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Physiological Transformation in Zebrafish (*Danio rerio*) *via* Silver Nanoparticle Diets: Growth and Health Assessment

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ABSTRACT

Silver nanoparticles were synthesized by the co-precipitation method and characterized using UV-Visible spectroscopy, FESEM, EDAX, FT-IR, and Zeta potential analysis. 1,2,3,4 and 5mg of chemically synthesized nanoparticles were incorporated into fish meal, groundnut oil cake, wheat flour, and tapioca flour. Feed utilization and growth parameters of Zebrafish, such as Feed Consumption, Feed Conversion Ratio, Feed Conversion Efficiency, Growth, Assimilation, Metabolism, Gross Growth Efficiency, and Net Growth Efficiency, and Haematological parameters of zebrafish, such as total RBC, Hb, Hct, WBC, and total platelets, were estimated. Biochemical parameters including protein, carbohydrate and lipid were evaluated to determine physiological and metabolic responses. Digestive enzyme activities, including amylase, protease, and lipase, were analyzed to assess digestion efficiency after a 21-day experimental study. The UV-Visible absorption spectra were measured in the wavelength range between 300 to 700nm and exhibited a strong absorption peak at 423nm. FESEM analysis revealed predominantly spherical nanoparticles. The EDAX spectrum showed two peaks, located between 0.2 keV and 3KeV. FT-IR analysis in the wavelength range from 500-4000cm^{-1} identified functional groups responsible for the reduction and stabilization of the nanoparticles. XRD analysis showed distinct diffraction peaks at 23.98°, 28.62°, 37.89°,46.49°, 64.66°, and 77.57°, confirming the crystalline nature, phase purity, and high crystallinity of the synthesized AgNPs. Zeta potential analysis revealed a surface charge of -32.1 mV, indicating excellent colloidal stability. All the feed utilization parameters were higher in feed IV. All the haematological, biochemical, and digestive enzymes were enhanced at Feed IV (3mg) supplementation levels.

Keywords: Co-Precipitation; Incorporated feed; Haematology; Biochemical; Digestive enzyme.

1. INTRODUCTION

Nanotechnology has emerged as an innovative approach for improving aquaculture production by enhancing feed quality, nutrient delivery, disease resistance, and overall fish health. (Thangapandiyan and Monika, 2020). With sizes ranging from 1to100 nanometers, nanoparticles (NPs) exhibit distinctive physical and chemical properties, including a high surface area to volume ratio and enhanced bioavailability. Their adaptable structural features enable researchers to tailor NPs for diverse aquaculture applications, resulting in notable improvements in feed utilization, fish growth, health, and disease resistance (Ahmed *et al.* 2024). Owing to their unique optical, mechanical, and electrical properties, nanomaterials continue to be extensively investigated as functional feed additives in aquaculture nutrition. Among the various nanomaterials, silver nanoparticles (AgNPs) have gained considerable interest because of their antimicrobial activity, stability and potential to improve feed quality and fish health. When incorporated into aquafeeds at appropriate concentrations, AgNPs enhance feed utilization, physiological performance, and disease resistance. Zebrafish (*Danio rerio*) is a well-established experimental model in aquatic biology because of its rapid growth, high fecundity, transparent embryos, and well-characterized physiology. Initially used in genetics and developmental biology, they now serve as models in various fields, including toxicology, neuroscience, and regenerative medicine. Their exponential growth in research utility highlights their significance in advancing scientific understanding across disciplines (Handy *et al.* 2008). Their growth and health are strongly influenced by nutrition, making them an ideal model for assessing the effects of nanoparticle-supplemented diets. Although several studies have investigated the synthesis and biological applications of silver nanoparticles, limited information is available regarding the effects of graded dietary levels of chemically synthesized AgNPs. The present study was undertaken to synthesize silver nanoparticles, incorporate different concentrations into experimental diets, and evaluate their effects on feed utilization, growth performance, haematological indices, biochemical composition, and digestive enzyme activities in zebrafish, with the aim of identifying the

most effective dietary supplementation level. (Dube, 2024). The novelty of the present study lies in the comprehensive evaluation of the dose-dependent effects of chemically synthesized AgNP-enriched diets on growth, feed utilization, hematological status, biochemical composition, and digestive activities in zebrafish, with the aim of identifying an effective dietary supplementation level for potential application in aquaculture nutrition.

2. MATERIALS AND METHODS

2.1 Materials

Silver nitrate ($AgNO_3$), Trisodium citrate ($Na_3C_6H_5O_7$), and sodium hydroxide (NaOH) were collected from the Department of Biology, The Gandhigram Rural Institute, Deemed to be University, Gandhigram, India. All these chemicals used for the synthesis of silver nanoparticles were analytical grade. All the glassware was washed with deionized water and dried before use.

