INFLUENCE OF SUPPLEMENTING NANO ZINC AND BLACK CUMIN ON HAEMATOLOGICAL PARAMETERS AND GROWTH PERFORMANCES 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

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Dedicated to My Beloved

Parents and

My Respected Guide

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Certificate

This is to certify that the work recorded in the thesis entitled "**Influence of supplementing nano zinc and black cumin on haematological parameters and growth performances of *Cirrhinus mrigala***" submitted by **Rita Joshi** 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.

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

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

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Abbreviations

FAO	:	Food and agriculture organizations
AD	:	Anno Domini
ha	:	Hectare
BW	:	Body weight
g	:	Gram
°C	:	Degree Celsius
P:E	:	Protein : Energy
AA	:	Amino acid
MOS	:	Mannan oligosaccharides
NPs	:	Nanoparticles
NMs	:	Nano materials
Se	:	Selenium
Cu	:	Copper
Fe	:	Iron
FeO	:	Ferrous oxide
ZnO	:	Zinc oxide

ml	:	Millilitre
Bp	:	Boiling point
Do	:	Dissolved oxygen
DNS	:	Dinitrosalicyclic acid
Nm	:	Nanometers
pH	:	Potential of hydrogen
ANOVA	:	Analysis of variance
ZnO	:	Zinc oxide
Lat.	:	Latitude
Long.	:	Longitude

ABSTRACT

Zinc is the necessary trace elements for aquatic animals that affects the biological processes and physiological functions. Thus, the supplement of ZnO-NPs can be used as an alternative method to overcome zinc deficiency. Nanoparticles have the potential to enhance the growth and health of the fish. The main aim of the present work is to find out influence of supplementing nanozinc with black cumin on growth performances and hematology of the *Cirrhinus mrigala*. Different zinc forms were administered in basal diet for the formation of different treatments for this present study. The control without any zinc supplementation and treatment feeds with an inorganic Zinc (ZnO T1), nanozinc oxide (ZnO, T2) and zinc oxide with black cumin (T3) and last was nanozinc with black cumin (T4) with the same concentration @ of 15 mg/kg of feed. Growth performances, hematological parameters and enzymatic performances of Mrigal were estimated after 56 days of feeding trial. Mrigal fed with T4 treatment feed showed higher Growth rate, DWG, SGR, showing good FCR indicated that when nanozinc combined with black cumin it enhances its performances. Hematological parameters values such as hemoglobin, RBC, Hematocrit, are also higher in T4 treatment as compare to control and other inorganic zinc (ZnO) and nano zinc having same concentration in all treatments (15 mg /kg of feed). MCV, MCH, and MCHC do not showed significant change ($p>0.05$) among treatments and WBC and platelets decreased with increase in zinc supplementation in feed. As it showed high values in control and lowest in T4. This study provides that 15mg of Zinc oxide nanoparticles with black cumin incorporated feed was suitable for the growth and hematological parameters of Mrigal and it would be used in the feed of fishes as micronutrients. the highest weight gain and specific growth rate (SGR), % per day), which was significantly different ($p < .05$) from the other experimental diets. Significantly ($p < .05$), higher activities of the digestive and metabolic enzymes were recorded in the fish fed ZnO-NP containing diets as compared to the diets containing inorganic Zn or control diet. The main aim of this study is to evaluate the growth efficacy of ZnONP-supplemented. The effects of zinc oxide nanoparticles show the higher improvement of growth and metabolic functions in Labeo rohita. These results suggest that the nanotechnology could apply for feed formulation technology and pave the way for the dietary supplementation of zinc oxide nanoparticles as safe ingredients for aquatic animals to overcome the zinc deficiency.

Keywords - Zno –nanoparticles, haematology, enzymatic performances, zinc, Black cumin

CHAPTER 1

INTRODUCTION

Agriculture has provided humans with terrestrial, animal source food for at least 5,000 years (Modlinska and Pisula, 2018), but fish and other aquatic animals have traditionally been caught from the ocean and inland waters. Aquaculture production surpassed capture fisheries production for human consumption in 2016 and it contributed 52% of the total harvest weight of aquatic animals for human consumption in 2018 (FAO, 2020). The world's hunger for fish is expected to almost double by 2050 due to growing affluence and populations, according to an assessment that anticipates the demand being fed by a big rise in farmed seafood. Meat is important in human diets because a dietary portion of it typically has a higher protein concentration with a better balance of essential amino acids than an equal amount of a plant-source food (Tilami and Samples, 2018). The supply of protein available to humans is a critical factor in global food security (Henchion *et al*., 2017). Intensification of aquaculture has become an important practice in recent years to satisfy hunger and needs. High density stocking, providing artificial feeds and fertilization of the pond water have become common husbandry practices in both fish and shrimp culture systems. A number of microbial components and plant products such as polysaccharides, lentinan, levamisole, schizophyllan, oligosaccharides, and muramyl dipeptide and yeast derivatives have been used as immunstimulants in aquaculture to stimulate immune system of fish (Mastan, 2015). These are used as adjuvant in fish vaccines and as additives in aqua feeds. Especially use of immunostimulants as a prophylactic agent to hinder prevalence of epidemics is began to popular. Use of meditional herb incorporated in fish feed has many therapeutic characteristics (Ziaee *et al*., 2012). Nutritional scientists used the medicinal plants and their bioactive principles or extracts for many different purposes in aquaculture farms. They exhibited various advantageous properties such as growth-promoting, antimicrobial, immunostimulant, antioxidants, and hepatoprotective activities (Latif *et al.,* 2021).

Black cumin (*Nigella sativa*) is one of the most popular and widely used because of the various nutritional and pharmaceutical properties in different regions of the world (Mollazadeh and Hosseinzadeh, 2014). Black cumin seeds are rich vitamins, minerals and many active compounds, such as antioxidants (thymoquinone and dithymoquinone), flavonoids, sterols, alkaloids, and unsaturated fatty acids (mainly represented by palmitic, oleic and linoleic acid) that make them desirable health-promoting food (Niroomand *et al.,* 2020).

Also, their administrations also improved the growth performance and immunity of fish (Yousefi et al., 2021). It is stated that most of the pharmacological and therapeutic properties of black cumin seed are due to the presence of thymoquinone (18.4 – 24.0% of the volatile oil) which is a major bioactive compound of its volatile oil (0.4–2.5% of the seed oil) (Dey *et al*., 2020). The growth performance, feed conversion, meat quality can be positively affected by the inclusion of black cumin or its active principles (i.e., thymoquinone) in fish diet. Additionally, the dietary supplementation of black cumin or its derivatives in fish diets, not only enhance of the immune responses, improve the biochemical profile in fish blood, protect against the invading pathogenic bacteria, but also help to ameliorate the oxidative stress responses of fish towards heavy metal toxicants that may be present in the aquatic environment (Abd_El-Hack *et al.*, 2021). Various studies are carried out to determine the effect of supplementation of black cumin seed in feed. Khondoker *et al.,* (2016) used black cumin seed in 2%, 4% and 6% doses and investigated immunological parameters such as bactericidal activity and phagocyte activity. Awad and Awad, (2017) used black cumin seed and recorded the importance of determining the ideal dose of medicinal plant to improve the immune system of fish and avoid the risk of immunosuppressant. Along with medicinal plant, minerals are also considered as vital nutrients as they play an important role in maintaining osmoregulation and formation of bones and scales. Trace minerals are mainly absorbed from water which makes it difficult to determine their requirements for fish (NRC, 2011). Zinc 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 and is required for growth, immune function, nutrition, metabolism, fertility, wound healing and maintenance of oxidative stress in animals (Zhao *et al.,* 2014). Zn absorption in animals is very less and differs with the sites of gastrointestinal tract and age of the animal (Swain *et al.,* 2016). 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. Zn deficiency caused reduced growth rate, increased mortality, low body weight, skeletal deformities, cataracts and fin and skin erosion in fish (Rider *et al.*, 2010).Therefore, adequate amount of Zn is required to avoid such deficiency symptoms.

Zinc may be included in the diets either as inorganic salts, viz. Zn oxide (ZnO) and Zinc sulphate (ZnSO4), or as organic chelates, for example Zn propionate, Zn gluconate and Zn acetate. 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). Metal-oxide nanoparticles (NPs) have entered human life in recent years in demand of the nanotechnology. The size of these materials ranges between 1–100 nm and contain metal ions mostly Ag, Ti, Cu, Zn, Fe and Al etc. NPs are characterized with their high surface to volume ratios, electronic properties, reactivity, surface structure and crystal characteristics (Hoseini *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.,* 2020). It has been reported with many advantages like tissue-specific targeting, dose and toxicity reduction, as well as increased bioavailability, Drug efficacy, and reduction of secondary adverse effects (Shah and Mraz, 2019). The physiochemical properties of nanomaterial's result in its wide application on food preservation, water treatment, and healthcare, among others (Ogunkalu, 2019). Nanotechnology has enormous potential to provide innovative improvements to aquaculture systems to reduce costs, increase efficiency and to reduce impact on the environment. With different approaches, use of nanotechnology to produce nano sized Zn called as nano Zn is a potential alternative to both organic and inorganic Zn sources. The use of nano Zn has shown to produce better results as compared with conventional Zn sources and less toxic (Sahoo *et al.* ,2014). Globally, ZnONPs have gained special attention because of their environmentally friendly characters with various excellent applications such as anticancer, antimicrobial, and photo catalysis, etc (Jiang *et al.,* 2018).

Fish occupying high tropic level in the aquatic ecosystem and being an important food source are considered as indicator of ZnO contamination in the aquatic environment. The Indian major carp Mrigal (*Cirrhinus mrigala*) is used of great commercial importance because it is the most common fish consumed by the largest population in India. It is often cultured in polluted water and thus susceptible to exposure to different metal oxides (Das *et al.,* 2017). Hence, for the present research *Cirrhinus mrigala* is used, as it is a good biological model for study with different effects on supplementing nanoparticles in the feed due to its diverse characteristics, such as high tolerance to stress and diseases and a wide variety of environmental conditions. The toxicants in an aquatic ecosystem are taken by fish and transported to the tissues and organs through the blood. Growth and hematological parameters are widely used as a health indicator. Feed consumption and feed conversion efficiency of Mrigal were higher in feed containing zinc oxide nanoparticles (Onuegbu *et al.,* 2018). Assimilation and metabolism of Mrigal were increased with an increase in ZnO nanoparticles in the feed. It released into the environment can harm aquatic creatures and pose a health risk to humans through the food chain. Under the current challenges of a high population, aquaculture presents a safe and healthy food source (Dawood *et al.*, 2021). However, sustainable production of aquatic organisms has to consider the intensive and super-intensive farming systems (Schumann and Brinker, 2020). Intensive systems could afford high production yields from the lowest possible unit of water (Van Doan *et al.*, 2020).

Under this background, present research on "**influence of supplementing nano zinc and black cumin on hematological parameters and growth performances of *Cirhinus mrigala***" intends to carry out with objectives listed as listed as follows:

I. Determining the role of nano zinc and black cumin on growth performances of *Cirrhinus mrigala*.
II. Assessing the effects of nano zinc alone or combination of black cumin on hematological parameters of *Cirrhinus mrigala*.

CHAPTER 2

REVIEW OF LITERATURES

2.1. General aquaculture

Fisheries and aquaculture play an important role in achieving FAO's Strategic Objectives of eliminating hunger, food insecurity and malnutrition. The edition of the State of World Fisheries and Aquaculture, people have never consumed so much fish or depended so greatly on the sector for their well-being as today. The demand for fish increases, the sector is also striving to be more productive and sustainable and to enable more inclusive and efficient systems while reducing rural poverty and enhancing the resilience of livelihoods to disasters, crises and climate change (FAO, 2014). Aquaculture is the process of cultivating aquatic animals together, mainly fish, crustaceans, mollusks, aquatic plants in controlled aquatic environments for any industrial, recreational or public purpose. Some types of intervention within the culture practices like stocking, feeding, reduction of disease occurrences, and protection from predators to enhance the production. (FAO, 1995). It is the fastest-growing food-production sector in the world. It supplies 50% of all fish consumed globally, and by 2030, it will be the prime source of fish. Fishing was prevailing within the time of Stone Age (Subasinghe *et al.*, 2009). Fan Lei, a Chinese politician turned fish culturist mentioned commercial fish culture in his book.

2.2. Classic of fish culture

The use and validation of fish health monitoring tools have become increasingly evident due to the expansion of aquaculture (Fazio, 2018). The principles of management of capture and culture fisheries are very different from each other. For capture fisheries has attempt to harvest maximum sustainable yield by regulating fishing effort and mesh after taking into account parameters of population dynamics. The earliest proof of fish culture supposedly comes from ancient Egypt, wherever Tilapia was powerfully coupled to Goddess Hathor and idea of rebirth In India reference of fish cultivation was found in Kautilya's Arthashastra.

Hora (1943) showed the proof of the strategy of fattening of fish in the chapter of Matsya-Vinod in Manasoltara (King Someswara, 1127 A.D.). Rath (2010) found the proof of introduction of exotic fish *Osphronemus gourami* from Java in 1841 in India. Ancient fish culture in tiny ponds and bundhs is thought to have existed for hundreds of years in Eastern India.

2.3. Indian scenario of fish culture

Globally India stands second in culture fisheries production. China, with world's one fifth of population produces one-third of total fish harvested and two thirds of fish cultivated (FAO, 2016). In India, this sector constitutes about 5% of the global fish production and 3% of the global fish trade. The per capita availability of fish in India has increased from 3 kg to 9.1. With a national per capita consumption of 11 kg, fish is recognized as one of the chief components in the domestic food security in India (Jayasankar and Barik, 2015). The three IMCs, namely Catla , Rohu and Mrigal 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) . Aquaculture resources India is blessed with rich natural sources for enhancing freshwater aquaculture production: 2.42 million ha of ponds and tanks; 1.07 million ha of beels, jheels and derelict waters; 0.12 million km of canals; 3.15 million ha of reservoirs and 0.72 million ha of upland lakes.

Freshwater aquaculture with a share of 34% in inland fisheries in mid 1980s has increased to about 80% in recent years (DADF, 2017). Regardless of the importance of freshwater aquaculture in Indian food sector, no extensive reviews have been made on the sector. (Jayasankar, 2014) Aquaculture sector in India has to come up with timely strategies to cope with the future challenges of increased fish demand, selective consumers' choices, production of safe and quality fish protein and tapping the export earning among the many challenges.

2.4. Global production

Capture fisheries and aquaculture contribute over 15 percent of animal proteins in human consumption and thus play an essential role in eradicating poverty and achieving sustainable development worldwide by 2030, an agenda set by the United Nations (Bernatchez *et al.,* 2017; FAO, 2018).

Remarkable success has been achieved in world fisheries and aquaculture during the past decades. According to the 2018 statement by the Food and Agricultural Organization of the United Nations , the average annual increase between 1961 and 2016 in global food fish consumption (3.2 percent) outpaced global population growth (1.6 percent) and exceeded that of meat from all terrestrial animals combined (2.8 percent) (FAO, 2018). The long-term sustainability of fisheries and aquaculture, however, faces many challenges, including overfishing, climate change, germplasm degradation, and diseases .The development of sequencing technologies and the advances of genomics are instrumental in addressing some of these challenges and can benefit sustainable fisheries and aquaculture (Bernatchez *et al.*, 2017).

Aquaculture is usually an intensive technology-based industry focused on development of new production technologies, diversification of production in support of new sustainable (ecological) production models (FAO, 2021). With the establishment of the SDGs, the international community is adopting a holistic approach to food production (Stentiford *et al.*,2020). The aquaculture sector needs to do the same and embrace new paradigms for evaluating the sector that goes beyond tones and dollars of product (FAO, 2017). Aquaculture can contribute to sustainable food systems by ensuring a nutritious product that benefits local communities. Governance structures need to embrace this new paradigm and incorporate aquaculture into national policies to ensure that the sector is developed with adequate resources and safeguards for local communities.

2.5. Present production of carp culture

India is the second largest producer of fish next to China and Indonesia ranks third in aquaculture production (FAO, 2014). In India, the major carps, Catla (*Catla catla*), Rohu (*Labeo rohita*) and Mrigal *(Cirrhinus mrigala*) 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 person 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). Since more than a decade ago, with increasing demand for rohu, farmers in India have shifted from three species to two species polyculture system with rohu and catla (FAO, 2018).

