower in 30mg/kg concentrated diet of nZnO compare to 60mg/kg concentrated diet of nZnO in Nile Tilapia.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The present experiment was carried out with the aim to find out the **"Influence of different quantity of zinc sources on the production performances of *Cirrhinus mrigala*".** The experiment was conducted in the laboratory conditions of the department of Aquaculture, Faculty of Fishery Sciences campus, West Bengal University of Animal and Fisheries Sciences, Chakgaria, Panchasayar, Kolkata-700094 (Lat. 22º 82'N; Long. 88º 20'E).

The whole research imbodied in thesis has been documented in seven chapters, *Viz. Introduction, Review of literatures, Materials and methods, Results and discussion, Summary and conclusion, Future scope of research and Bibliography* comprise the entire study that is included in this thesis.

1. The *Introduction* chapter highlights the importance of research on importance of zinc and zinc supplemented as nano particle feed additive and its role in growth performance, haematological and enzymological changes in mrigal. This chapter also highlights the objectives of the present research work.
2. In the next chapter i.e. *Review of literatures*, the information about current status of aquaculture and emergence of nano technology for betterment of aquaculture production and fulfil the demand of food security. In this study we reviewed the efficiency of different zinc source in nano as well as normal form on different fish species and animal nutrition and their role on growth performance, haematology and enzyme activity.
3. In the chapter *Materials and methods*, the protocols taken during experiment was noted clearly.

- ✓ Mrigal fish was stocked @15 each in every glass aquaria capacity of 243 litter (L120cm×W45cm×H45cm). Tanks were organised in triplicate manner.
- ✓ The basal feed was prepared with different ingredients and the control feed was prepared without any zinc source. The treatment feeds were prepared with normal zinc of 30mg/kg (T1), nano zinc 30mg/kg (T2), nano zinc 15mg/kg (T3) and nano zinc 7.5 mg/kg (T4). With the help of hand pelletizer machine, the pellets are prepared and they were dried at room temperature.
- ✓ Fishes are fed control and four treatment feed (C, T1, T2, T3, T4) @3% of body weight. Feeding was done twice a day (9 AM and 4 PM).

- ✓ Water quality parameters were taken 1st day of experiment, then 28th day and 56th day of interval.
- ✓ Growth data was taken from 1st day to 56th day in weekly interval. Growth data was recorded 8 times during experiment.
- ✓ At the end of the experiment the fish blood was collected to analyse the haematological parameter (Hb, RBC, WBC, PCV, MCV, MCH, MCHC).
- ✓ Also, blood serum was prepared to analyse enzyme activity (Amylase, Lipase, ALT, AST)
- ✓ ANOVA Statistical analysis was performed to identify the significant and non-significant difference among the control and treatments diets result on mrigal growth, haematology and enzyme activity.

4. In the chapter *Results and Discussion*, the results part was noted clearly and discussed with previous results done earlier by researchers.

Water quality parameters

Temperature, pH, dissolve oxygen, total alkalinity, total hardness, total ammonia -nitrogen was recorded 1st day, 28th day and 56th day of the experiment. The average temperature of water ranged from 26.6 °C to 26.85 °C. pH of water ranged from 7.51 to 7.65. The Average dissolve oxygen of water ranged from 5.66 to 5.76 mg/l. The average alkalinity of water ranged between 135 to 137.1 mg/l. The average of total hardness varies between 634 to 658 mg/l and the average of total ammonia-nitrogen was ranged from 0.01 to 0.04 mg/l.

- ✓ Water quality parameters were found nonsignificant ($P > 0.05$) between control and treatment tanks.
- ✓ Temperature, DO, pH, Hardness, alkalinity and ammonia showed normal range. There was no significant changes by the effect of nano zinc supplemented feed.

Growth parameters

The growth of fish in term of weight were observed weekly basis. During experiment period the sampling weight was recorded 8 times. Different growth parameters such as average weight, average weight gain, average daily weight gain, specific growth rate, FCR were estimated during experiment period.