2.1.1 Synthesis of Silver Nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) were synthesized by the co-precipitation method using silver nitrate ($AgNO_3$) as the metal precursor and trisodium citrate ($Na_3C_6H_5O_7$) as both the reducing and stabilizing agent. 1% Trisodium citrate was prepared by taking 1g in a 100 mL standard flask and making up to volume with deionized water. AgNPs were prepared by adding 20 mL of Trisodium citrate solution to 100 mL of 1 mM AgNO3 solution, kept under agitation on a magnetic stirrer at a temperature of about 80°C and gently stirred for an hour. The solution gradually changed from colourless to yellowish-brown, indicating the formation of silver nanoparticles. The reaction was centrifuged for 10 min at 9000 rpm; the pellet was washed three times with distilled water, followed by a wash with ethanol to remove residual reactants and impurities. The purified AgNPs were dried in a hot air oven at 80°C for 8 hours to obtain dry nanoparticle powder. The dried AgNPs were stored in airtight glass vials at room temperature until further characterization and feed preparation.

2.2 Characterization of Silver Nanoparticles

Nanoparticles were systematically characterized to assess their optical, structural, morphological, elemental chemical, and surface charge properties using dried nanoparticle powder. The optical properties were first investigated by UV–visible spectroscopy (Thermo Scientific GENESYS 180), where AgNPs dispersed in deionized water by ultrasonication exhibited a characteristic surface plasmon resonance (SPR) peak within the 300-700nm range, confirming the nanoparticle formation. The surface morphology of silver nanoparticles (AgNPs) was examined using scanning electron microscopy (SEM, CARL ZEISS EVO 18, Germany). For imaging, the dried powder was mounted on aluminum stubs with conductive carbon tape, sputter-coated, and observed at 15KV to evaluate particle shape and surface details. Elemental composition was confirmed by energy-dispersive X-ray spectroscopy (EDAX, BRUKER Flash 6130) attached to the FESEM, confirming silver presence and detecting associated elements. Surface functional groups bound to AgNPs were identified using Fourier-transform infrared spectroscopy (FT–IR, Jasco FT/IR-4700), with spectra recorded between 4000–400 cm^{-1} at 4 cm^{-1} resolution to reveal stabilizing groups. Crystallinity, phase purity, and structural parameters of silver nanoparticles were determined by X-ray diffraction (XRD, BRUKER D8 ADVANCE, Cu K_α radiation). Diffraction patterns were compared with JCPDS Card No. 04-0783 reference data, and crystallite size was calculated using Scherrer's equation based on lattice plane reflections. Finally, colloidal stability and surface charge of AgNPs were assessed by zeta potential analysis (HORIBA SZ-100). In this electrophoretic method, nanoparticle powders were dispersed in distilled water with NaCl electrolyte, adjusted to pH 7, and shaken before measurement. The resulting zeta potential values, expressed in millivolts, reflected particle mobility under an applied electric field and provided a measure of suspension stability (Fernandez Montesinos *et al.* 2009).

2.3 Collection and Acclimation of *Danio rerio*

For the present work, healthy zebrafish (*Danio rerio*) with an average body weight of (1±0.05g) were collected from aquagardens, Kadachananthal, Madurai, Tamil Nadu, India. The fish were visually inspected to ensure they were free of external lesions, deformities, and signs of disease before being transported to the laboratory in oxygen-filled polythene bags. All the experimental procedures involving animals were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC). The Gandhigram Rural Institute (Deemed to be a university), Gandhigram, India (Approval No: 001/2026). The fish were acclimated for 15 days in concrete tanks before the feeding trial. During acclimation, the fish were fed a basal diet prepared from fish meal, groundnut oil cake, wheat flour, and rice bran twice daily to apparent satiation.

2.3.1 Physico-chemical parameters of experimental water

Table 1. Physico-chemical parameters of experimental water

S. No.	Parameter	Analytical Method	Reference
1	pH	pH (Electrometric method)	APHA 2012
2	Temperature	Thermometer (Direct measurement)	,,

S. No.	Parameter	Analytical Method	Reference
3	Dissolved oxygen	Winkler's iodometric titration method	"
4	Dissolved Carbon dioxide	Acid–base titration method	"
5	Total Alkalinity	Acid–base titration method	"
6	Total chloride	Mohr's argentometric titration method	"
7	Total Hardness	EDTA complexometric titration method	"