2.6. REVIEW ON MRIGAL

2.6.1. HISTORICAL BACKGROUND

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).

2.6.2. Mrigal

Systematic position

Kingdom:	Animalia
Phylum:	Chordata
Class:	Actinopterygii
Order:	Cypriniformes
Family	Cyprinidae
Subfamily	Labeoninae
Genus:	*Cirrhinus*
Species:	*Mrigala*

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. Mrigal 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, along with rohu labeo and the catla. 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 (Dev *et al.*, 2011).

2.6.3. Identity

2.6.4. Biological features

Body bilaterally symmetrical and streamlined, its depth about equal to length of head; body with cycloid scales, head without scales; snout blunt, often with pores; mouth broad, transverse; upper lip entire and not continuous with lower lip, lower lip most indistinct; single pair of short rostral barbels; pharyngeal teeth in three rows, lower jaw with a small post-symphysial knob or tubercle. The origin of dorsal fin nearer to end of snout than base of caudal; dorsal fin as high as body with 12 or 13 branched rays; last unbranched ray of dorsal fin non-osseous and non-serrated; pectoral fins shorter than head; caudal fin deeply forked; anal fin not extending to caudal fin; lateral line with 40-45 scales.

2.6.5. 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. It has a stenophagous feeding habit ; detritus and decayed vegetation form its principal food components; while phytoplankton and zooplankton comprise the rest of its components. 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 100000-150 000 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 24-31 ºC (FAO, 2009).

2.6.5. Reproduction

They are 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.

2.6.6. 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 conquences 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 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 reduce 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 (*Catla catla*) and rohu (*Labeo rohita*). 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.

2.6.7. 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 percent 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, behavioral and morphological criteria the optimum vitamin C requirement for newly hatched mrigal is between 650 and 700 mg/kg diet.

2.6.8. Feed additive in aquaculture

Globally, the rapid progress of aquaculture industry with a variety of farmed fish and other aquatic animal is result to increased stress, reduced growth performance and increased infectious diseases in cultured species (Dawood and koshio, 2016).

Several alternative approaches have been successfully applied to overcoming the pathogenic diseases (such as, fungal, viral and bacterial diseases). Chemotherapy is the most commonly applied method, using large quantities of chemical materials and antibiotics. Ages ago, antibiotics are applied in aquaculture practice to control or to prevent infectious diseases before they occur by treating the host via water or food (Christensen *et al.*, 2006)

However, the overusing of antibiotics has several disadvantages, the overuse of antibiotics could produce antibiotic resistance bacteria (Angulo, 2000). Recently, numerous sustainable routes are available to control intestine micro biota and health status of fish by providing the diets with additives or functional foods. These functional foods including: immune-stimulants, probiotics, prebiotics, synbiotics, organic acids, enzymes and mycotoxins binders.

The target of some of “feed additives” are the feed quality, containing; pellet binders, feed preservatives and antioxidants (antimicrobial and antifungal products) as concluded by(Bharathi *et al.,*2019). Further additives are applied in aqua feeds to increase the fish performances and health. The idea of functional nutritional additives in aquaculture is a developing new design to improve feeds for fish. Dietary supplementation of probiotics has been shown significantly modulates the intestine microflora, and thereby assists in balancing a micro flora that has been settled and play an essential role in competing beneficial. Pathways by how this is performed consist of the inhibitory complexes generation against potential invaders (viz. lysozyme, bacteriocins, lactoferrin,), or the mode of action is direct competition for vital nutrients and binding sites, virulence genetic expression inhibition, the providing of essential enzyme and nutrients led to enriching nutrition, and the modification of interactions with the environments and the stimulation of immune response (Merrifield *et al.,* 2010).

Organic acids can be used directly into feed components and complex feed using different routes. Solid acids and acid salts are directly supplemented in diet or through specific premixtures, whereas, other organic acids such as blends or liquid acids are sprayed onto the diet. The organic acids achievement in the gut consist of two activities include decreasing- pH action of organic acids in gastrointestinal tract, this contributes to improve activity of digestive enzymes and produces an inhibition the growth of the pathogens via the separation of the acids and generation of anions in the microbial cells (Luckstadt ,2008). Fish larvae have tremendous growth potential, displaying relative growth rates that may exceed 70% a day (Conceicao *et al.,*2003) and are necessarily sustained by high protein deposition rates. In addition, it is well established that AA are a major energy source during the larval stage of most marine teleost species (Ronnestad *et al.,* 2003).

Thus, fish larvae AA requirements are expected to be higher than those of juveniles or adult fish, usually falling in the range of 50 to 60% (NRC, 2011). Any AA losses due to dietary deficiencies have a large negative impact in larvae, more than in juveniles (Conceiçao *et al.,* 2003). Therefore, optimizing the formulation of dietary protein for fish larvae is of paramount importance to make the most of larvae growth potential.

The reported effects of MOS supplementation on cultured fish points to this component as a useful tool to produce healthier fish and improve fish production. MOS supplementation reinforces epithelial barrier, stimulates the immune system, promotes growth and feed efficiency and effectively enhances disease resistance. These combined effects suggest that the stimulation of the immune system together with the reinforcement of the integrity and functionality of the intestinal barrier are the primary modes of action of MOS in fish.

2.6.9. Nanoparticles in aquaculture

Nanotechnology has gained potential opportunities in various fields to produce better materials and products. According to the National Nanotechnology Initiative (NNI), "Nanotechnology is defined as research and technology development at the atomic,molecular,ormacro molecule a level susinga length scale of approximately one to one hundred nano. 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 (Gornatietal, 2009). Nan materials are corner stone's 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 human. Nanomaterials has gained a huge popularity because they exhibit unique optical, magnetic, electrical and other properties at nanoscale (De Crozals *et al.,* 2016). 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 involves the application of materials at the nanoscale to produce new products or processes, and with many potential benefits to society (Roco and Bain, 2005). 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 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, and 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

2.6.10. Different zinc forms and use in aquaculture-

Zinc 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).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.

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) and seawater fish (Sunda, 1984) have 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). 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 (Zn-AA), 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, 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).

2.6.11. Supplementing Nanoparticles in the fish feed-.

The biggest challenge that threatens sustainable aquaculture development is the availability and affordability of high-quality feed. The basic nutrient requirements of fish include protein (32%), dietary energy (8.5-9.5%), fat (4-6%), carbohydrate (20-35%) and fiber (< 4%), but vary in accordance with age and species. The main objective for fish industry is to make bigger cost-effective fish feed (Baruah et *al.*, 2007). Fish meal is the vital nutrients for growth element in fish feed. The suitable fish feed ingredients are helpful to enhance for fish growth, disease resistance, immunity and health promoting factors (Zhou *et al.*, 2004). Feed supplementation has been more familiar administration method in fish cultivating technique and many supplements have been shows that a capable to enhance the fish immune system or regulating the harshness of infections, (Menanteau-Ledouble *et al.*, 2015). Nanotechnology plays an important role in the future areas of research in animal nutrition (Bunglavan et al, 2014). The capability of nanoelements is improve protein stability may affect in number of biological functions such as digestion, metabolism and nutrient uptake (Sharma *et al.,* 2007). Nowadays nanotechnology received great attention in agriculture and related fields including aquaculture and fisheries. In aquaculture, nanotechnology involves the preparation and utilization of various nanoparticles is very helpful for many ways like, nutrient supplements, therapeutic agents and gene delivery, etc. (Dar *et al.,* 2020).

Several combinations of plant and animal ingredients have been used to have optimal nutrient requirements in fishes. Consequently, minute concentrates of some soluble organic/inorganic materials have been used as additives in fish feed either to augment deficient nutrients or act as catalyst to improve nutrient bioavailability. Recent advances in nanotechnology are currently revolutionizing different aspects of science and technology, including aquaculture. The capability of nanoelements is improve protein stability may effects in number of biological functions such as digestion, metabolism and nutrient uptake (Sharma *et al.,* 2007). Many investigators reported that dietary feed additives of nanoelements are consuming numerous beneficial effects in nanomaterials such as iron, zinc, selenium, cobalt, etc as well as these elements fulfill the requirement of fish species (Dar *et al.,* 2020). Every trace mineral factors are contain their exact role in immunity of cultured animals, but the crucial trace metals such as Zn, Mn, Cu and Se that have been linked with an enhancement in immunity or role that maintain immunity. The immune system is used several methods to detoxify these foreign factors or antigens (Basuini *et al.,* 2016). The small components have mainly strengthened by the significance of their functions, immune protection and antioxidative defence.

Feed supplements in nano forms are evaluated to find the different properties between improving growth and immunity through antioxidant consequence to their use in small amount than its bulk counterparts, which increases ration criteria (Rajendran, 2013). Different types of metal nanoparticles (NPs) such as Se, Fe, Cu, FeO, and ZnO are utilized in aquaculture responsibility (Srinivasan *et al.,* 2016). The metal oxide nanoparticles mainly, trace metal elements are limited with useful immunostimulants in feed additives to fish, it is a novel delivery system for improving the immune capability and infection resistance against to harmful microbes). Dietary additives of chitosan nanoparticles level (1.0 g/kg diet) are utilized to enhance antioxidants and natural immunity response of Nile tilapia (Abdel-Tawwab et *al.,* 2019). Dietary feed with supplementation of iron nanoparticles is used to improve growth and better feed efficiency of Nile tilapia (*Oreochromis niloticus*) fish compared to the control group (Roedar and Roedar, 1968). Dietary additives of various nano elements such as selenium, zinc and manganese are used to enhance stress resistance and bone mineralization of gilthead sea bream (*Spares aurata*) (Izquierdo *et al.,*2017). The fisheries and aquaculture industries can be revolutionized by using nanotechnology with new tools, such as rapid diagnosis of diseases which will enhance the ability of cultivable organisms to uptake drugs like hormones, vaccines and essential nutrients etc. The metal nanoparticles (NPs), such as Fe, FeO, Se, Zn, ZnO, Cu, and MgO etc., play a crucial role in aquaculture operations. It has been reported that iron NPs when fed to young carp, *Carassius auratus* and sturgeon, *Acipenser gueldenstaedtii* showed a faster growth rate, 30% and 24% respectively (Srinivasan *et al.,* 2016).

Different Se sources (nano-Se and Seleno methionine) supplemented diets improved the growth, antioxidant status and muscle Se concentration of Crucian carp. Nanotechnology is in constant development and its applications are ever more varied and specific, with a high potential for improving livestock production and animals in general. Nanoparticles like Se is supplemented is an important consideration), various forms of Se supplementation have been studied in fish species including hybrid striped bass (Cotter *et al.,* 2008), common carp (Jovanovic *et al.,* 1997), crucian carp (Wang *et al.,* 2007) and common barbel (Kouba *et al.,* 2014).

Recently, Zhou et al., (2009) showed that selenium nanoparticles (nano-Se) are more efficient in increasing muscle Se content compared to organic Se (seleno methionine) in the crucian carp. Since materials at the nanometer dimension exhibit novel properties (Wang *et al.,* 2007), nano-se as the novel form of Se has attracted attention.

2.6.12. Effect of zinc oxide nanoparticles on hematological parameters of fish

Hematological examination is one of methods commonly used to evaluate fish physiological status and health (Fazio, 2019). Routine hematological analyses include evaluation of blood cell counts and other cell-related parameters as well as measurements of biochemical indices: concentrations or activities of plasma compounds. Red blood parameters include: erythrocyte count (RBC), hemoglobin concentration (Hb), hematocrit value (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). White blood parameters include leukocyte count (WBC) and sometimes also differential leukocyte count – (DLC) evaluation of percentage or number of various types of leukocytes: lymphocytes (Lym), neutrophils or heterophils (Neu), monocytes (Mono), eosinophils (Eos) and basophils (Bas). Thrombocyte count (TC) and blood cell morphology are rarely evaluated.

Manual hematological analysis requires knowledge, skills and experience to obtain reliable and reproducible results. The results of hematological analyses in fish depend on many intrinsic and extrinsic factors: fish stress level (Carbajal *et al.*, 2019), method of blood sampling (Bojarski *et al.,* 2018), preanalytical factors such as anticoagulant use , temperature and time of blood storage , and analytical procedures including type of diluents , correctness of blood cell classification etc.

Among freshwater fishes, 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. It is often cultured in polluted water and thus susceptible to exposure to different metal oxides (Das *et al.,* 2017). Hence, Cirrhinus mrigala acts as a good biological model for toxicological studies due to diverse characteristics, namely, high tolerance to stress and diseases and a wide variety of environmental conditions. The toxicants in an aquatic ecosystem are taken by fish and transported to the tissues and organs through the blood. Growth and hematological parameters are widely used as a health indicator in ecotoxicological studies because these parameters react before the toxicants enter the body of fish. Fish blood is a suitable way to determine and diagnose the toxicity of metal and metal oxide nanoparticles (Sevcikova et al., 2016, Ates *et al.,* 2016) and hematological analysis is excellent to assess the stress condition of aquatic organisms (Bahmani *et al.,* 2012) .

This study provides information about the toxic potential of zinc oxide nanoparticles. -15mg of Zinc oxide nanoparticles incorporated feed was suitable for the growth of Mrigal. Growth performance is a factor reflecting environmental toxicity in fish, and even a small concentration of heavy metals has a negative effect, triggering physiological changes such as growth and metabolism and reducing health and survival rates (Husain *et al.,* 2010)

One of stress-related effects that may disturb blood sampling, result in analytical errors or even make blood analyses impossible is increased blood coagulability. To minimize stress, fish should be harvested immediately before blood sampling and blood collection should be done as fast as possible. If stressing fish is inevitable, e.g. they were harvested earlier, subjected to stressful experimental procedures or are seriously ill (e.g. infected by pathogens), decision about sedation or anesthesia should be taken into consideration and/or the use of higher concentration of anticoagulant may be necessary to prevent stress induced hypercoagulability. Stress-induced hematological changes depend on type of stressor, its magnitude and time of action (Burgos-Aceves *et al.,* 2019). In fish it may involve erythrocyte swelling increase in MCV and Ht), release of splenic erythrocyte reserve or – in a longer period of time – increased erythropoiesis (increase in Ht, Hb and RBC). On the other hand, stress may affect white blood parameters. Short-term stress sometimes results in an increase in WBC but chronic and strong stress usually causes leucopenia (Tort, 2011). Stress may also affect differential leukocyte count causing shift from lymphocytes to neutrophils and monocytes (Grzelak *et al.,* 2017). According to different studies and investigations stated that both acute and chronic thermal stress affected all red and white blood parameters in a different way depending on acclimation temperature.

2.6.13. Effect of nanozinc supplementation in performances of fish.

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 nanostructure thin films. Due to its easy processing in various forms, it is used in various applications from optoelectronics to energy conversion, photo catalysis, and sensors (Pislaru-Danescu *et al.,* 2018).

ZnO NPs having exciting properties such as high stability, anti-corrosion, photo catalytic, 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 (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. 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).

Large-scale production and huge use of zinc oxide lead 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.,* 2009). 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. 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. Faiz *et al*., (2015) reported that the feed conversion ratio was higher in ZnO nanoparticles incorporated feed of juvenile grass carp.

The assimilation and metabolism of Mrigal were increased with an increase in ZnO nanoparticles in the feed. It is widely used in sunscreens, cosmetics, paint, construction materials, and other products. It released into the environment can harm aquatic creatures and pose a health risk to humans through the food chain. ZnO-NPs are toxic to fish. Furthermore, ZnO-NPs could increase malondialdehyde (MDA) level, lessen superoxide dismutase (SOD) level, and elevate the level of neutrophil_extracellular traps (NETs). However, there is a consensus that metal dietary exposure is the main route of chronic exposure of metals to fish in aquatic ecosystems (Shaw and Handy, 2011).

Most studies have focused on assessing toxic effects of waterborne exposure to ZnO NPs (Franklin *et al.,* 2007), whereas data on magnification and potentially toxic effects through dietary routes are limited and published studies point out the need for more knowledge in this area. Reliable ecological risk assessments require access to toxicity data on risks of bioaccumulation and on any potential physiological and biochemical disturbances found in different species.