- ✓ There was significant ($p<0.05$) difference in average weight of fish. The growth rate differed in different treatment tank. After completion of the experiment the highest average body weight was recorded 21.62±1.22(g) in T3 tank and lowest body weight was recorded 15.33±0.63(g) in control tank. From experiment, experienced that mrigal obtained weekly growth significantly. But there was no significant growth variation among T1, T2 and T4. There was retarded growth in control group compared to treatments during experiment period.
- ✓ The average body weight gain did not show significant differences with in T1 and T4 but, there was significant difference among other groups. Highest average body weight gain was observed in T3 tank (17.93±1.20g) and lowest was recorded in control (11.65±0.65g). T3 tank showed highest peak in between 4^{th} and 5^{th} week and then decreased and again increased during 6^{th} and 8^{th} week. The ABWG did not show significant differences within T1 and T4 but, there was significant differences among other groups ($P<0.05$).
- ✓ Present study showed that daily weight gain has no significance differences among the control and treatment groups. Highest daily weight gain was recorded 0.32g and lowest was 0.2 g. T1, T2 and T4 tank DWG was 0.25g, 0.26g and 0.24g respectively.
- ✓ In specific growth rate there was no significant difference among treatment groups but there was significant difference of T3 with other three treatments and control group. There were no significant differences ($p>0.05$) among treatment groups of T1, T2 and T4.
- ✓ Better FCR was recorded in T3 (1.54±0.01) which was significantly differed from other groups ($p<0.05$). The highest FCR was found in control group (2.06±0.01). There was no significant difference between T1 (1.72±0.01) and T2 (1.60±0.02). The significant difference was found in T4(1.96±0.01) with other treatments.
- ✓ Control group showed 100% survivability but survivability was high in T3 (99.31%) against other treatment groups.

Haematological parameters

- ✓ Present study showed increased and decreased count of blood parameters in effect of nano zinc oxide against control. Haemoglobin, RBC, platelets count and PCV showed increased value in treatment than control and these parameters showed highest value in T3 (8.0±0.11g/dl).

- ✓ Haemoglobin, RBC, platelets count and PCV showed increased value in treatment than control.
- ✓ WBC was decreased in treatment tank than control tank. Some of the parameters were remain minor changes like monocytes, eusinophil, MCV, MCH, MCHC counts.

Enzyme parameters

- ✓ Present study reveal that amylase activity is highest in T3 treatment and lowest amylase activity was recorded in control tank. After blood serum analysis highest amylase activity was recorded 51.00±0.22u/l in T3 tank and lowest amylase activity was recorded 32.00±0.15u/l in control. Highest result was founded in T3 treatment containing nano ZnO concentration of 15 mg/kg.
- ✓ The highest lipase activity was recorded in T3 (76.50±0.18u/l) and lowest lypase activity was recorded in control (47.00±0.15u/l). But there was no significant difference in T3 and T4.
- ✓ Also, ALT and AST value was recorded which reveal that T3 perform better than control.

Conclusion

Present study on the **"Influence of different quantity of zinc sources on the production performances of *Cirrhinus mrigala*."** embodied in this thesis has been able to meet the objectives of the study to a bigger scope with some impressive findings. The findings of the research will be helpful in understanding the zinc supplementation effect on mrigal growth performance and nano zinc concentration of 15mg/kg in mrigal feed will show the best performance in respect of growth and FCR. So, it is recommended to incorporate the said doze while preparing the feed for mrigal. The findings of present study reveal that zinc oxide nano form having the potential of increasing the production performance of mrigal which can help in achieving the animal nutritional demand and eliminate the global hunger problem.

CHAPTER 6

FUTURE SCOPE OF RESEARCH

6. FUTURE SCOPE OF RESEARCH

In spite of several important findings, the present study has limited with several shortcomings. Therefore, the further studies require on

- ✓ The study indicated that scope of nanotechnology for the production of healthy fish, although nano science is still at its initial stage in the field of mineral nutrition,
- ✓ So future work should mainly focus on to know the effect of nano-zinc, their mechanism, site of absorption and finally mode of action.
- ✓ It can be also used to know the effects of nano zinc on diseases aspects also.
- ✓ It is necessary to know the toxic impact of nano zinc at recommended level is there or not.
- ✓ This study opens the scope to know the impact of nano zinc on immunological research as well as disease related research.
- ✓ It helps on the study on the impact of nano zinc on water quality parameter which is varying much vital for fish growth and survivability.
- ✓ The field trial is also required to know the impact of nano zinc also.
- ✓ The research work may be required to know the effect of nano zinc on flesh quality of fish also.