2.4 Acute Toxicity Test

Before initiating the feeding experiment, an acute toxicity study was conducted using chemically synthesized AgNPs at concentrations ranging from 1to10 mg/L to evaluate the toxicity profile and identify an appropriate concentration. Dose-dependent effects were investigated, and the dietary supplementation level associated with the most favorable biological responses in zebrafish was identified. An acute toxicity test is a short-term bioassay employed to assess the immediate effects of a substance on test organisms, generally within an exposure period of 24 to 96 hours. In fish, such tests are commonly performed to determine the median lethal concentration (LC_{50}), which represents the concentration of a chemical or compound capable of causing mortality in 50% of the exposed organisms under controlled experimental conditions. This test provides important information regarding the toxic potential of the compound and serves as a basis for further sub-lethal and chronic toxicity studies. During the experiment, mortality, behavioral alterations, and physical responses of the fish were monitored at regular intervals, while the test water remained unchanged throughout the exposure period. The required quantities of chemically synthesized silver nanoparticles (Ch-AgNPs) were dispersed separately in distilled water using an ultrasonic bath sonicator and sonicated at 40 kHz for 30 minutes to obtain homogeneous stock suspensions. Subsequently, different concentrations of the prepared test solutions, namely 0 (control), 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg/L of Ch-AgNPs, were introduced into experimental tanks. Ten uniformly sized *Danio rerio* (1.5 ± 0.5g) were introduced into each concentration of chemically synthesized silver ranging from 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg. The experiment was conducted in triplicate. Dead fish were removed immediately to avoid contamination of the test medium. Based on visual observations of concentrations that produced no mortality, at least five experimental concentrations, including control, sub-optimal, requirement range, above requirement, and maximum limit, were selected for the growth study in accordance with the OECD guidelines for the testing of chemicals (2000). The acute toxicity study showed no mortality and visible adverse effects at AgNp concentrations upto 10mg/L.

2.5 Selection of Feed Ingredients and Experimental Feed Preparation

Feed ingredients were selected based on nutrient content, with protein levels determined by the Micro-Kjeldahl method and ration formulated using Pearson's Square (Aguirre, 2023). Fishmeal (anchovy) and groundnut oilcake served as protein sources; wheat and tapioca flour as carbohydrate sources; sunflower and fish oil as lipid sources and binders; Supplevite mix (Virbac Chelated Agrimin® Forte) as vitamins and minerals; and sodium chloride and sodium benzoate as preservatives. All components were dried, powdered, and sieved (425 µm). Major ingredients were mixed with 130–150 mL distilled water, autoclaved at 121 °C (15 psi, 30 min), and cooled. Minor ingredients, including oils, supplements, and silver nanoparticles, were added at levels of 1,2,3,4 and 5mg per 100g of feed. The mixture was mechanically blended until a homogeneous dough was obtained to promote uniform incorporation of the nanoparticles throughout the feed. The dough was then pelletized into pellets (1mm die) using a pelletizer suitable for zebrafish, dried at room temperature, and stored in airtight containers at room temperature.

2.5.1 Design for Growth Studies

For the present study, a uniform size of zebrafish, Danio rerio (1.34±0.54g), was selected, and then ten fish were introduced into each rectangular glass tank (45cm L × 22 cm B × 22 cm H) having a capacity of 18 liters. For each treatment, triplicate samples were maintained. During rearing, the fish were fed an ad libitum diet of prepared feed twice a day for 1 hour each at 9-10 am and 4-5 pm. The unfed were collected after one hour of feeding without disturbing the fish. The unfed were dried to constant weight. The fecal matter was collected daily before changing the water with the least disturbance to the fish and dried at 95˚C in a hot air oven. Approximately 70% of the tank's water was replaced with tap water. The experiment was continued for 21 days. On the 21st day, the length and weight of the fish were measured in vivo to calculate growth parameters.

2.6 Growth Performance of *Danio rerio*

2.6.1 Survival Rate (SR)

The survival rate of the fish was determined in order to evaluate mortality. This parameter was expressed as the percentage of fish that remained alive compared to the initial number of individuals stocked in each tank at the beginning of the trial.

$$\text{SR}\ (\%) = \frac{\text{Initial number of fish}}{\text{Number of surviving fish}} \text{X}100$$

2.6.2 Condition Factor (K)

The condition factor (K) is widely recognized as an index for evaluating the overall health, vitality, and robustness of fish. It is derived from the relationship between body weight and length, thereby serving as an indicator of growth performance and providing valuable insights into the nutritional status of the species.

$$\text{Condition factor (K)} = \frac{\text{w(g)}}{\text{L(cm)3}}$$

2.6.3 Feed Consumption (FC)

Feed consumption was estimated by subtracting the dry weight of the uneaten feed from the initial dry weight of feed supplied. The result was expressed as the quantity of dry feed consumed per gram of live fish over the 28-day experimental period.

$$\text{Feed Consumption (FC)} = \frac{\text{Feed given} - \text{unfed}}{\text{Number of fish}}$$

2.6.4 Feed Conversion Efficiency (FCE)

Feed Conversion Efficiency (FCE) is an index that evaluates how effectively fish are able to transform the ingested feed into body mass. It is calculated as the reciprocal of the Feed Conversion Ratio (FCR) and expressed in percentage terms. Higher FCE values are indicative of improved feed utilization and enhanced growth performance in the fish.