ZnO NPs are known to exhibit toxicity through oxidative stress responses (Ng *et al.,* 2017). However, the underlying mechanisms associated with ZnO NPs toxicity remain to be elucidated. One mechanism of toxicity involves dissolution in biological fluid (Cho *et al.,* 2011). Zinc ions released from ZnO NPs may lead to an increase in local concentrations of toxic ions in exposed tissues (gastrointestinal tract) and in other internal organs, subsequently inducing oxidative damage as described for heavy metal toxicity. However, exposure induced mild histological changes in kidney and seems to affect liver functioning in regards to changes in liver enzyme activity after a recovery period. Observations indicate that the liver and kidneys may be the most sensitive to ZnO NPs exposure through the gastrointestinal route. Whether observed changes in particular organs represent part of an adaptive organism response to ZnO NPs toxicity or merely a reflection of such toxicity levels must be addressed.

2.6.13. Black cumin seed in aquaculture

The usage of antibiotics in aquaculture owing to the occurrence of many drug-resistant bacterial strains, which are deposited in fish organs and affect the aquatic environment (Maron *et al.,* 2013). Because of emerging resistance to many antimicrobial agents (Tartor *et al.,* 2018), natural remedies have gained tremendous attention as dietary supplements in aquaculture. Currently, there are noticeable predilections for the usage of alternatives safe available cheap natural plants and their extracts as a growth enhancer, immune stimulator, for disease control, against toxicity, and antioxidant promoter (Hamed and Tawwab, 2021). Among natural plants, Black cumin is belonging to the family ranunculaceae and it has been known since 1400 years ago (Botnick *et al.,* 2012).

Due to its diversity and potential properties, this plant is broadly cultivated and used in various regions all over the world (Tembhurne *et al.*, 2014). Also, it is a promising medicinal plant that possesses astonishing properties including immunomodulation, antioxidant and antitoxic. This herb are rich in thymoquinone, thymol, and carvacrol which are substantial pharmacologically active compounds. Black cumin powder and its oil exhibit strong antibacterial activity against Aeromonas hydrophila, columnaris disease, and Pseudomonas fluorescents in freshwater fishes (Dey *et al.,* 2020). Additionally, it can act as an antimicrobial drug which exaggerates the immune response and elevates the survival percentages in farmed fish particularly those reared under high stocking densities like *Anabas testudineus.* Altogether, due to being available, low cost, and efficacious, the usage of black cumin and its oil in aquaculture have been recommended as a feed supplement to improve fish health rendering them more resistant to bacterial infections (Khatun *et al.,* 2015). Enriched diets with black cumin either oil or seeds enhance the immunity through an elevation in total protein level and lysozyme activity in Rainbow trout (Awad *et al.,* 2013) and promote the hemoglobin, hematocrit and globulins values of blood samples of Nile tilapia (Hussein *et al.,* 2020).

2.6.14. Effect of Black cumin seed in fishes

The use of Soybean meal (SBM) in fish diets generates several undesirable effects on the liver, intestinal villi structure, digestive enzymes, and gut microbiota because of numerous anti-nutritional substances (Mohammadi *et al.,* 2020). For the sustainability of aquaculture practices, nutritionists must find and use alternative feed ingredients to formulate cheap and nutritionally balanced feeds. so there are several studies on alternative protein sources, evaluation of more affordable industrial by-products as a protein source in fish diets is still necessary (Sezgin and Aydın, 2021). Studies have been carried out on alternative feed ingredients, mostly with plant-based and industry byproduct (Aydın and Gumus, 2020), Researchers have continue to explore less expensive and easily available feed ingredients in fish feeds.Now, various studies aimed to investigate the efficiency of black cumin seed or its extract as a feed additive in aquatic species (Niroomand *et al.,* 2020).

The growth performance of carp fingerlings in this study was negatively affected by the increasing dietary black cumin seed (BCS). The reduced weight gain (WG) and specific growth rate (SGR) observed with BCS based diets could be related to reduced feed intake because of the poor palatability of BCS. Similarly, it is stated the high inclusion level of plant-based ingredients such as olive cake, fenugreek seed, rapeseed and sesame meal results in decreased feed intake in some fish species, resulting in growth suppression of fish (Saleh, 2020). It is reported that BCS has a specific flavor characteristic, such as a typical neutral smell, moderately bitter taste, and black-brown color (Naumova *et al.,* 2020). The reason for the decrease in feed intake in BCS based treatments compared to control could be due to smell and bitter taste of black cumin seed (cetin *et al.,*2008). Black cumin seed was shown to cause a decrease in feed intake and growth performance of fish, and these effects may vary depending on its administration levels, and fish species.

Feed intake and growth performance of fish fed with BCS -based diet might be increased by using feed additives such as yeast, protein hydrolysates and plant extracts to increase the attractiveness and palatability of the diet (Bilen *et al.,* 2019). The result showed that the BCS palatability for carp fingerlings is lower than SBM. These observations were consistent with previous studies that showed replacing SBM with high-level blend plant protein and sesame seed meal significantly reduce feed intake of fish because of poor palatability (Saleh, 2020). Several studies have shown that serum biochemical parameters are important indicators to assess the nutritional and health status of fish. The cholesterol and triglyceride levels in carp fingerlings were significantly decreased by dietary BCS (Niroomand *et al.*, 2020). The fish groups fed diets with black cumin seed (*Nigella sativa*) showed slightly abnormal behavior changes due to reduction of dissolved oxygen. These signs were reported by Doudoroff and Shumway (1970). Moreover, the control fish group exhibited remarkable signs of distress, including fast swimming, erratic movement, great surfacing frequency to gulp atmospheric air, loss of body equilibrium and decreased attention to the feeding. At the end, the fish died with opened mouth. In contrast, fish groups maintained at the same condition of oxygen deficiency and supplemented with dietary black cumin showed less above-mentioned abnormal behavior than the control group. Such response to oxygen deficiency may be due to increase of respiration rate and heart rate (Mason, 1991). Also, reduction of water dissolved oxygen content which may be attributed to the inhibiting effect of photo systems I and Π and community respiration (Ojala, 1966). The improvement of dietary N. sativa on Nile tilapia (Oreochromis niloticus) behavior under oxygen deficiency may supported by (Chanock *et al.*, 1994), who reported that phagocyte activation is usually associated with abrupt rise in oxygen consumption, leading to production of reactive oxygen species (ROS) which play an important role in the host defense.

Dorucu *et al.*, (2009) used black cumin at a rate of 1, 2.5 and 5% for 21 days and determined serum protein and total immunoglobulin level, hematocrit (Hct), leucocyte levels, glass-adherent NBT positive cell activation of O. mykiss. Khatun *et al.*, (2015) studied effect of Black cumin seed oil (Nigella sativa) on enhancement of immunity in the climbing perch, *Anabas testudineus* and investigated immunological parameters such as bactericidal activity and phagocytic activity. Also (Khondoker *et al.*, 2016) used black cumin 2%, 4% and 6% doses and investigated immunological parameters such as bactericidal activity and phagocytic activity. Awad *et al.*, (2013) studied Effect of black cumin seed oil in a rate of 1%, 2% and 3% and nettle extract (Quercetin) on enhancement of immunity in rainbow trout. For the role of black cumin on mortality rate of *O. niloticus* under oxygen deficiency. it was obvious that high level (2%) of N. sativa has significant effect on mortality rate where it reduced the mortality rate, from 100% in the control to 20% in 2% black cumin. While, the treatment received low level (1%) of black cumin showed high mortality rate (40%).

CHAPTER 3

MATERIALS AND METHODS

The present experiment was carried out with the aim to find out the influence of supplementing nano zinc and black cumin on haematological parameters and growth performances of *Cirrhinus mrigala.*

3.1. Experimental site

The experiment was conducted in the laboratory conditions of the Department of Aquaculture, Faculty of Fishery Science campus, West Bengal University of Animal and Fisheries Sciences, Chakgaria, Kolkata (Lat. 22^{o} 82'N; Long. 88^{o} 20'E).

3.2. Preparation of tanks

Rectangular glass tanks used for the experiment of (120 cm × 45 cm × 45 cm) size (243L) capacity, fifteen (15) in number are placed in the laboratory conditions and experiment was carried out in triplicate condition. First the tanks were scrubbed, cleaned with chlorinated water (200 ppm), flushed thoroughly with bleached water, sun dried for 3 days. After one day kmno4 treatment was given in all the tanks to protect it from disinfectant. All the tanks were filled with clean water to a volume of 162 L each and conditioned for three days.

3.3. Stocking of fish and acclimatization

Healthy and uniform mrigal fingerlings of weight **(**3.67±0.06g) irrespective of sex were used for all the experiments. The fish 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 2 ppm $KMnO_4$ solution for 10 min. Keeping them unfed for 24 hours, they are fed with floating pelleted control feed (28.37%protein) for 7 days @ 4% body weight with proper aeration facilities, where they got acclimatized fully before experimentation. The experiment fish from the acclimatized stocks of 225 fishes (3.67±0.06g) were stocked with the stocking density of 15 fishes per tank in triplicate condition. The tanks were labelled and covered with nylon netting for adequate protection. All tanks were supplied with continuous aeration through air-stones, connected to a central air compressor. Chlorine-free aerated tap water was used throughout the experiment. Fecal matter and unutilized feed materials were siphoned out and 40-50 % 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 (3.67±006 g) respectively 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.

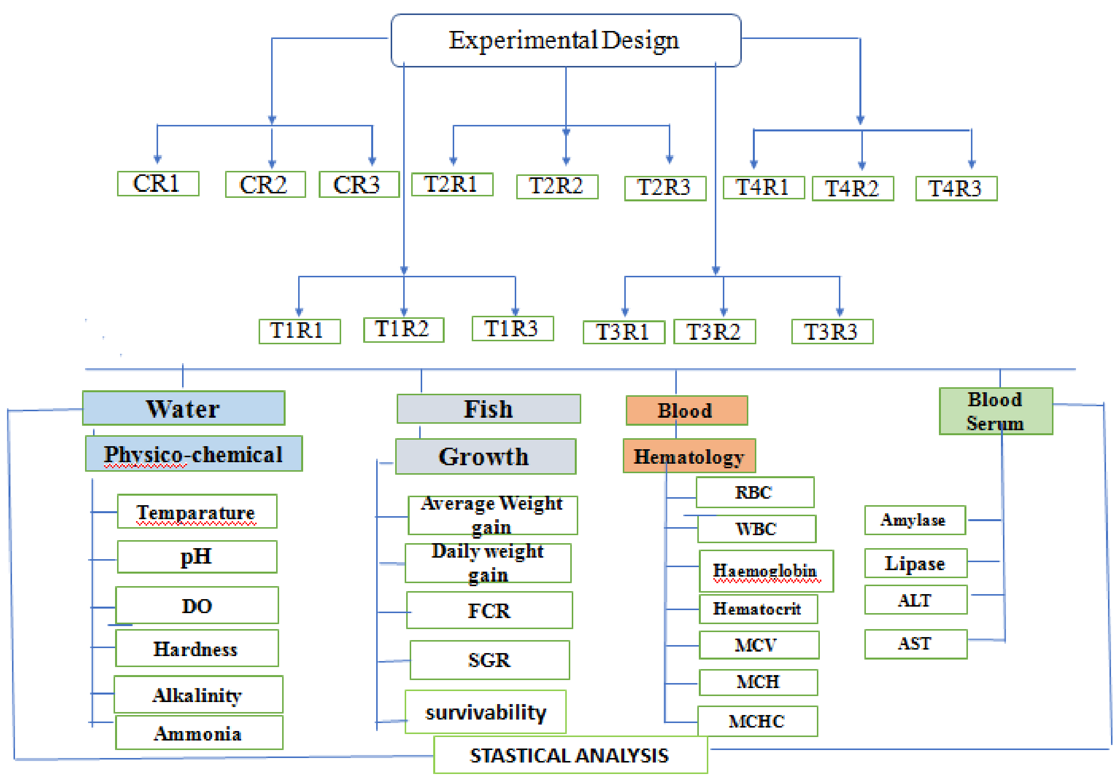


Fig.1: Experimental protocol followed in the present investigation

3.5. Experimental feed preparation

The feeds were produced in the feed mill facilities of ICAR CIBA, Kakdwip, West Bengal, India. Feed formulation is presented in table. All feed ingredients including black cumin seeds were purchased from local market. Different zinc inorganic form as ZnO and Zinc oxide nanoparticles (ZnO-NP) were taken from fish nutrition department The raw materials are selected based on the ability to supply nutrients. Soybean meal and mahua oil cake were used as a protein sources; wheat flour was used as carbohydrate sources, fish oil and sunflower oil were used as lipid source, anda mineralwas also added. 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 100^0C and later on cooled.

After cooling, experimental diet such as T_1, T_2, T_3, T_4) were prepared with the addition of (ZnO, ZnO –NP, ZnO + Black cumin, ZnO –NP + Black cumin) respectively @ 1.5 and cumin @ 1 % per kg of feed mixed with the ingredients along with this fish oil, sunflower oil, lecithin, mineral mixtures were also added (table 1). Then it was then extruded with the help of a hand pelletizer. The pellets (1.5 mm) were dried at room temperature. The formulated feed was kept in an airtight container at −20°C until used to prevent contamination (Rajan and Rohini, 2021).

The experimental diets were subjected for chemical analysis of crude protein (CP), crude fat (CF), total ash and moisture are measured by Weende system of proximate analysis and it was adopted for estimation of chemical composition of feeds. The proximate analysis of feed was done in the laboratory of Department of fish processing technology, faculty of fishery sciences, WBUAFS, Kolkata.

Table 1: Ingredients of treatment feed (dry matter basis)

Ingredients (g)	Control	T1	T2	T3	T4
Wheat flour	37	37	37	37	37
Mustard cake	25	25	25	25	25
Soyameal	26	26	26	25	25
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	**100**	**100**	**100**	**100**	**100**
ZnO (mg)	0.0	1.5	0.0	1.5	0.0
nZnO (mg)	0.0	0.0	1.5	0.0	1.5
Black Cumin	0.0	0.0	0.0	1.0	1.0

3.6. Duration of experiment and feeding schedule -

Fishes were fed the diets at 4% of (w/w) body weight per day (twice daily in equal proportion; at 10.00a.m and 4:00p.m daily. The experiment was continued for 8 weeks. At the end of the 8 weeks feeding trial, final sampling was carried out for the analysis of different digestive enzymes (Lipase and amylase), metabolic enzymes (ALT, AST) and hematological parameters.

3.7. Proximate composition of feed ingredients

3.7.1. Crude protein (CP)

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. Five ml aliquot of digested feed/ fish muscle/ faeces 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%) 12mL, bromocresol green (1%) 6mL, dehydrated alcohol 200mL and distilled water 782 mL}. The nitrogen trapped in boric acid was estimated by titrating it against N/100 sulphuric 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:

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

$$\text{Crude protein (\%)} = \text{\% Nitrogen} \times 6.25$$

3.7.2 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. 60–800C). At 1000C, 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 Dry matter basis using the following formula:

Wt. of dry sample = (Wt of thimble + Oven dried sample) – (Wt. of thimble)

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

$$\text{Crude fat (\%)} = \frac{\text{weight of crude fat}}{\text{weight of dry sample}} \times 100$$

3.7.3 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 600^0C 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:

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

3.7.4. Moisture

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

$$\text{Moisture (\%)} = \frac{\mathit{Initial\ weight} - \mathit{final\ weight}}{\mathit{Initial\ weight}} \times 100$$

3.8 Physico-chemical analyses of water sample

Weekly sampling 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, and Total alkalinity were analyzed. The method for different analysis and the instrument 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	APHA ,2005
Dissolved oxygen (DO) (ppm)	Winkler's method	Jhingran et al.,1969
Total hardness (ppm)	Titration method	APHA, 2005
Total alkalinity (ppm)	Titration method	APHA, 2005

3.8.1 Temperature

Water temperature of all aquariums was measured by centigrade thermometer on spot and expressed as (°C).

3.8.2 pH

The pH of water sample was estimated by pH meter.

3.8.3 Dissolved Oxygen (DO)

Dissolved oxygen of water sample was estimated following the Wrinkler's methods (APHA, 2005). water sample collected in a 300 ml sampling bottle avoiding any air bubbles then 1 ml of Wrinkler's a (Manganous Sulphate Solutions) followed by 1 ml of Wrinkler's B (potassium Iodide) were added. When precipitated settled sufficiently to a level, 1 ml of concentrate sulphuric acid (H_2 SO_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 colorless.

The volume of the titrant was recorded and the result was expressed as mg/L Dissolved oxygen was calculated as follows:

$$DO = \frac{0.2\ \text{Of Na2S2O3}}{\text{mL Of Sample taken}} \times 1000$$

3.8.4 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 1998) as indicated. The value was expressed in mg/l.