CHAPTER 7

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7. BIBLIOGRAPHY

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Kherwal Raj Kisku

B.F.Sc, M.F.Sc Scholar, Faculty of Fishery Science, WBUAFS, Kolkata.

Contact No**:-8768618064**

E-mail:- krkisku56@gmail.com

CAREER OBJECTIVE

Appearing M.F.Sc candidate with six months of internship experience in B.F.Sc, both industrial and academic, focused in aquaculture, food processing and technology, engineering, resource management, aquatic environmental management, extension and aquatic animal health studies. I am looking for career in a premier institute to apply my knowledge and skills, so that I can contribute to the success of the organization and keep myself abreast with the latest trends and technologies.

BASIC ACADEMIC CREDENTIALS

DEGREE	UNIVERSITY/BOARD	Year	O.G.P.A.
M.F.Sc (appearing)	West Bengal University Of Animal And Fishery Sciences, Kolkata-37,West Bengal	2022	
B. F.Sc	West Bengal University Of Animal And Fishery Sciences, Kolkata-37,West Bengal	2020	**7.36%**
Higher Secondary (W.B.C.H.S.E)	West Bengal Council of Higher Secondary Education	2015	**53.4%**
Secondary (W.B.B.S.E)	West Bengal Board of Secondary Education	2011	**67.75%**

EXPERIENTIAL LEARNING (TRAINNING PROGRAM)

- **Institute Name** :- ICAR- CENTRAL INSTITUTE OF FRESHWATER AQUACULTURE (Rahara, Kolkata)
- **Duration** :- 05.08.2019 to 17.09.2019

- **Farm Name** :- Freshwater Fisheries Research & Training Center (Kulia)
- **Duration** :- 19.08.2019 to 06.09.2019

- **Farm Name** :- Government Fish Technological Station
- **Duration** :- 09.09.2019 to 24.09.2019

- **Farm Name** :- Sasya Shyamala Krishi Vigyan Kendra (Narendrapur)
- **Duration** :- 21.10.2019 to 31.10.2019

- **Farm Name** :- North 24 Parganas Krishi Vigyan Kendra (Ashok Nagar)
- **Duration** :- 01.11.2019 to 18.11.2019

- **Farm Name** :- Fisheries Experimental Learning, Dept. of Aquaculture

- **On-Farm Training Program** at Kakdwip Research Centre of ICAR-CIBA (Central Institute of Brackish water Aquaculture), West Bengal.

KEY SKILLS

- Basic academic knowledge in subjects of Fishery Science.
- Thorough idea about feed formulation, feed processing technology, feeding management, feed testing and feeding trials.
- Integrated farming system.
- Management aspect of aquatic environment and different resources, extension works.

SOFT SKILLS

- Operating Systems Packages: WINDOWS, Microsoft office.
- Opereting editing softwares: Photoshop, Premiere pro

INTERPERSONAL SKILL

- Ability to rapidly build relationship and set up trust.
- Ability to cope up with different situations.
- Confident and Determined
- Hard working

EXTRA-CURRICULAR ACTIVITIES

Passion for Football, Video Editing, Travelling, explores remote places.

PERSONAL DETAILS

- **Father's Name** :- Bishnupada Kisku
- **Permanent Address** :- Vill – Goalapara, P.O – Dangardih, P.S – Boro, Dist – Purulia, PIN- 713131
- **Date of Birth** :- 25th June 1995
- **Language Known** :- Bengali, English, Santali & Hindi
- **Nationality** :- Indian

DECLARATION

I declaration hereby that all above mentioned facts in this resume are true. In case of any dispute, I will be responsible.

Place: Kolkata, West Bengal
Date: 02/02/2023

Kherwal Raj Kisku.

(Signature)