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{W1} - \text{W2}}{\text{Feed COnsumed}} \text{X100}$$

2.6.5 Feed Conversion Ratio (FCR)

The feed conversion ratio (FCR) was determined by calculating the total feed intake in relation to the increase in wet body weight of the fish. The gain in wet weight was measured as the difference between the initial weight recorded at the start of the trial and the final weight obtained at the end of the experiment.

$$\text{Feed conversion Ratio (FCR)} = \frac{\text{W1} - \text{W2}}{\text{Feed COnsumed}} \text{X100}$$

2.6.6 Growth

Growth is calculated by subtracting the initial wet weight from the final wet weight.

$$\text{Growth} = W_1 - W_2$$

2.6.7 Percentage Growth (PG)

Percentage growth expresses the daily increase in fish weight relative to their body mass. It serves as a standard measure of growth efficiency over a set period. Higher PR values reflect quicker weight gain and stronger overall performance.

$$\text{PG (\%)} = \frac{\text{Growth}}{\text{W0}} \text{X100}$$

2.6.8 Specific Growth Rate (SGR)

Specific Growth Rate is calculated by the formula given below.

$$\text{Specific Growth Rate} = \frac{\text{Growth}}{\text{Experimental Period (Days)}} \text{X100}$$

2.6.9 Gross Growth Efficiency (GGE) (%)

Gross Growth Efficiency (GGE) was determined as the percentage of feed converted into body mass related to the total feed consumed, calculated using the following equation.

$$\text{Gross Growth Efficiency (GGE) (\%)} = \frac{\text{Growth}}{\text{Feed COnsumed}} \text{X100}$$

2.6.10 Assimilation (A)

Assimilation (A) is calculated by subtracting the fecal matter from feed consumption.

Assimilation (A) = Feed consumed - Faecal matter

2.6.11 Metabolism (M)

Metabolism (M) is calculated by subtracting the growth rate from assimilation (A).

Metabolism (M) = Assimilation – Growth rate.

2.7 Haematology Parameters

Haematological indices are key indicators of fish health, stress, and physiological status in aquaculture. In this study, blood was sampled from five fish per tank after anesthesia with clove oil (50 µl/L). Samples were collected from the caudal vein and divided into EDTA vials for hematology and plain vials for serum preparation. Serum was separated by tilting, centrifugation (2000 rpm, 15 min, 4°C), and stored at –20°C. Analyses covered hemoglobin (Hb), red and white blood cell counts (RBC, WBC), hematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

2.7.1 Haemoglobin (Hb)

The oxygen transport pigment in RBCs is measured using a haemoglobinometer by acid dilution and color comparison.

$$\text{Hemoglobin concentration (gm/dl)} = \frac{\text{OD of absorbance test}}{\text{OD ofabsorbance standard}} \text{X16.31}$$

2.7.2 Red Blood Cells (RBC)

Nucleated fish erythrocytes counted via haemocytometer.

$$\text{No.of RBC (million/cu.mm of blood)} = \frac{\text{No of RBCX Dilution factor}}{\text{Area counted X Depth of } fluid}$$

2.7.3 White Blood Cells (WBC)

Immune cells counted in haemocytometer corner squares.

$$\text{No. of leucocytes (1000/cu.mm of blood)} = \frac{\text{No of leucocytesX Dilution factor}}{\text{Area counted X Depth of } fluid}$$

2.7.4 Haematocrit (PVC)

Ratio of packed RBC column to total blood column using microhaematocrit centrifugation.

$$\text{PCV}(\%) = \frac{\text{Height of Packed RBC column}}{\text{Total Height Blood Column}} \text{X100}$$

2.8 Biochemical Parameters

Biochemical markers in fish blood and tissues serve as reliable indicators of metabolic activity, organ function, and adaptive responses to diet or environmental stress. In this study, gill, muscle, and liver tissues were dissected, rinsed with 80% saline, weighed, and stored at –20 °C for analysis. These parameters are widely used in aquaculture nutrition, toxicology, and health monitoring to detect sublethal effects of pollutants, feed additives, and therapeutic agents.

2.8.1 Total Protein (Lowry Method)

Tissue homogenates were prepared in Tris buffer, centrifuged, and supernatants reacted with alkaline copper solution and Folin reagent. The resulting blue complex was measured at 660 nm (Lowry *et al.* 1951).

$$\text{Total protein} = \frac{\text{OD of the sample}}{\text{OD of the standard X concentration of standard}} \text{X100}$$

2.8.2 Total Carbohydrate (Anthrone Method)

Tissue extracts were treated with anthrone reagent in concentrated H_2SO_4, heated, and cooled to form a green complex measurable at 620 nm. Glucose standards were used for calibration (Carroll *et al.* 1956)

$$\text{Total carbohydrate} = \frac{\text{OD of the sample}}{\text{OD of the standard X concentration of standard}} \text{X100}$$

2.8.3 Total Lipid (Folch Method)

Lipids were extracted from tissues using chloroform–methanol, followed by phase separation. The chloroform layer containing lipids was evaporated, and the residue was weighed (Folch *et al.* 1957).

2.9 Digestive Enzyme

Digestive enzyme assays provide insight into nutrient digestion and absorption efficiency in fish. Key enzymes include protease (protein hydrolysis), amylase (starch breakdown), and lipase (fat digestion). Enzyme activity is measured from intestinal or hepatopancreatic extracts and expressed in units per milligram of protein.