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

3.8.6 Total alkalinity

Total alkalinity was calculated through titration method (APHA ,1998). 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 NH_2SO_4 till the colour disappear .then the burette reading was noted down and that sample ,2 drops of methyl orange indicator were added and the solution turned into orange colour . The titration was continued with 0.02 NH_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.

$$\text{Total alkalinity (mg/L)} = \frac{\text{Volume of H2SO4}}{\text{Volume of sample taken (ml)}} \times 100$$

3.8.7. Total ammonia –nitrogen

After proper filtration of the sample, phenol solution, sodium solution and oxidizing solution were added to the sample. The sample were then wrapped with paper and kept at room temperature (22-27°C) in subdued light for at least 1 hour. A blue colour appeared which was stable for 24 hrs. The ammonia concentration pf the samples was directly estimated through a double a double beam UV –Vis –Spectrophotometer (CECIL CE -4002) at 543 nm wavelength.

3.9. Growth performance analysis

3.9.1. 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. Five fish were caught using scoop net from each replicate tank during every sampling and blotted on absorbent paper to record the weight using electronic balance. Average weight of fishes of each replicate was then calculated separately for each tank. The different growth indices were calculated using the following formula -

Indices used to evaluate growth performance –

1. Daily weight gain $= \frac{\text{final weight} - \text{Initial weiht}}{\text{No of days}} \times 100$
2. Average weight gain $= \sum \frac{\text{Final WeighT} - \text{Initial Weight}}{\text{Number of replicates}}$
3. Feed conversion ratio (FCR) $= \frac{\text{TotaL Feed intake(g)}}{\text{Total wet weight gain (g)}}$
4. Specific growth rate (SGR) $= \frac{\text{Log Final weight} - \text{Initial Weight}}{\text{Experimental period}} \times 100$
5. Survival (%) $= \frac{\text{Number of fish survived}}{\text{Initial number of fish}} \times 100$

3.10. Estimation of hematological parameters

The changes in haematological parameters like total erythrocyte count, total leucocyte counts, thrombocyte counts, haematocrit values, haemoglobin levels, red blood cell indices, i.e., mean corpuscular volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), differential leucocyte counts and serum biomarkers like aspartate aminotransferase (AST), alanine aminotransferase (ALT), were monitored periodically. The blood samples from all the tanks were collected.

3.10.1. 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)

Dilution = 200 area counted = 5×0.04 = 0.2 mm^2

3.10.2 Estimation of Haemoglobin (Hb)

The haemoglobin level of blood was estimated by the cyanomethemoglobin method using Drabkin's fluid [Drabkin, (1950]. The haemoglobin concentration was then calculated using the following formula:

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

3.10.3Hematocrit (Packed Cell Volume)

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

3.10.4. Mean Cell Volume

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

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

3.10.5. Mean Cell Haemoglobin

The mean cell haemoglobin (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).

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

3.10.6. 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 hematocrit (Stoskopf, 1993).

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

3.10.7. Leucocyte Count

Leucocytes were counted by using the hemocytometer. 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

3.11. 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% ethylene diamine 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.

3.12. Collection of serum

Blood without anticoagulant was allowed to clot at about 30°C by keeping the syringe in a slanting position and then kept at 4°C overnight. The serum samples were collected by centrifugation at 2500 rpm for 15 min, transferred to Eppendorf tubes, and stored at -20°C for further analysis of biomarkers.

3.12.1. Blood smear preparation, fixation, and staining

3.12.1.1. Blood smear preparation -

A drop of blood without anticoagulant was placed on one end of a chemically cleaned slide. A second slide was held at an angle of 45° 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.

3.12.1.2. 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.

3.12.1.3. Staining

The methanol-fixed slides were then stained by the May Grunwald Giemsa method (Hayhoe *et al.*, 1964). 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.

3.13. Biochemical parameters

3.13.1 Estimation of digestive enzymes

3 fish from each tank (3fish/replicate) were dissected to obtain the intestine for digestive enzymes analysis and kept in an ice-cold plate and washed with physiological cold saline (0.85%), For digestive enzymes, the fish were fed 30 min prior the sampling and then the gut was dissected out. The pooled gut sample of each treatment group was macerated with 5% 0.25 M chilled sucrose solution using a mortar and pestle. This mixture was then centrifuged at 5000 rpm in 4^0C for 20 min and the fat free supernatant was immediately used as sample for digestive enzymes analysis (Naskar et al., 2022). The collected supernatant was used as a crude enzyme source to estimate the amylase, lipase enzymes activity.

3.13.1.1 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 25^0 c for 5 minutes prior assay.
B) DNS reagent: - 1gm 3, 5 dinitrosalicylic acids was dissolved at room temperature in 50 ml of ddH_20 then added 20 ml of 2MNaOH solution and 28.2 gm Rochelle salt. Finally made up to 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 amvlovtic 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.

3.13.1.2 Lipase estimation (Ogunbivi and Okon, 1976)

Apparatus:-Test tube, micro pipette with tips, incubator, and 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) Phoaphate 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 .

3.13.1.3. 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).

3.14. Statistical analyses-

The data were presented as a mean ± standard deviation. The data on Growth, daily weight gain, SGR, FCR, survibability, haematological parameters, and biochemical parameters were analysed by one-way ANOVA test and confirmed the significance of difference among the treatments with Duncan post-hok analysis. 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 1: Different activities during experiments(Experimental set up, tank cleaning, conditioning of fishes and drying of feed, package feed)

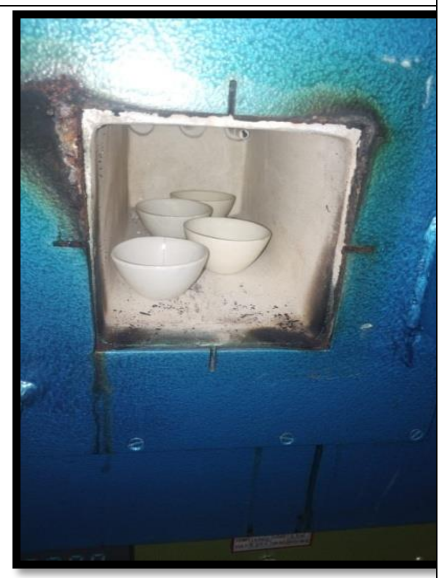
(a) Sampling of fish

(b) Blood collection

(c) Blood collection

Ash estimation

Fat estimation



Moisture estimation

Plate 2: Different activities during experiments (Sampling of fish, Blood collection, ash content, fat estimation etc.)

Plate 3: Different activities during experiments (Sampling of fish, Blood collection etc.)

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Physico-chemical parameters of water

Productivity of any fish culture mainly depends on the physical, biological and chemical characteristics of water and soil. Good water quality is important for survival and growth of fish. Water quality parameters were observed throughout the experimental period of 60 days. The parameters were temperature, pH, dissolved oxygen, alkalinity, total hardness, and total ammonia.

4.1.1. Temperature

Temperature is an important component that has a direct impact on important factors like the water temperature, growth, oxygen demand, food requirement and food conversion ratio (Adhikari, 2011). The temperature of experimental tanks water did not differ so much among the treatments during the periods of investigation. The temperature of all tanks water did not show significant changes and it ranges between 26.4°C to 26.85°C. The highest temperature value was recorded in 56th day in T2 tank and lowest temperature value was recorded 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 56th 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 56th day. The temperature changes of the T4 were not changed significant 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.

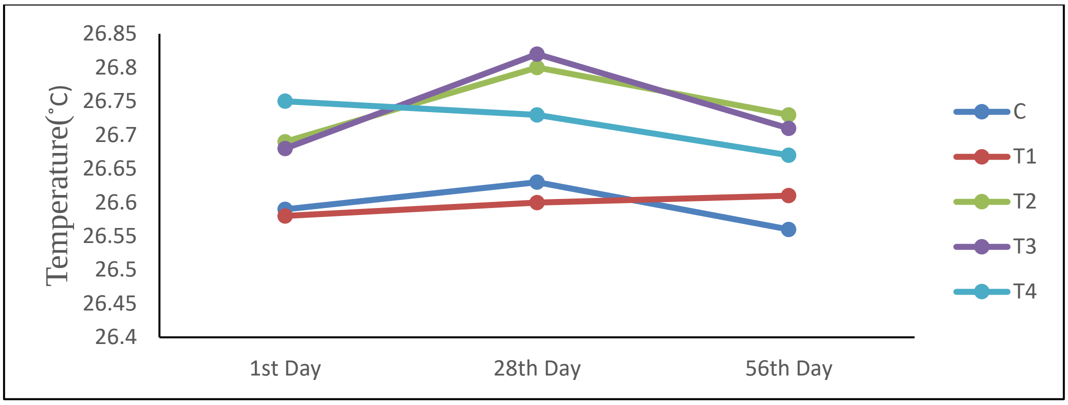


Fig.-2: Temperature variation of experimental tanks at 1st, 28th and 56th day

4.1.2.PH

The pH is negative logarithm of hydrogen ion (H^+) concentration. It is an important limiting factor in fish culture. 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 were not changed significantly ($p<0.05$). The pH variation of experimental tanks at 1st, 28th and 56th day was presented in figure-3.

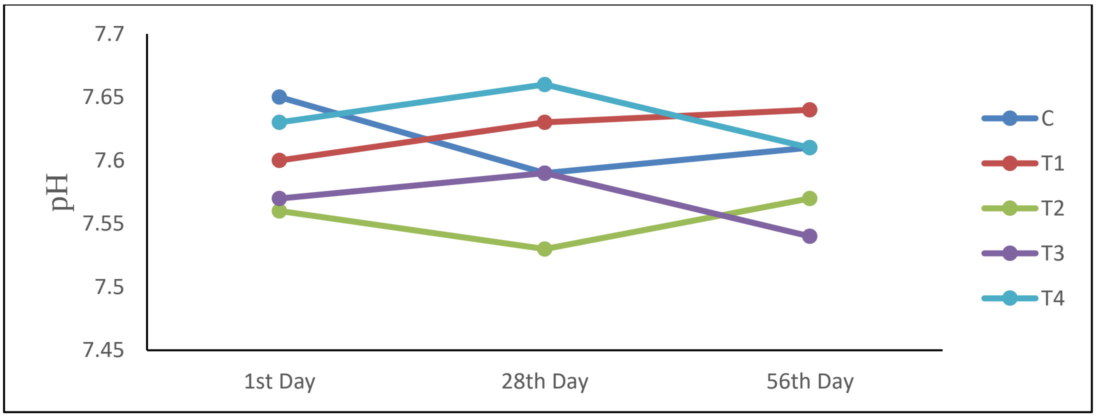


Fig.-3: pH variation of experimental tanks at 1st, 28th and 56th day

4.1.3. Dissolved Oxygen

The dissolved oxygen (DO) is considered the most important for the survival of organism under aquaculture. The temporal trend in the values of DO, in general exhibited a more or less steady state condition under all the treatments. However, in T2 and T4, it declined 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 in

T4 treatment it increased after 28th day. But in control tanks, DO increased 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, 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 figure4.The DO values showing insignificant ($p>0.05$) differences throughout the experimental period and also it was maintained in the same level without fluctuation.

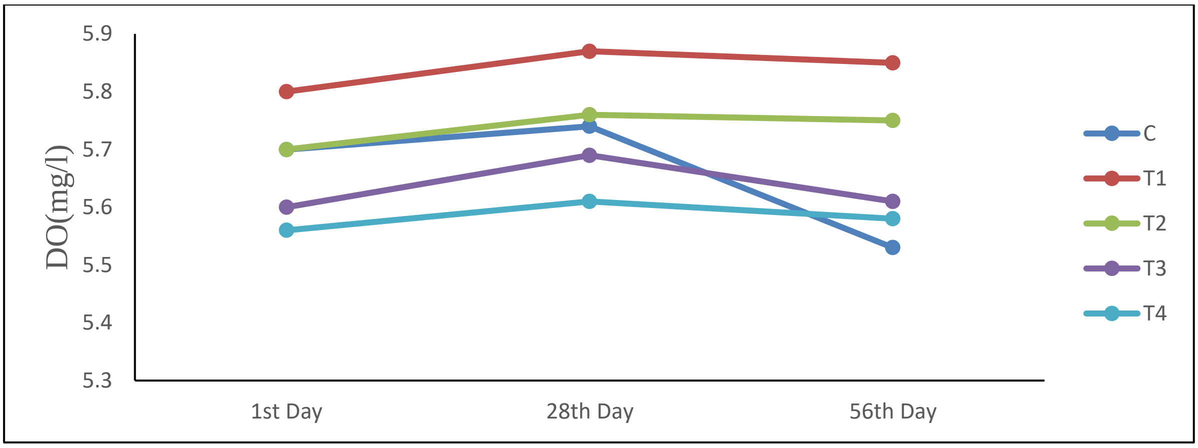


Fig.-4: Dissolved oxygen variation of experimental tanks at 1st, 28th and 56th day

4.1.4. Total alkalinity

Total alkalinity of water intended to increase over time in all the treatments groups at 28thday. 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 experimental period. The overall mean value of alkalinity was highest at T3 (137.1 mg/l), and lowest at T4 (135mg/l). There was no 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 indicated in figure 5.

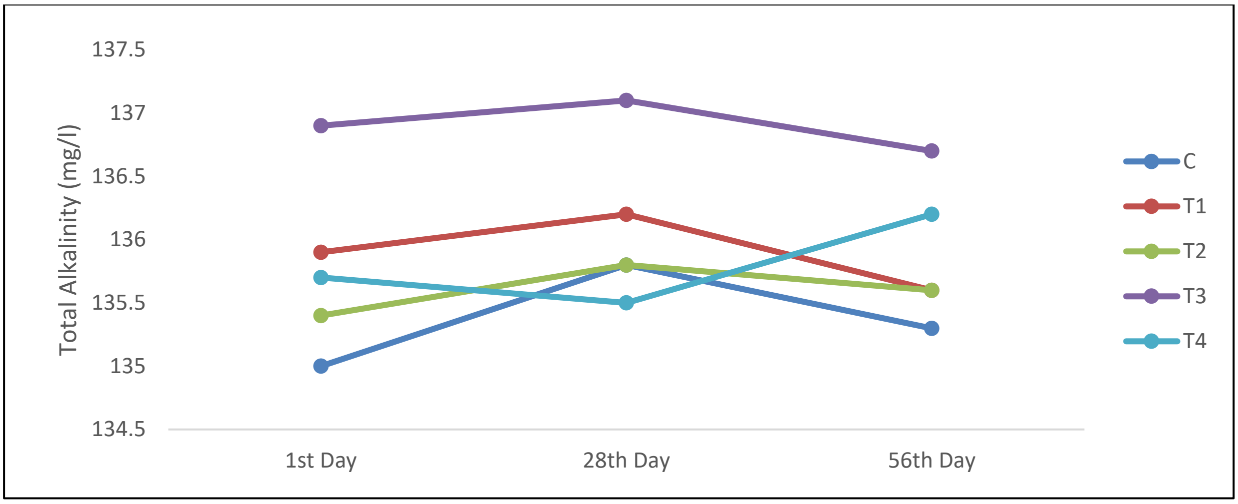


Fig.-5: Total alkalinity variation of experimental tanks at 1st, 28th and 56th day.

4.1.5. Hardness

The temporal variation in the concentration of hardness was observed and those were not showing significant differences ($p<0.05$). Figure 6 showed the hardness value ranged in between 630 to 660 mg/l. In control group, hardness decreased slightly in the 28th day and 56th day. During experiment observed the lowest value was 634 mg/l and the highest was 658 mg/l. In control groups, hardness value decreased from 1st to 28th day. The 1st day value was 658 mg/then 28th day value was 651mg/l and then it became 647 mg/l in 56th day.T1 increased in between 1st and 28th day from 645 to 647 mg/l and then decreased to 644 mg/l.

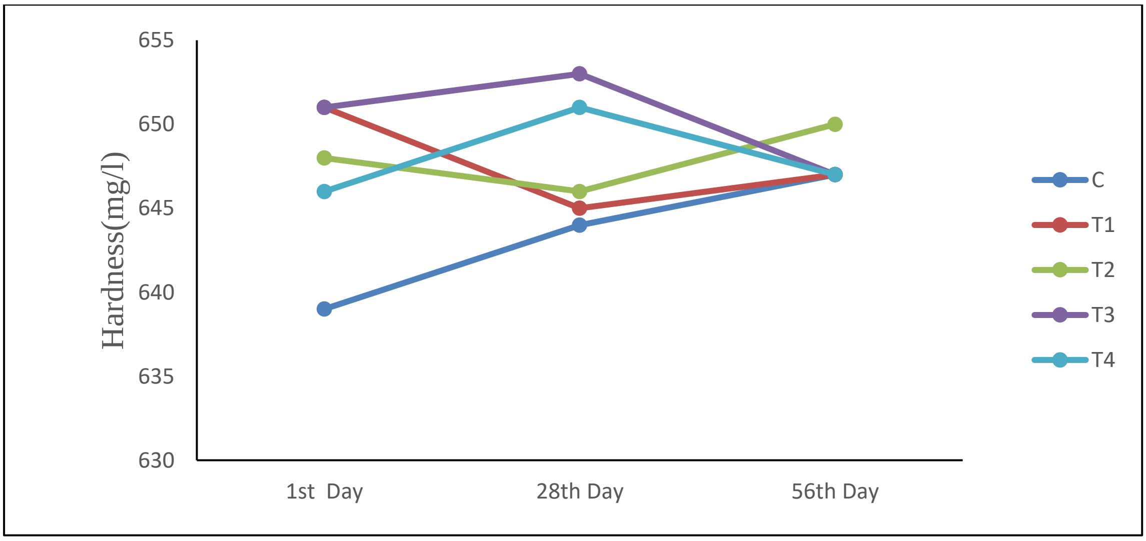


Fig.-6: Total hardness variation of experimental tanks at 1st, 28th and 56th day

4.1.6. Total ammonia

There was no significant change of total ammonia value among the treatment groups ($p<0.05$). It ranged between 0.03 to 0.05 mg/l. The highest value observed was 0.05 mg/l in T3 group and the lowest was 0.02 mg/l in T1and T4. T1 and T4 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.

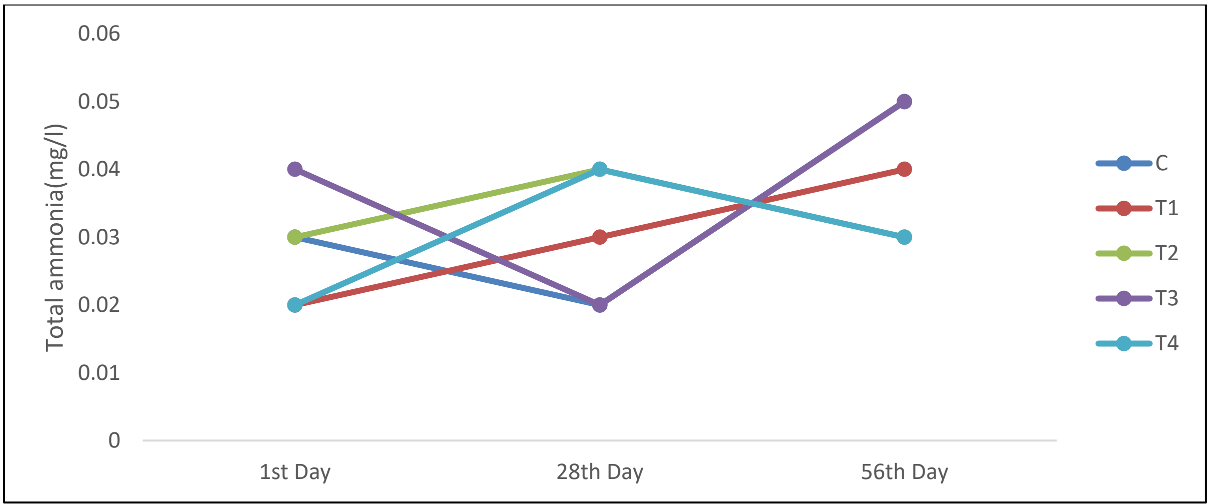


Fig.-7: Total ammonia variation of experimental tanks at 1st, 28th and 56th day

4.2. Proximate composition of experimental diets

The proximate composition of different experimental diets on dry weight basis are presented in Table -3. The moisture (%) content of all the experimental diets varied from 8.4 to 8.8. Highest moisture content was found in T1 and T4 feed valued 8.8 % and lowest moisture value was recorded in T3 and T4 feed valued 8.4 %. The crude protein (%) content ranged from 28.37 to 28.41. Highest crude protein percentage recorded 28.41 in T3 feed and lowest crude protein percentage was recorded 28.35 in T4 feed. The fat value ranged from 9.26 to 9.88 %. Highest fat content was recorded 9.88 % in T1 feed and lowest fat content was recorded 9.26 % in T4 feed. The ash (%) content ranged from 9.34 to 9.86 %. Highest ash value was recorded 9.86 % in T4 feed and lowest ash value was recorded 9.34 % in T3 feed.

Table-3: Proximate composition of experimental diets (on wet weight basis)

Treatment	Ash	Crude Fat	Moisture	Crude Protein
Control	9.49	9.80	8.5	28.37
T1	9.43	9.88	8.8	28.40
T2	9.68	9.23	8.8	28.38
T3	9.34	9.81	8.4	28.41
T4	9.86	9.26	8.4	28.35

4.3. Growth performance

The growth performance and feed efficiency parameters of the experimental fish (*Cirrhinus mrigala*) such as, average body weight (ABW), 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. Average body weight

The average body weight of fish tended to increase over time, however the pattern of growth differed in the test fish among different treatment tanks. The growth rate differed in different weeks of the experiment period. The highest average body weight (20.88g) was founding T4 treatment and the lowest average weight (15.22g) was at control groups. From ANOVA analysis, there was significant difference shown among the treatments ($p<0.05$).

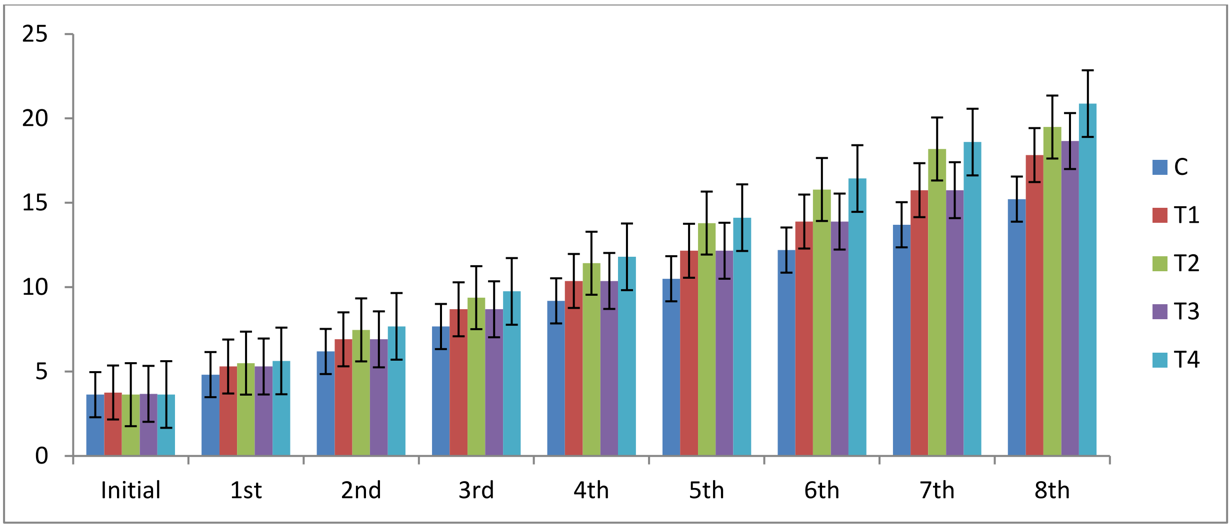


Fig -8: Variation of average body weight (g) of *Cirrhinus mrigala* treatment wise.

It was clear from the Table 4 that the weekly growth obtained of *Cirrhinus mrigala* showed significantly difference among all the treatments including control ($p<0.05$). But there were variations of growth obtained within the treatments.

Table.4. The weekly average body weight (g) observed of *Cirrhinus mrigala* treatments wise.

Weeks	C	T1	T2	T3	T4
Initial	$3.63^{A}\pm0.042$	$3.76^{A}\pm0.092$	$3.63^{A}\pm0.014$	$3.68^{A}\pm0.064$	$3.64^{A}\pm0.007$
1st	$4.82^{aB}\pm0.078$	$5.3^{bB}\pm0.078$	$5.5^{cB}\pm0.099$	$5.3^{bB}\pm0.078$	$5.63^{bB}\pm0.007$
2nd	$6.19^{aC}\pm0.078$	$6.91^{cC}\pm0.113$	$7.47^{dC}\pm0.049$	$6.91^{bcC}\pm0.113$	$7.68^{bC}\pm0.099$
3rd	$7.67^{aD}\pm0.035$	$8.69^{dD}\pm0.049$	$9.38^{eD}\pm0.099$	$8.69^{cD}\pm0.049$	$9.75^{bD}\pm0.106$
4th	$9.19^{aE}\pm0.078$	$10.37^{dE}\pm0.163$	$11.42^{eE}\pm0.488$	$10.37^{cE}\pm0.163$	$11.8^{bE}\pm0.035$
5th	$10.5^{aF}\pm0.0283$	$12.16^{bF}\pm0.127$	$13.8^{cF}\pm1.167$	$12.16^{bF}\pm0.127$	$14.12^{bF}\pm0.141$
6th	$12.2^{aG}\pm0.177$	$13.89^{bG}\pm0.191$	$15.79^{cG}\pm1.386$	$13.89^{bG}\pm0.191$	$16.44^{bG}\pm0.184$
7th	$13.7^{aH}\pm0.46$	$15.75^{bH}\pm0.071$	$18.19^{cH}\pm2.022$	$15.75^{bH}\pm0.071$	$18.6^{bH}\pm0.17$
8th	$15.22^{aI}\pm0.75$	$17.83^{bI}\pm0.410$	$19.49^{cI}\pm1.018$	$18.66^{bI}\pm0.629$	$20.88^{bI}\pm0.24$

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

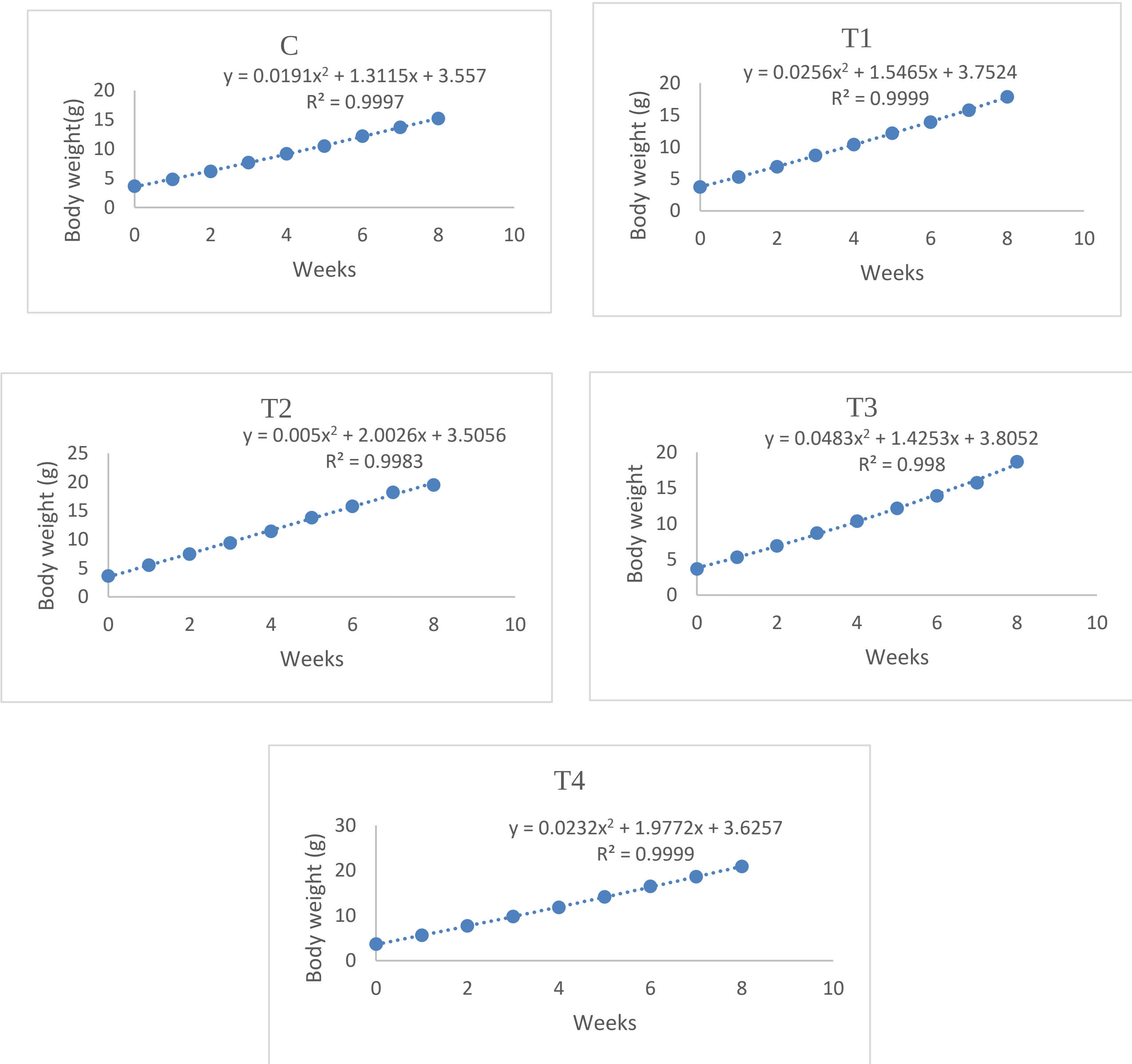


Fig 9. Relationship of body weight (g) week-wise of different treatment groups of *Cirrhinus mrigala* (C, T1, T2, T3, T4).

At 1st week, there was no significant change in growth among different group from control. But from the 2nd week onwards, there was significant change in growth among the treatment as compared to control group. The poor growth was observed in all the weeks in control as compare to all other treatments groups (figure 8). 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. There is strong relationship found as the R^2 value is nearer to 1.

4.3.2. Average body weight gain

Weight gain was to increase with the advancement of the study and average body weight gain (ABWG) attained by the fish recorded in weekly basis. In the present study, ABWG attained by the experimental fish was recorded and all findings were put in figure 10. The highest weight gain found in T4 (17.24g), and lowest weight gain was recorded in control group (11.59 g) as compared to other treatment groups. From Table.5, it was seen that there was significant difference ($p<0.05$) among C, T1/T3, T2 and T4 in the average body weight gain. But no significant difference found between T1 and T3.

Table-5: Average body weight gain (ABWG), daily weight gain (DWG), specific growth rate (SGR) and feed conversion ratio (FCR) of *Cirrhinus mrigala*.