2.9.1 Protease

Intestinal extracts incubated with BSA substrate; undigested protein precipitated with TCA, followed by ninhydrin color reaction at 570 nm. Activity expressed as glycine released per mg protein (Anson *et al.* 1938).

$$\text{Protease activity(U/ml)} = \frac{\text{OD of assay X total volume of assay}}{\text{Volume of enzyme x volume used in colorimetric determination}}$$

2.9.2 Amylase

Extracts incubated with soluble starch reaction stopped with DNSA reagent, boiled, and absorbance measured at 540 nm. Activity expressed as maltose produced per mg protein (Bernfeld *et al.* 1955).

$$\text{Amylaseactivity(U/ml)} = \frac{\text{OD of assay X total volume of assay}}{\text{Volume of enzyme x volume used in colorimetric determination}}$$

2.9.3 Lipase

Extracts incubated with olive oil emulsion and $CaCl_2$; reaction stopped with acetone–ethanol; fatty acids quantified by titration with NaOH using phenolphthalein indicator. Activity expressed as fatty acids released per mg protein (Bier *et al.* 1955).

$$\text{Lipase activity (U/ml)} = \frac{\text{Volume of NaOH consumed X 0.1 (normality of NaOH)}}{\text{Volume of sample}}$$

3. STATISTICAL ANALYSIS

Data analysis was carried out using IBM SPSS (Version 20). Parameter values for fish-exposed groups were expressed as mean ± SD from triplicate measurements at each concentration. One-way ANOVA followed by Duncan's multiple range test was applied to assess differences between treatments and controls, with statistical significance set at $P < 0.05$. Growth parameters of *Danio rerio* were analyzed through one-way ANOVA, and Duncan's test was used to identify significant variations among means at the 5% probability level.

4. RESULTS AND DISCUSSION

4.1 Characterization of Silver Nanoparticles

4.1.1 UV-Visible Absorption Spectroscopy

UV-Visible absorption spectroscopy is a widely used technique for examining the optical properties of nanosized particles. The absorbance spectra of silver nanoparticles were measured at wavelengths between 300-500nm. It exhibits a strong absorption Surface plasmon resonance (SPR) at 423nm, as shown in Fig. 1a. A similar peak at 423nm for silver nanoparticles was also

reported. The appearance of this characteristic peak is attributed to Ag^{+} ions on metallic silver nanoparticles (Ag^{0}). The relatively sharp and well-defined absorption band indicated the formation of nanoparticles with a fairly uniform size distribution and minimal aggregation. The absorption peak of green-synthesized silver nanoparticles was at 420 nm. A sharp peak of absorbance at 345 nm, which indicates an almost uniform size of Ag nanoparticles (Cui *et al.* 2023). The UV-Vis spectra of AgNPs synthesized using Silver nitrate and Trisodium were observed at 380nm. The maximum absorption wavelength of the synthesized AgNPs was found to be 430 nm (Ali *et al.* 2023)

4.1.2 Field Emission Scanning Electron Microscopy (FESEM)

The morphology of the synthesized AgNPs was examined using FESEM. The image revealed predominantly spherical nanoparticles with slight agglomeration, a common phenomenon due to high surface energy and attractive forces between nanoparticles. Although clustered, the particles retained distinct boundaries, indicating that individual nanoparticles were successfully formed. (Fig 2). The FESEM image of AgNPs synthesized by silver nitrate was spherical and similar to the above, whereas the FESEM image of AgNPs was spherical. (Khan *et al.* 2011).

4.1.3 Energy Dispersive X-ray Spectroscopy (EDAX)

The elemental composition of the synthesized silver nanoparticles was determined using EDAX analysis. The EDAX spectrum of the AgNPs shows three peaks at 0.3 keV, which correspond to the characteristic emission of elemental silver and confirm the presence of metallic Ag (Fig 3) (Khan *et al.* 2011). Minor peaks corresponding to carbon (C) and oxygen (O) were also detected. These elements are attributed to surface-bound organic molecules, atmospheric adsorption, or the carbon-coated sample holder used during analysis. (Yerragopu *et al.* 2020). The EDAX spectrum of the silver nanoparticles shows two peaks between 2 and 4 keV (Gudikandula and Charya, 2016).

4.1.4 Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR spectroscopy was performed over the range of 4000-500cm^{-1} to identify the functional groups associated with the synthesized AgNPs (Fig 4). The broad absorption band at 3357 cm^{-1} corresponds to O-H/N-H stretching vibrations, indicating hydroxyl or amine groups. The peak at 1735cm^{-1} is assigned to C=O stretching of carbonyl groups, while the band at 1513cm^{-1} represents C=C stretching of aromatic compounds. The absorption at 1379cm^{-1} corresponds to C-N stretching or O-H bending vibrations. The peak at 1097cm^{-1} is attributed to C-O stretching of alcohols or ethers, and the band at 841cm^{-1} represents aromatic C-H bending vibrations, indicating the presence of capping agents during AgNPs synthesis (Dasaradhudu *et al.* 2020).

4.1.5 X-ray Diffraction (XRD)

The XRD technique is used for structure and phase analysis of all compounds. The XRD diffraction peaks of silver nanoparticles are indexed at 23.98°, 28.62°, 37.89°,46.49°, 64.66°, and 77.57° (2θ), which are represented in Fig. 5. The clear and sharp diffraction peaks confirmed that the prepared compounds are pure with a high degree of crystallinity. The characteristic diffraction peaks correspond to the crystalline planes of metallic silver, while the narrow peak widths indicate good crystallinity and successful formation of crystalline AgNPs. No significant impurity peaks were detected, suggesting the synthesized nanoparticles are predominantly composed of crystalline silver (Zamana *et al.* 2022).

4.1.6 Zeta Potential Analysis

The surface charge and colloidal stability of the synthesized AgNPs were evaluated by zeta potential analysis (Fig.6). The nanoparticles exhibited a potential of −32.1 mV, confirming strong colloidal stability with low aggregation. Multiple charge populations (−14.6, −33.4, −55.9 mV) indicated heterogeneous fractions, while biomolecule capping contributed to the negative surface charge, which is attributed to adsorbed oxygen-containing functional groups identified by FTIR, which act as capping agents and stabilize the nanoparticles (La spina *et al.* 2020).

Table 2. Physico-chemical parameters of experimental water

S. No.	Parameter	Result	Acceptable Limit
1	pH	6.6 ±0.1	6.5-8.5
2	Temperature	26± 1.03	15-35
3	Dissolved oxygen	7.12± 0.1	≥5
4	Dissolved Carbon dioxide	0	≤5
5	Total Alkalinity	20± 0.1	5-5000
6	Total chloride	82.7± 0.1	4-160
7	Total Hardness	290 ±0.3	≥20

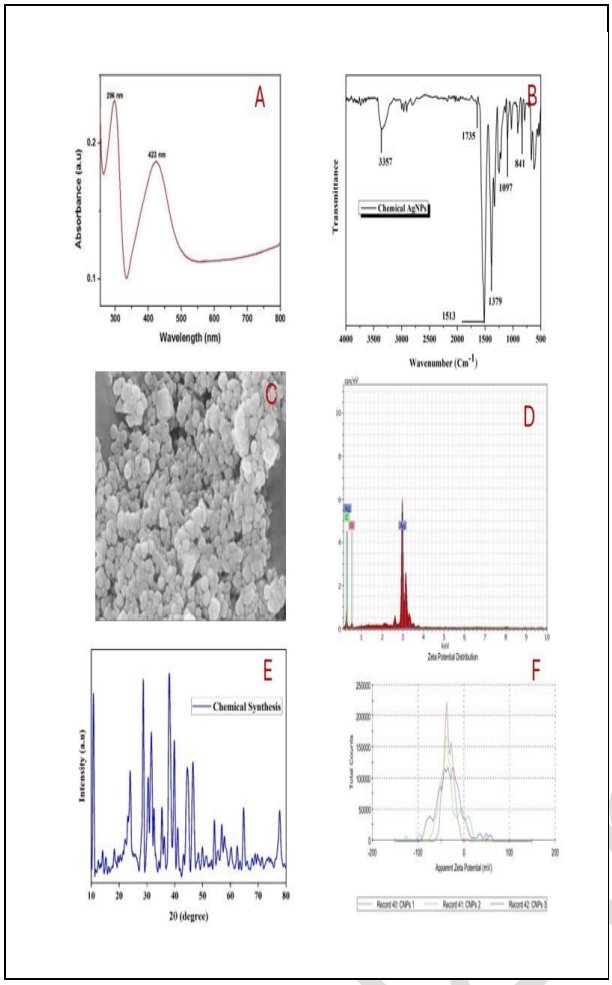

Fig. 1: (A) UV-Visible absorption spectrum of silver nanoparticles (B) FESEM image of silver nanoparticles (C) EDAX spectrum of silver nanoparticles (D) FT-IR spectrum of silver nanoparticles (E) X-ray diffraction (XRD) of silver nanoparticles and (F) Zeta potential analysis of silver nanoparticles

5. CONDITION FACTOR

The Condition factor (K) of zebrafish was estimated for comparative purposes to assess the feed. The final condition factor of zebrafish was increased in all the feed supplements with silver nanoparticles (Table 1). The final condition factor of koi carp increased in CuO nanoparticle-incorporated feed. The condition factor of Freshwater prawn Macrobrachium rosenbergii post-larvae increased when fed with 40g/ kg^{-1} of copper nanoparticles in the feed.