Treatment	ABWG (g)	DWG (g)	SGR (%)	FCR
C	11.59±0.02^{a}	0.206±0.002^{a}	2.55±0.001^{a}	1.371 ± 0.001^{c}
T1	14.07±0.02^{b}	0.251±0.001^{b}	2.77±0.001^{b}	1.289± 0.001^{b}
T2	15.86±0.01^{c}	0.283±0.001^{c}	3.00±0.001^{c}	1.196±0.002^{a}
T3	14.98±0.01^{b}	0.267±0.002^{b}	2.89±0.001^{c}	1.261±0.001^{b}
T4	17.24±0.02^{d}	0.307±0.002^{c}	3.11±0.001^{c}	1.125±0.002^{a}

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

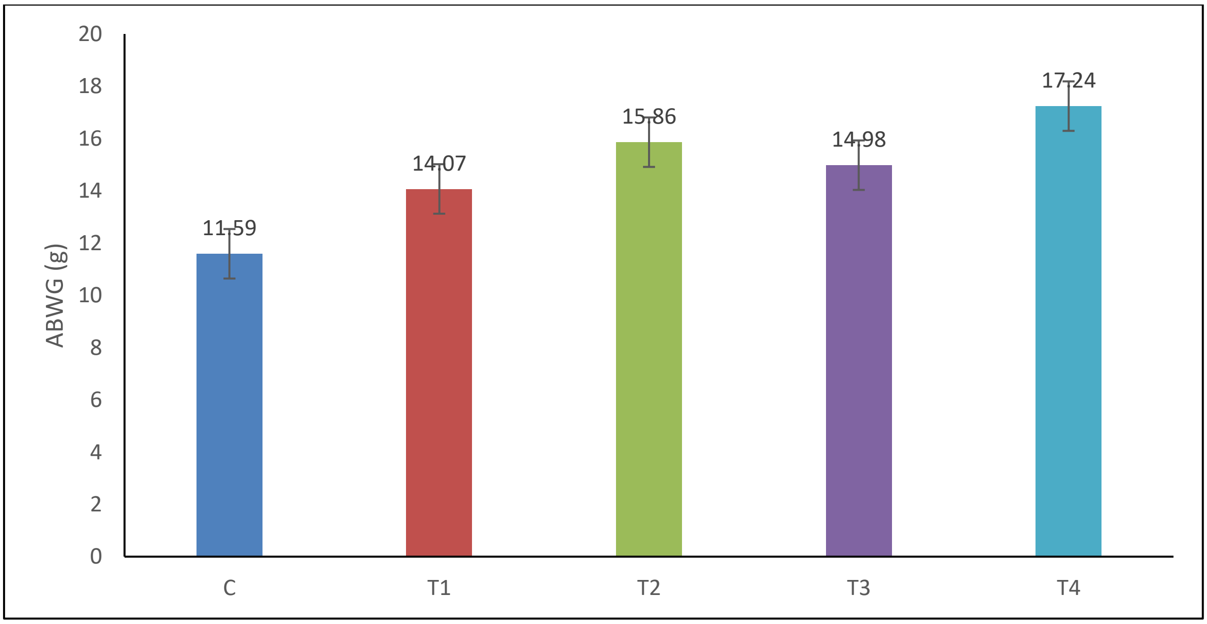


Fig.-10: Variation of average body weight gain (g) of *Cirrhinus mrigala* treatment wise

Present study showed that fish fed with T4 treatment feed which is combination of ZnO NP along with black cumin, recorded more ABWG as compared to other treatment feed. This study is similar with previous study which concluded that rohu fingerlings fed diet containing 15 mg/kg Zno –NP has the highest weight gain, which was significantly different ($p<0.05$) from the fish fed other experimental diets including the control group (Mondal *et al*., 2020).

By combination of zinc oxide nanoparticles with black cumin it enhanced the growth rate of mrigal.Similar to present results, Al-Dubakel*et al*., (2012) concluded that the growth performances of *Cyprinus carpio* fingerlings fed on 1% black seed supplemented diet enhanced growth performance. The growth rate of *Oreochromis niloticus* fed on 2% black seed supplement diet improved growth therefore increase the body weight (Diab *et al*., 2008). Oz *et al*., (2018) concluded that *Onocorhynchus mykiss* juveniles fed on 1% and 1.3% black cumin seed supplemented diets at the rate of (0.1%, 0.4%, 0.7%, 1% and 1.3%) improved their growth rate. All these earlier mentioned studies supported the present findings.

4.3.3. Daily weight gain (DWG)

From the present study we found that DWG showed significant difference($p<0.05$) among C, T1/T3, T2/T4 treatment groups during 56 days of experimental period and it is observed from the Table.3. But there was no significant difference between T1 and T3; T2 and T4 treatment groups. T4 having maximum daily weight gain (0.307g) followed by T2 (0.283g) and then T3 (0.267g), T1 (0.251g) and lowest daily weight gain found in control group (0.206g). It was observed that there was an increasing trend of DWG from control to T2 and then it was declined trend from T2 to T3 and then increasing growth trend from T3 to T4 (figure 11).

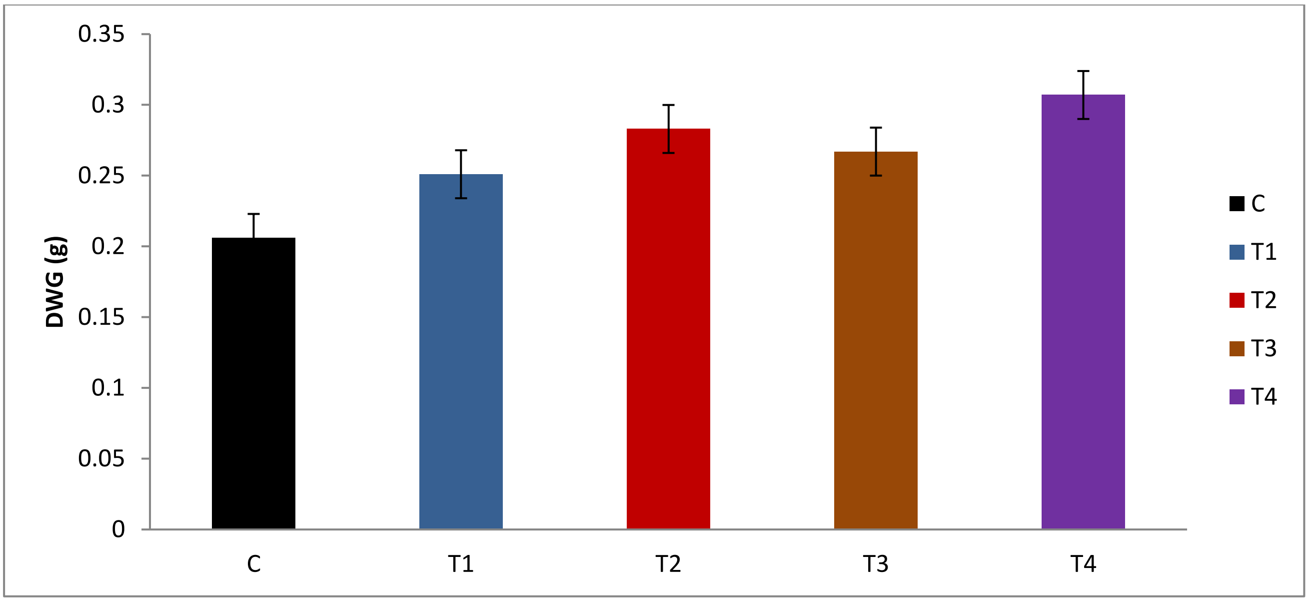


Fig.-11: Variation of DWG in different treatments

4.3.4. Specific growth rate (SGR)

Specific growth rate is the most commonly used indices in growth. The SGR value of different treatment group are mentioned in table 5 showed significant difference ($p<0.05$) among the treatment groups. The highest SGR value was recorded in T4 (3.11%) and lowest in control (2.55%) group. There were no significant differences ($p>0.05$) among treatments group T2, T3and T4 as shown in table 2 and figure 12. But there was difference between all these treatments when compare to control and T1.

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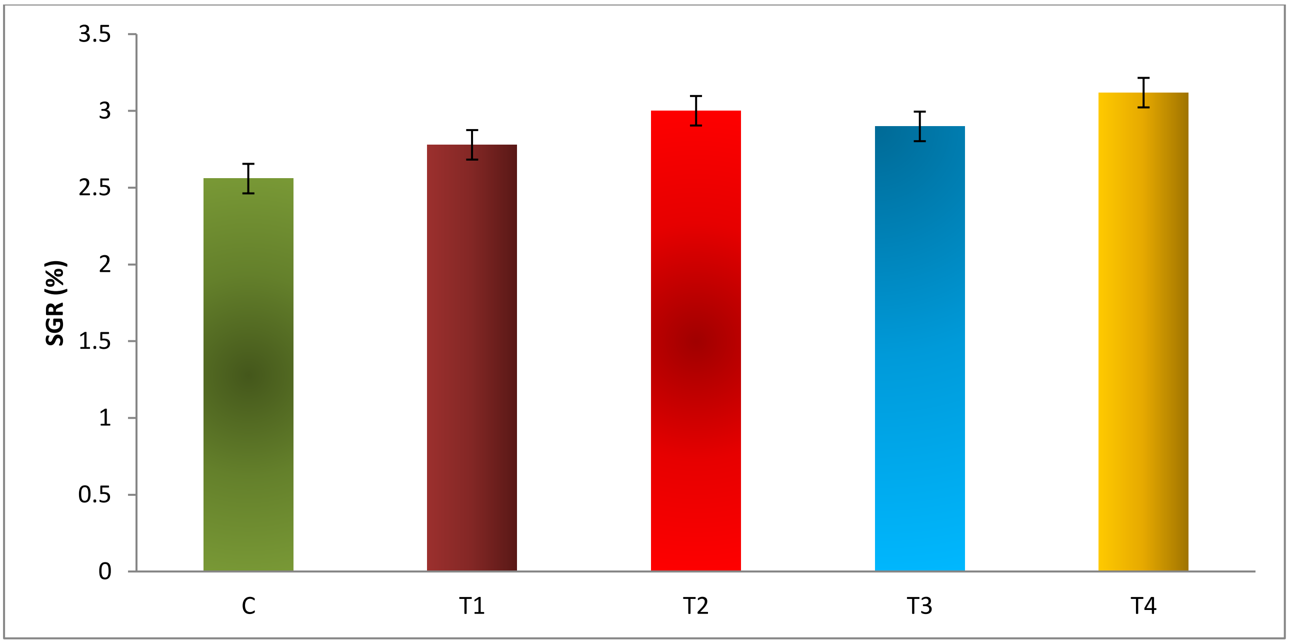

Fig.-12: Treatment wise variation of SGR of *Cirrhinus mrigala*

The present study indicated that T4 have higher SGR value when compare to other treatment groups and there was significant difference between value of SGR of T4 when compare to control andT1 groups.

Previous studies of Aziz *et al*. (2015) reported a higher SGR of *Labeo rohita* fingerlings fed a diet containing 15 mg ZnO-NP/kg as assimilation and metabolism of mrigal were increased with an increase in ZnO nanoparticles in the feed. Swain *et al*. (2018) reported that 10 mg /kg zinc oxide nanoparticles –supplemented diet fed *Labeo rohita* performs increased growth rate, as we considered here that experimental fish (mrigal) also following the same growth performance as it comes under the major carp. So, these earlier mentioned previous studies supported present study.

4.3.5. Feed conversion ratio (FCR)

FCR gives the amount of feed required to produce a unit weight gain of fish. It is calculated from feed intake and weight gained during the experimental period. The FCR of control group of fishes was (1.37), whereas that of the treated group of fishes was vary from (1.289-1.125), indicating superiority of treated groups to control ones.

The FCR of different treatment groups are indicated in figure 13 and table 5. Good FCR was recorded in T4 (1.125 g) and the FCR values was varied significantly among C, T1, T3 and T2,T4 ($p<0.05$). The highest FCR was found in control as compare to other treatment groups. Relationship of FCR with body weight (g) treatment wise was shown in figure 14.

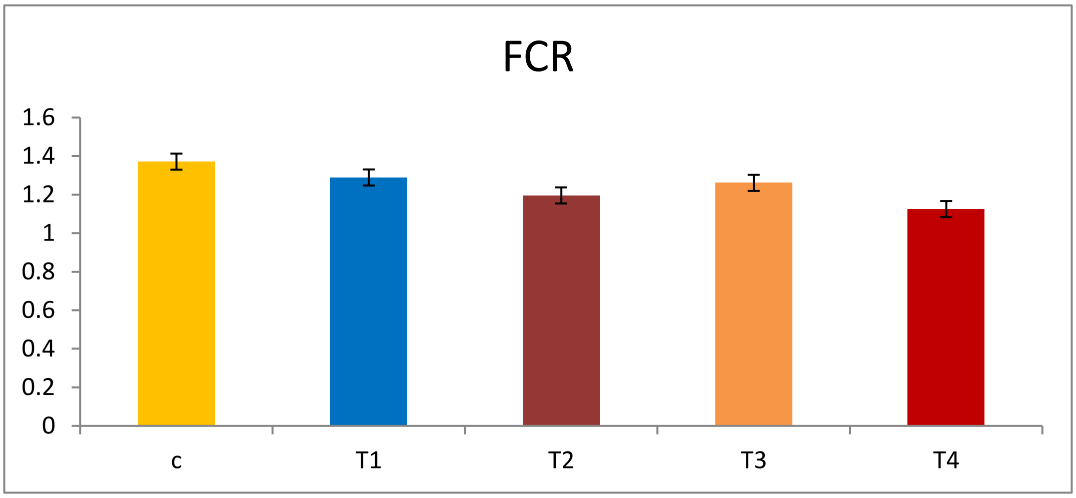

Fig.-13: Variation of FCR of *Cirrhinus mrigala*

Present study indicated T4 treatment containing ZnO–NP @15 mg /kg concentration diet achieve low FCR and similar study suggested by Mondal *et al.*, (2020) reported that 20 mg/kg concentration of ZnO –NP showed low FCR in rohu. Faiz*et al.*, *(*2015) reported that the feed conversion ratio was higher in ZnO nanoparticles incorporated feed of juvenile grass carp. Feed consumption and conversion efficiency of mrigal were higher in feed containing 15 mg of Zinc oxide nanoparticle. Onuegbu *et al*., (2018) reported an increase in the concentration of ZnO nanoparticles with feed consumption decreased feed conversion ratio. These all supported that experimental diet containing nano ZnO showed better FCR as compared to normal ZnO and control groups.

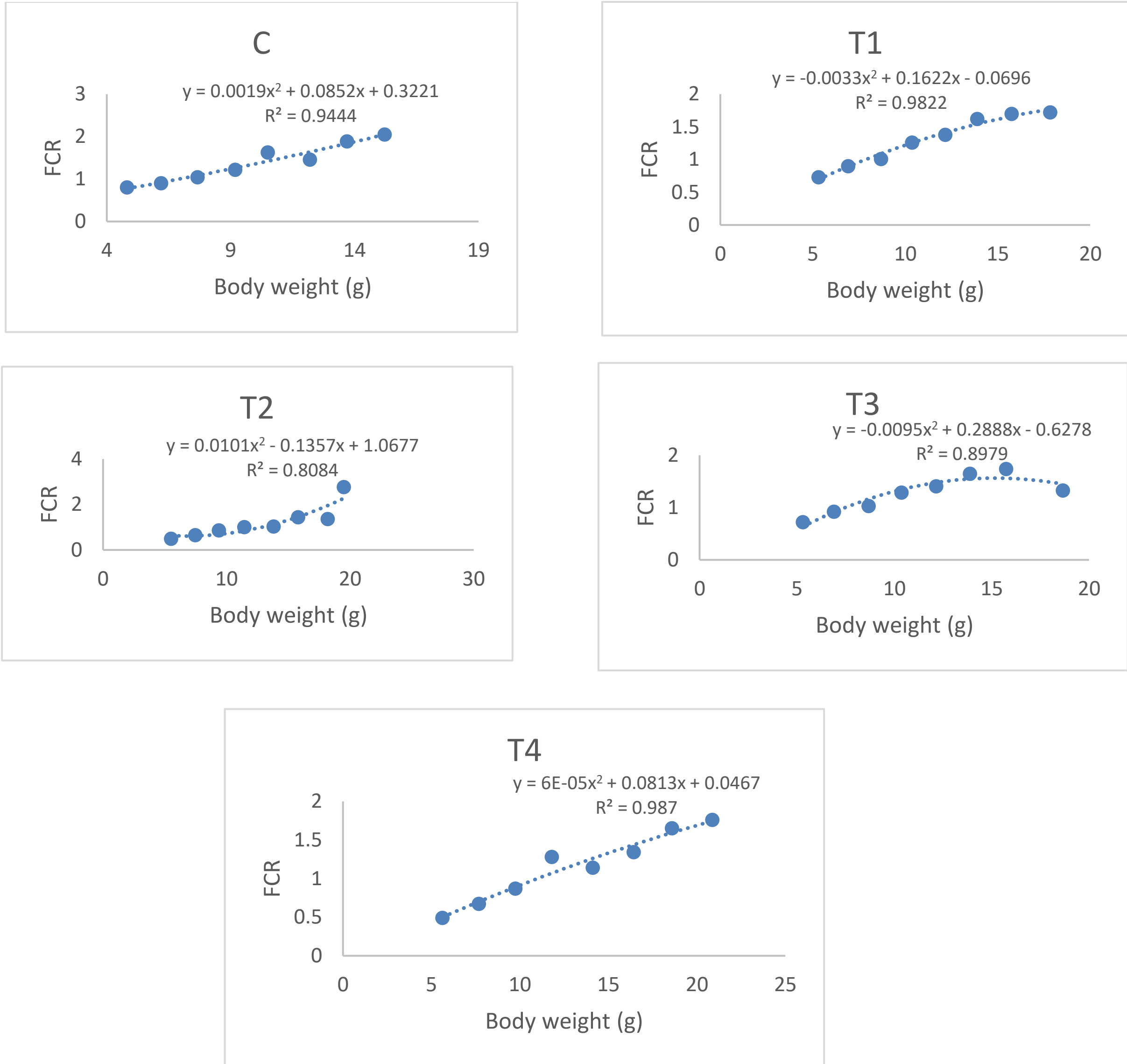

Fig.-14: Relationship of FCR with body weight (g) treatment wise (C, T1, T2, T3, T4, T4)

4.3.6. Survivability

It is defined as the ratio of the fish survived with initial number of fish which is described in percentage (%). In the present study, survivability was observed on weekly basis up to 8th week of experimental period. Control group having 100% survivability and T1, T2, T3 and T4 group having survivability 93.75%, 96.53%, 95.83% and 98.96% respectively. The highest survivability was observed in T4 and lowest survivability in T1 among the treatments groups excluding the control group as shown in figure 15.

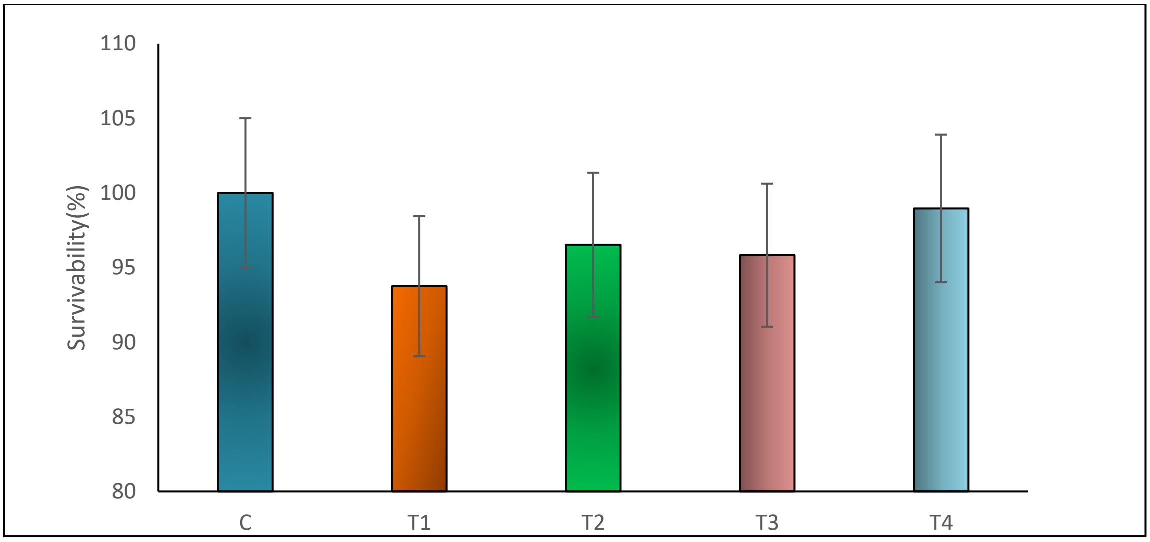

Fig.- 15: Survivability (%) variation of *Cirrhinus mrigala* at different treatments.

Awad *et al.*, (2019) reported that in 15 mg/kg diet of nano ZnO showed survivability between 95-100% in Nile tilapia. Latif *et al*., (2021) concluded that black cumin containing feed @ 0, 1, 2 and 5 % then there is the survivability between 95-100%, which supported present study of having survivability of black cumin added feeds T3 and T4 was 95.83% and 98.96% respectively.

4.4. Hematological parameters

The hematological analysis are very helpful in judgment 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). In the present study, RBC, Hb, Platelets, WBC of mrigal showed significant ($p<0.05$) difference among the treatment. From the table 3. it indicated that Hb and RBC showed highest value in T4 (11.50±0.19 mg/dl) and (3.80±0.17 million/cu-mm) respectively and lowest value showed in Control group (6.30±0.15 mg/dl) and (2.10 million/cu-mm) respectively. Platelets and WBC value showed highest value in control group (0.64±0.002 $x10^5$) and (3900 no/cu-mm) respectively and lowest platelets value in T1 groups (0.45±0.001 $x10^5$) and WBC value in T2 (1300 no/cu-mm) respectively. There was significant difference of hemoglobin content among C, T1/T2/T3 and T4 ($p<0.05$). But there was significant difference of platelets count among T1, C/T2/T4 and T3 ($p<0.05$) and significant difference of WBC among T2, T3, T4, C, T1.

It was also indicated that, ESR, PCV, M.C.V, M.C.H and M.C.H.C parameters were not showed significant ($p>0.05$) difference among the treatment groups. Result showed that WBC count is higher in case of black cumin supplemented group with zinc oxide T3 and T4 as compare to non-supplementation of black cumin with zinc oxide group T2 except control as indicated in table 6 and figure 16. The relationship of body weight (g) with RBC, hemoglobin and platelets were presented in figure 17. There is strong relationship of RBC and haemoglobin content with body weight as R^2 is nearer to 1. But there is poor relationship of platelets count with body weight ($R^2 = 0.321$).

Table 6. Different hematological parameters treatment-wise of *Cirrhinus mrigala*

Parameters	Control	T1	T2	T3	T4
Haemoglobin	6.30 ± 0.15^a	6.70 ± 0.11^b	6.80 ± 0.17^b	6.50 ± 0.21^b	11.50 ± 0.19^c
RBC (Million/Cu-mm)	2.10 ± 0.18^a	2.20 ± 0.19^a	2.20 ± 0.21^a	2.10 ± 0.22^a	3.80 ± 0.17^b
Platelets count ($x10^5$)	0.64 ± 0.002^b	0.45 ± 0.001^a	0.60 ± 0.0025^b	0.76 ± 0.003^c	0.62 ± 0.0018^b
WBC (no/Cu-mm)	3900 ± 23.94^d	3600 ± 24.89^d	1300 ± 19.01^a	2100 ± 21.64^b	2600 ± 25.59^c
ESR (mm in 1st Hour)	14±0.1	16±0.2	29±0.1	32±0.2	8±0.1
PCV(%)	18.9±0.19	20.1±0.23	20.4±0.24	19.5±0.22	34.5±0.17
M.C.V (FL)	90±3.28	91.3±3.31	92.7±3.32	92.8±3.32	90.7±3.22
M.C.H (Pg)	30±1.02	30.4±1.14	30.9±1.04	30.9±1.09	30.2±1.11
M.C.H.C (g/dl)	33.3±0.09	33.3±0.17	33.3±0.18	33.3±0.89	33.3±0.21

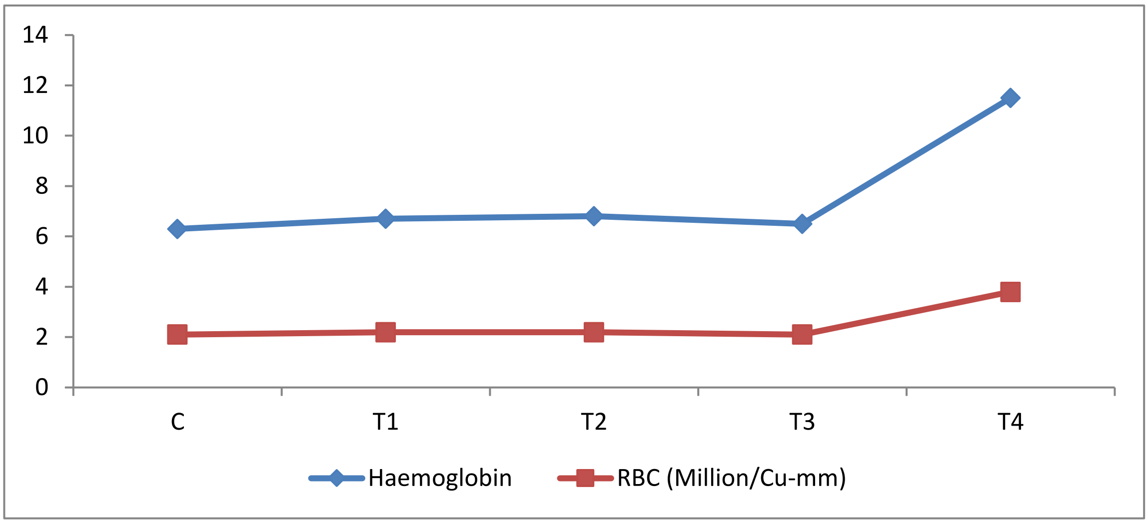

Fig.- 16: Variations of haemoglobin content (mg/l) and RBC (million /cu-mm) treatment-wise.

As similar to Present study, Firat (2007) reported 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 by introduction of toxicant and also in reverse of present result. Akbary *et al.* (2018) reported Hb, PCV and RBC count decreased and WBC count significantly increased compared to control in grey mullet exposed to sublethal concentration of copper oxide nanoparticles.

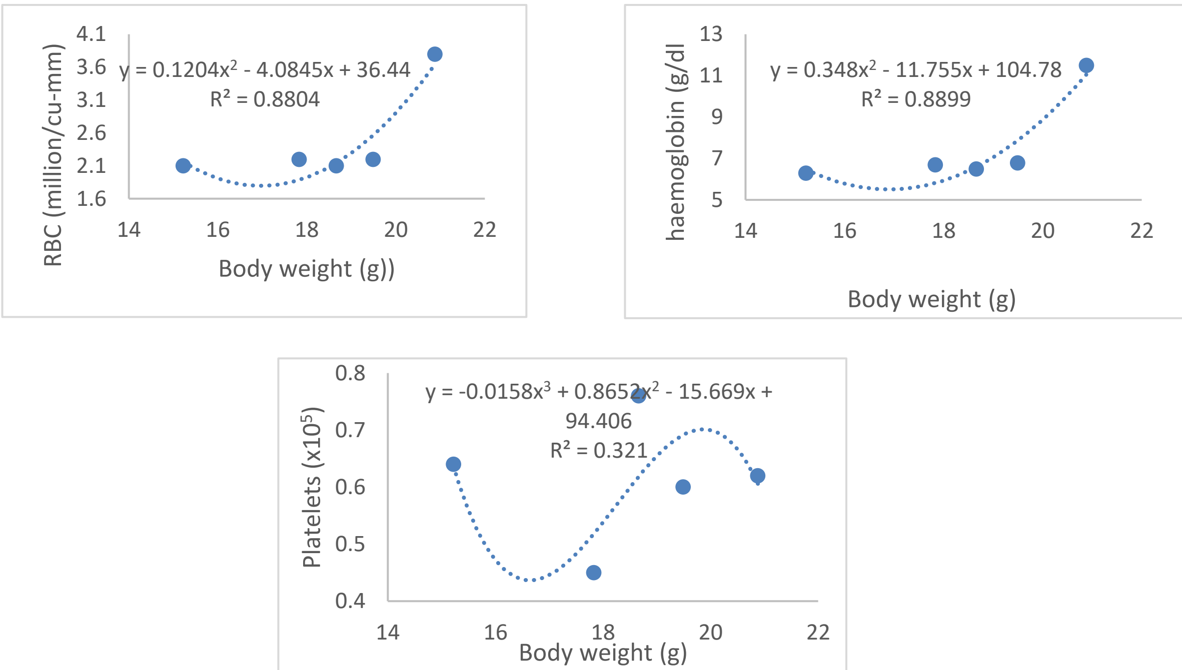

Fig.-17: Relationship of body weight (g) with RBC, haemoglobin and platelets.

Ali *et al.* (2015) reported significant variation in blood parameters when compare to control with high concentration of selenium nanoparticles supplementation feed to African catfish (*Clarius gariepinus*). Previous study of Novreen *et al. (*2018) reported significant variation blood parameters such as MCH, MCV, MCHC, PCV and RBC levels of common carp exposed to bulk and CuO NPs when compare to control group after 14 days of exposure. Chupani *et al. (*2018) reported that when common carp (*Cyprinus carpio*) exposed to diet having ZnO nanoparticles had no effect on hematological parameters.

Shah and Altindag (2005) reported that hematological parameter such as PCV, Hb, RBC and WBC were used to assess the functional status of oxygen carrying capacity of the bloodstream and have been as an indicator of metal pollution in the aquatic environment. Decrease in platelets value of grass carp in response to all Zn supplemented diets in the previous study indicate hemodilution in response to zinc (Celik *et al*.,2013).

A comparison of blood parameters of fish fed different form of different dietary inorganic sources of Zn revealed that all blood parameters were significantly higher for fish fed diet containing ZnO nanoparticle at lower rate (30 mg/kg) as compare as compared to zinc supplemented diets. It seems that Zn in nanoform was more efficiently absorbed, utilized and showed no negative impact on the absorption and bioavailability of other trace elements (Buentello *et al.,*2009). Present study result are in harmony with Bektas *et al. (*2018) who recorded that higher HCT levels were found at doses of 0.5, 2.5, and 10 g /kg *N. sativa*. The significant hematocrit values in *O. niloticus* fed with 3% *N. sativa* was reported by John *et al.* (2007). We observed significant decrease in WBC count in all groups of fish fed Zn supplemented diet as compare to basal diets like our result, other scientists also reported the decrease in WBC count in magur.

4.5. Enzymological performance

From the table 7, it showed that amylase activity of mrigal is highest in T4 treatment group fed with ZnO nanoparticle @ 15 mg/kg with 1% of black cumin. After blood serum analysis highest amylase activity was recorded (52.00±0.22u/l) in T4 tank and lowest amylase activity was recorded in control group (30.00±0.15u/l). The pattern of enzymological changes was shown treatment wise (figure 19 and 20). There was no significant difference($p > 0.05$) between T2 and T4 and same with T1 and T4 but they showed significant difference among all other groups($p<0.05$). Like amylase, lipase activity was also followed the same trend and the highest activity was recorded in T4 (75.00±0.18u/l) and lowest activity was (45.00±0.15u/l) in control group. It was also cleared from the table, lipase activity had higher value than the amylase activity. Metabolic enzyme (AST) varied as there is no significant variation between control and T1 and also T2 and T3 but T4 showed significant difference ($p>0.05$) with all other treatment groups and highest value recorded was (30.50±0.53u/l) in T4 and lowest activity recorded was 14.00±0.53in control group but in case of ALT, no significant difference($p>0.05$) were observed between T1 and T3 and also T2 and T4 but they varied significantly ($p<0,05$) with other treatments groups and also highest activity recorded was(45.00±0.62u/l) in T4 and lowest activity was (29.00±0.51 u/l) in control group. Relationship of body weight (g) with amylase and lipase enzyme was shown in figure 20. There is very strong relation of body weight change with amylase level (R^2=0.8099) and with lipase (R^2=0.9993). Relationship of ALT and AST with body weight(g) was shown in figure 21. There is medium relation of body weight with ALT (R^2=0.9998) and with AST (R^2=0.9836).