Table 3. Condition factor (K) of zebrafish

Feeds	Initial	Final
I	0.77±0.001	0.8±0.02
II	0.8±0.026	0.87±0.0004
III	0.6±0.032	0.67±0.001
IV	0.5±0.057	0.80±0.0072
V	0.7±0.05	0.88±0.001
VI	0.8±0.0052	0.83±0.002

5.1 Feed Utilization and Growth Parameters

The different feed utilization and growth parameters are presented in Table 2. The ANOVA (Analysis of Variance) of growth parameters (Feed consumption, Growth, Gross Growth Efficiency, Net Growth Efficiency) is presented in Table 3. Zebrafish feed consumption was higher with feed IV. The feed consumption of Mrigal was higher in feed IV, which contained 15 mg of zinc oxide nanoparticles (Dawadi *et al.* 2021). The feed conversion efficiency of Zebrafish was higher in feed IV. A low feed conversion ratio was observed in a diet supplemented with 60mg ZnONPs in Macrobrachium rosenbergii. Among the silver nanoparticle-incorporated feeds, growth and percentage growth were higher in feed IV. The growth performance metrics of L. rohita administered different dietary amounts of AgNPs demonstrate that the final body weight in AgNPs-treated fish was considerably greater ($P<0.05$) than in control fish (Thirunavukkarasu *et al.* 2019). Also reported that the growth of L. rohita is higher in T3, containing 10 µg/kg of Ag NPs-incorporated feed (Rajan and Jenifer, 2024). Higher growth of Penaeus vannamei juveniles fed diets supplemented with 100 mg/kg of silver nanoparticles is also reported (Hedayati *et al.* 2019). The prawns fed with a diet supplemented with 3–18 mg MnO NPs/kg showed enhanced growth performance, including final weight and weight gain (WG), and also showed significant differences in feed conversion ratio (FCR) in prawns fed with different diets (Caloudova *et al.* 2018). The assimilation and metabolism of the Zebra fish were higher in feed IV. Gross growth efficiency and the net growth efficiency of Zebra fish were also higher in feed IV. The assimilation, metabolism, gross and net growth of Mrigal were higher in feed V containing iron oxide nanoparticles (Kaleeswaran *et al.* 2024). Although Feed V and Feed VI also supported normal growth, no additional improvement beyond Feed IV was observed. Physiological benefits of dietary AnNPs reached an optimum level at 3mg per 100g feed, beyond which further supplementation did not produce proportional increases in growth. Once the nutritional and physiological requirements of the fish were adequately supported, additional AgNP supplementation offered further enhancement in growth performance (Asaikkutti *et al.* 2016).

6. HAEMATOLOGICL PARAMETERS

Haematological parameters of zebrafish are presented in Table 4. Haematological parameters are beneficial in assessing the health condition of fish species. The WBC count of zebrafish gradually decreases

as the quantity of AgNPs increases from feed I to feed VI. Platelet count decreases as silver nanoparticle concentration in the feed increases. The RBC, haemoglobin, MCV, and Hct values showed a significant ($P < 0.05$) decrease in the level on exposure of Oreochromis mossambicus to different concentrations of silver nanoparticles (Kaushik *et al.* 2016). The haematological parameters such as haematocrit, Hb, RBC, and WBC are used to assess the functional status of the oxygen-carrying capacity of the bloodstream and have been used as indicators of metal pollution in the aquatic environment (Muthuswami and Rajan, 2021). In the present study, the haematological parameters significantly decreased as the quantity of silver nanoparticles in the feed increased. The haematological parameters of Zebrafish decreased with increasing amounts of silver nanoparticles. (Muralisankar *et al.* 2014).

Table 4. Feed utilization and growth parameters of zebrafish concerning different quantities of silver nanoparticles. (Each value is the average (±SD) performance of five individuals in triplicate reared for 21 days)

Parameters	Experimental			Feed		
	Feed I (Control)	Feed II (1mg)	Feed III (2mg)	Feed IV (3mg)	Feed V (4mg)	Feed VI (5mg)
Feed consumption (FC) (g/g live wt/21 Days	1.6±0.5	14.9±1.8	13.5±1.3	12.9±0.4	11.5±0.80	8.3±0.5
Feed conversion Efficiency (FCE)	4.3±1.5	4.9±0.3	2.5±0.2	2.2±0.4	1.9±0.3	1.6±0.08
Feed conversion Ratio (FCR)	1.1±0.1	12.94±1.7	10±0.7	9.1±0.6	6.8±0.3	5.9±1.6
Growth (G)	22.4±1.4	2.0±0.3	1.7±0.5	1.2±0.5	1.2±0.5	1.2±0.2
Percentage Growth (PG)	1.3±0.1	40.0±0.5	35.0±1.1	25.0±1.1	25.0±0.2	20±0.5
Assimilation (A) (g/g live wt/21 days)	1.3±0.1	3.3±0.28	3±0.2	2.3±0.2	1.9±0.05	1.5±0.1
Metabolism (M) (g/g live wt/21days)	0.3±0.2	1.3±0.2	1.2±0.2	1.1±0.05	0.7±0.1	0.4±0.05
Gross Growth Efficiency (GGE) (%)	7.4±1.4	18.0±0.5	13.0±0.9	12.2±0.17	10.6±0.5	8.0±0.1
Net growth Efficiency (NGE) (%)	94±0.9	61±1.5	61±1.0	56±0.8	52±1.1	41±1.0