Table 7. Different enzymological performance of *Cirrhinus mrigala.*

Treatment	Amylase(u/l)	Lipase(u/l)	AST/SGOT(u/l)	ALT/SGPT(u/l)
C	30.00±0.15^a	45.00±0.15^a	14.00±0.53^a	29.00±0.51^a
T1	35.00±0.25^b	52.50±0.19^b	15.00±0.41^a	34.00±0.47^b
T2	49.00±0.22^c	70.00±0.22^d	27.00±0.51^b	41.00±0.32^c
T3	39.00±0.21^b	61.00±0.21^c	21.00±0.39^b	35.50±0.77^b
T4	52.00±0.22^c	75.00±0.18^d	30.50±0.53^c	45.00±0.62^c

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

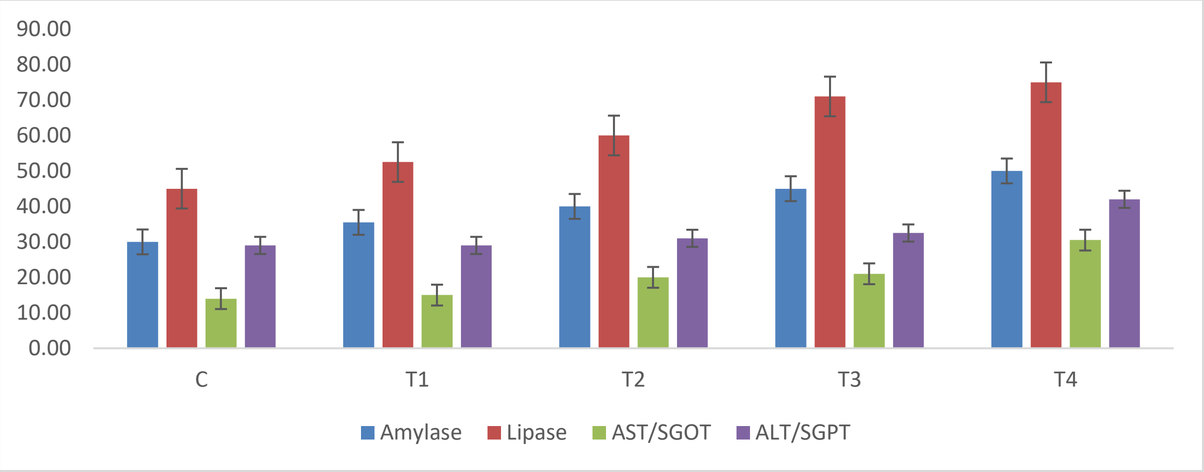

Fig.- 18: Enzymological variations treatment –wise of *Cirrhinus mrigala*

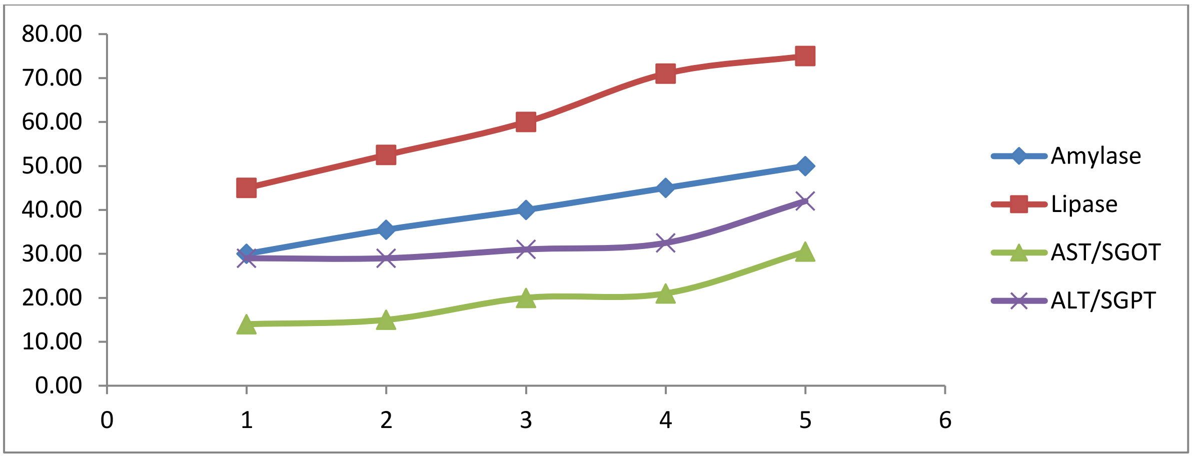

Fig.-19: Enzymological variation treatment –wise of *Cirrhinus mrigala*

Previous research revealed that the digestive enzyme activities changed consistently with the feeding regimes and feed composition (Hu *et al.*, 2014). Previous research indicated that Zn acts a vital function in formation and control of enzyme activity and metabolism of nutrients through their binding to specific receptors and control of their action (Wain, 2016). AST and ALT activity is often used as a diagnostic tool for monitoring and evaluation of hepatic health, and AST and ALT increase is the most reliable marker of liver damage in aquatic animals (Mirghead *et al.*, 2018).

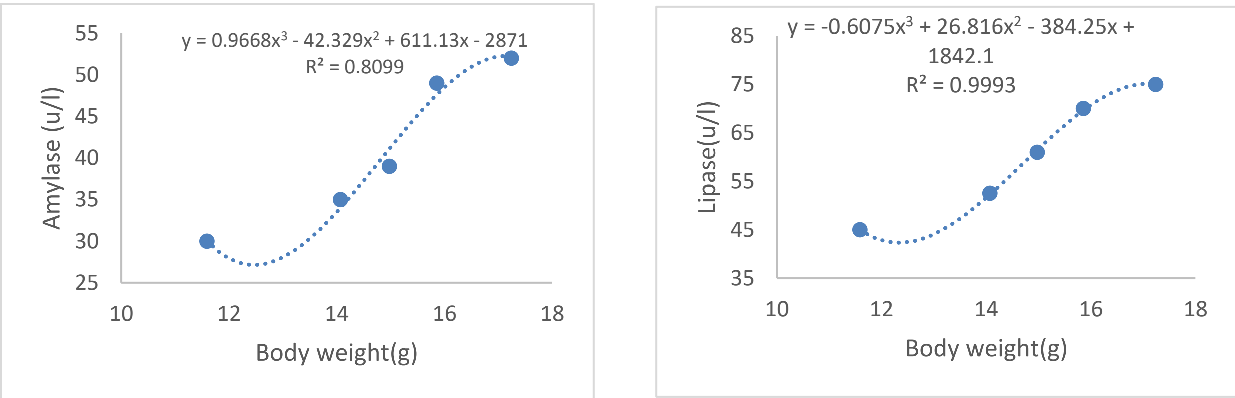

Fig.-20: Relationship of Body weight (g) with amylase and lipase enzyme

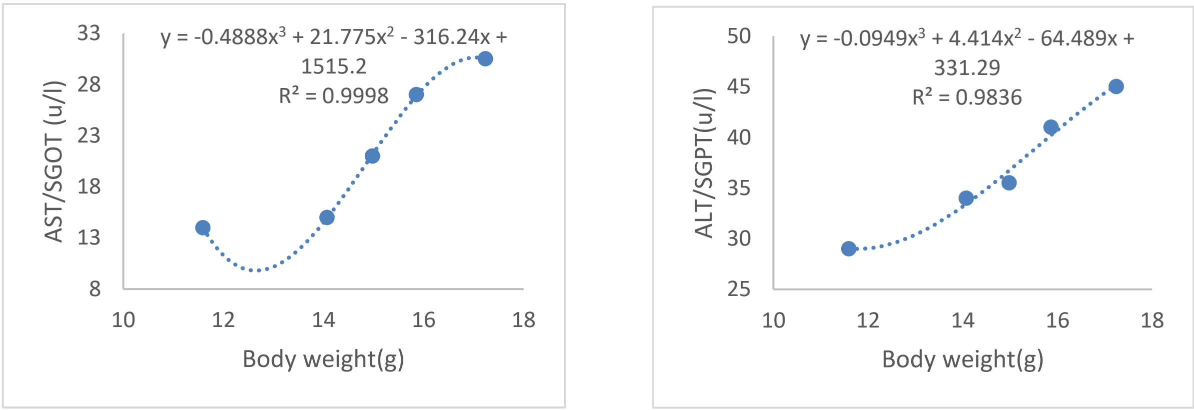

Fig.-21: Relationship of Body weight (g) with AST and ALT enzyme

In the present study, activities of lipase and amylase were higher in T4 and T2 treatments group fed with zinc oxide nanoparticles as compare to other treatments and control. The present results were in parallel with previous study which concluded that inclusion of nano zinc @30 and 60 mg /kg nano-Zinc oxide recorded the higher activity of digestive enzyme in tilapia when compare with control and inorganic ZnO (Ibrahim *et al.*, 2021). The present results could be credited to the function of Zn as a cofactor of many digestive enzymes as amylase and lipase that stimulate their activities (Watanabe *et al.*, 1997).

On the contrary to the present study, decreased AST and ALT levels in serum was reported in rohu fed diets with black cumin seed (Latif *et al.*,2021).The metabolic enzymes AST, ALT varied significantly in a dose-dependent manner in common carp (*Cyprinus carpio*) fed with different levels of black cumin with control fish displaying the highest levels and fish fed with the highest level of black cumin seed (1%) displaying the lowest AST(Yousefi *et al.*, 2021), which is in opposite to present result. As the present research carried out in *Cirhinus mrigala* but it also belongs to carp group so we assumed that they follow the same pattern as well.

CHAPTER 5

SUMMARY AND CONCLUSIONS

5. SUMMARY AND CONCLUSION

Studied on “influence **of supplementing nano zinc and black cumin on hematological parameters and growth performances of *cirrhinus mrigala*** has been carried out in indoor experimental facilities in the laboratory of the Department of Aquaculture, Faculty of fisheries sciences, WBUAFS. The compilation of the study embodied in this thesis consists of 7 chapters viz. Introduction, Review of literature, material methods, Result and discussions, summary and conclusion, Future scope of research and bibliography. The major findings of the work are presented here in:

i. Water quality parameters of the experimental condition measured on 1 st , 28 day and 56 the day of experiment and it did not showed significant difference among treatments ,all parameters have maintained in their optimal condition for the the research work to carried out. Such as temperature of all tanks water did not showed significant difference and it ranges between 26.4°C to 26.85°C , highest pH value was 7.65 and lowest pH value was 7.51, DO was 5.6-5.78 mg/l ,alkalinity was 134.5 – 137.5 mg/l ,hardness was 625-640 mg/l, total ammonia was 0.02- 0.05 mg/l.

ii. The body weight of fish tended to increases over time, however the pattern of growth differed in the test fish among different treatment tanks. The growth rate differed in different weeks of the experiment period. The highest average body weight was (20.88g) in T_4 treatment and the lowest average weight was (15.22g) at control groups. From ANOVA analysis, there was significant difference shown ($p<0.05$).

iii. In the present study, ABWG attained by the experimental fish was the highest weight gain found in T4 (17.24g), and lowest weight gain was recorded in control group (11.59 g) as compared to other treatment groups.

iv. We found that daily weight gain (DWG) shows significant difference ($p<0.05$) among the different treatment groups during 56 days of experimental period T4 having maximum daily weight gain (0.3070g) followed by T2 (0.28g) and then T3 (0.26 g) ,T1 (0.25g) and lowest weight gain value found in control group (11.59g) and we observed that there is an increasing trend of DWG from control to T2 and then it is minimally decrease from T2 to T3 and then increasing growth trend from T3 to T4.

v. Specific growth rate is the most commonly used indices in growth. The SGR value of different treatment group is mentioned in table 2. Showed significant difference ($p<0.05$) among the treatment groups. The highest SGR value was recorded in T4 (3.12 %) and lowest in control (2.56) group. There were no significant differences ($p>0.05$) among treatments group T2, T3 and T4, But there was difference between all these treatments when compare to control and T1.

vi. FCR gives the amount of feed required to produce a unit weight gain of fish. It is calculated from feed intake and weight gain during the experimental period. The FCR of control group of fishes was (1.37), whereas that of the treated group of fishes was vary from (1.28-1.25), indicating superiority of treated groups to control ones. Good FCR was recorded in T4 (1.125) and all FCR values was varied significantly ($p<0.05$) from other treatments groups. The highest FCR was found in control as compare to other treatment groups.

vii. In the present study, survivability was observed on weekly basis up to 8 the week of experimental period. Control group having 100% survivability and T1, T2, T3 and T4 group having survivability of 93.75%, 96.53%, 95.83% and 98.96% respectively. The highest survivability was observed in T4 and lowest survivability in T1 among the treatments groups excluding the control group.

viii. In the present study, RBC, Hb, Platelets, WBC of mrigal showed significant ($p<0.05$) difference among the treatment. It indicated that Hb and RBC showed high value in T4 (11.5) and (3.8) respectively and lowest value showed in Control group (6.3) and (2.1) respectively. Platelets and WBC value showed highest value in control group (0.064) and (3900) respectively and lowest value in T4 groups (0.62) and (2600) respectively. All other treatments groups also showed significantly difference with each other in RBC WBC, Hb and platelets.

ix. It is also indicated that, ESR, PCV, M.C.V, M.C.H, M.C.H.C parameters were not showed significant ($p>0.05$) difference among the treatments groups. Result showed that WBC count is higher in case of black cumin supplemented group with zinc oxide T3 and T4 as compare to non-supplementation of black cumin with zinc oxide group T2.except control.

x. It showed that amylase activity of mrigal is highest in T4 treatment group fed with ZnO nanoparticles @ 15 mg/kg with 1% of black cumin. After blood serum analysis highest amylase activity was recorded (52.00±0.22 u/l) in T4 tank and lowest amylase activity was recorded in control group (30.00±0.15 u/l). There was no significant difference ($p> 0.05$) between T2 and T4 and same with T1 and T4 but they showed significant difference among all other groups ($p<0.05$).

xi. Like amylase, lipase activity was also followed the same trend and the highest activity was recorded in T4 (75.00±0.18 u/l) and lowest activity was (45.00±0.15 u/l) in control group, lipase activity had higher value then the amylase activity.

xii. Metabolic enzyme (AST) varied as there is no significant variation between control and T1 and also T2 and T3 but T4 showed significant difference ($p>0.05$) with all other treatment groups and highest value recorded was (30.50±0.53u/l) in T4 and lowest activity recorded was 14.00±0.53 in control group but in case of ALT ,no significant difference($p>0.05$) were observed between control and T1 and also T1 and T3 but they varied significantly ($p<0,05$) with other treatments groups and also highest activity recorded was(45.00±0.62 u/l) and lowest activity was (29.00±0.51 u/l).

Conclusion

The results of the present study gave a new insight on the use of combining black cumin seed with different zinc forms enhances its properties in fish as natural growth promoter aqua feed of mrigal. The study levels of black cumin seed percentage (1%) and nano zinc concentration (15 mg/kg of feed) is safe and has positive effects on the growth performance, hematology, and enzymological performances of mrigal. It is suggested as a potential candidate to be used as feed additive in intensive fish culturing practices losses and ultimately to enhance the overall fish production. On the other hand we can say that as compare to inorganic zinc (ZnO), nano form is showing the higher improvement in growth of *Cirrhinus mrigala.*

CHAPTER 6

FUTURE SCOPE OF RESEARCH

6. FUTURE SCOPE OF RESEARCH

- 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 recognized the effect of nano-minerals, their mechanism, site of absorption and finally mode of action.
- There can be also scope to carried out further research on pretreatment method to minimize the smell or bitter taste of black cumin seed so that it can be used by fish easily and enhances its performances.
- We can do experiment on black cumin at high cumin percentage as low cumin percentage does not significantly showed changes in the initial period of experiment.

- There can also scope of combining black cumin with nanozinc at different concentration level and also different zinc forms so that we can easily compare its different effect with so we can draw a clear cut conclusion about different concentration of nanozinc and try to find the effect of inorganic zinc with black cumin.

- To find out the effect of black cumin in immunological parameter, disease resistance to certain microorganism, black cumin in proximate composition, antioxidant and histo - Biochemical parameters of experimental fish is also can be done in future to research.

- We can also found in haematological and innate immune parameters of *O. mossambicus* exposed to zinc concentrations, compared to those in the control group. It was also found that zinc accumulation increased significantly in liver, gill and muscle tissue according to the concentrations of the ambient, depending on the duration of exposure

- It can be also used for haematological and innate immune parameters can be used in the follow-up of health status of fish species exposed to Zn concentrations. These parameters can be used to predict the effect of metals such as zinc on fish populations.

CHAPTER 7

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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	(upto 3rd semester)
B. F.Sc	Govind ballabh pant university of Agriculture & Technology pantnagar	2020	**7.313**
Higher Secondary	Central Board of Secondary Education	2015	**79%**
Secondary	Central Board of Secondary Education	2013	**8.0**

EXPERIENTIAL LEARNING (TRAINNING PROGRAM)

- Internship in fish processing industry.
- Internship in shrimp farm.
- Internship in trout farm.
- I worked with my batch mates in the carp projects in ug college.
- Internship in trout farm.

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, Excel.

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

Reading novel, singing, playing, explore new adventure, travelling.

AWARDS/ACHIEVEMENT

- NTS Scholarship in M.F.Sc by ICAR.
- Sports secretary of college and hostel.

PERSONAL DETAILS

- **Father's Name** :- Umapati joshi
- **Permanent Address** :- West khera gaulapar ,kathgodam ,haldwani .Dist. Nainital,uttrakhand pin code -263126.
- **Date of Birth** :- 15 th may 1996
- **Language Known** :- English& Hindi,kumauni
- **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
Date:02.02.2023 **(Signature)**