Table 5. ANOVA (analysis of variance) of growth parameters (feed consumption, growth, gross growth efficiency, net growth efficiency) of Zebra fish (*Danio rerio*)

S. No.	Parameters	Source of Variation	Sum of Squares	DF	Mean Squares	F	SIG
1	Feed consumption	Between Groups	115.224	5	23.045	17.451	0.005 S
		Within Groups	15.847	12	1.321		
		Total	131.071	17			
2	Growth	Between Groups	1.961	5	0.392	26.221	0.005 S
		Within Groups	0.179	12	0.015		
		Total	2.140	17			
3	Gross Growth Efficiency	Between Groups	246.61	5	49.292	82.832	0.005 S
		Within Groups	7.141	12	0.595		
		Total	253.602	17			
4	Net Growth Efficiency	Between Groups	4706.667	5	941.333	736.969	0.005 S
		Within Groups	15.333	12	1.278		
		Total	4722.000	17			

7. DIGESTIVE ENZYME ANALYSIS

Table 6. Haematological parameters of zebrafish

Blood Parameters	Feed I	Feed II	Feed III	Feed IV	Feed V	Feed VI
RBC (millions/cumm)	0.34	0.3	0.2	0.2	0.14	0.09
Hemoglobin (gm/dl)	1.01	1.0	0.8	0.7	0.6	0.4
Haematocrit (PCV) (%)	2.81	2.4	1.8	1.5	1.3	0.8
WBC count (Cells/cumm)	16,100	16,200	12,400	10,000	7,000	5,100
Platelet count (Lakhs/cumm)	1.28	1.29	99,000	79,000	55,000	47,000

Amylase activity showed a steady dose-dependent increase, progressing from 1.88 in F_0 to 3.99 in F_5, with the highest treatment group achieving more

than a two-fold rise compared to the control, indicating improved carbohydrate breakdown. Lipase activity also showed a pronounced elevation, rising from 89.7 U/L in F_0 to 321 U/L in F_5, indicating enhanced lipid digestion and metabolic efficiency. (Khorshidi *et al.* 2023). Collectively, these findings suggest that silver nanoparticle supplementation stimulates digestive enzyme production and activity, thereby promoting nutrient assimilation and feed utilization in zebrafish.

8. BIOCHEMICAL ANALYSIS

Danio rerio fed AgNPs-incorporated diets showed significantly elevated biochemical levels in muscle, gill, and liver tissues. The F_3 feed (3 mg AgNPs) produced the highest concentrations of total protein, carbohydrates, and lipids, with values markedly greater than those in other feed groups. Dietary supplementation (Mahmud and Haque, 2025).

9. CONCLUSIONS

This study demonstrates that dietary inclusion of chemically synthesized silver nanoparticles can modulate growth performance and physiological outcomes in zebrafish, with the magnitude of the effects varying with supplementation level. Among the tested treatments, the diet containing 3 mg of silver nanoparticles per 100 g feed yielded the most favorable improvements in growth and feed efficiency under the present experimental conditions. These findings suggest that carefully optimized supplementation may serve as a nutritional strategy to enhance growth and nutrient utilization in aquaculture species. However, the observed decline in hematological parameters at higher nanoparticle concentrations suggests that excessive intake may trigger adverse physiological responses, underscoring the need to balance growth-promoting benefits against potential toxicity. Thus, silver nanoparticle supplementation should be evaluated not only for its capacity to enhance growth but also for its implications on overall physiological health and safety. From an aquaculture standpoint, the results provide preliminary support for the use of silver nanoparticle-based feed additives while highlighting the need for dose optimization. Future research should involve extended feeding trials, detailed histopathological and molecular analyses, assessment of oxidative stress and immune responses, and comprehensive safety evaluations to establish long-term impacts. Additionally, validation of optimal dietary levels in commercially important aquaculture species is essential before practical application.

ABBREVIATIONS

The following abbreviations are used in this manuscript:

Abbreviations	Expansion
UV-Vis	UV-Visible spectroscopy
SEM	Scanning Electron Microscope
EDAX	Energy Dispersive X-ray Spectroscopy
FT-IR	Fourier Transform Infrared Spectroscopy
Ag NPs	Siver Nanoparticles
RBC	Red Blood Corpuscle
WBC	White Blood Corpuscle
Hb	Haemoglobin
keV	Kiloelectron Volts
nm	Nano Meter
mg	Milli gram
SS	Sum of square Variation
DF	Degree of Freedom
F	Critical Probability Value
cu. mm	Cubic Millimeter
gm/dl	Grams Per Decilitre
%	Percentage

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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