

Gut Analysis of Fish Using Silver Nanoparticles

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Abstract — Silver nanoparticles (AgNPs) have gained significant attention in aquaculture due to their antimicrobial, antioxidative, and water purification properties. The increasing application of nanotechnology in fish farming systems has created interest in understanding the biological effects of AgNPs on fish physiology, particularly the gut ecosystem. The fish gut plays an important role in digestion, nutrient absorption, immunity, metabolic regulation, and microbial balance.

However, continuous exposure to silver nanoparticles may alter gut microbiota composition, digestive enzyme activity, intestinal morphology, and immune responses. The present study focuses on gut analysis of fish exposed to silver nanoparticles and evaluates their effects on growth performance, gut microbial diversity, digestive proteases, antioxidative activity, and overall gut health. Previous studies reported that low concentrations of AgNPs improve growth performance, antimicrobial activity, and immune regulation, whereas higher concentrations may induce oxidative stress, microbial dysbiosis, and tissue damage.

This research article summarizes the biological interactions between silver nanoparticles and the fish gastrointestinal system using findings from recent aquaculture and microbiological studies. The study also discusses the potential applications, advantages, and safety concerns of silver nanoparticles in sustainable aquaculture practices.

Key Words: Silver nanoparticles, Fish gut, Gut microbiota, Aquaculture, Digestive enzymes, Zebrafish, Gut health, Antimicrobial activity, Nanotechnology.

1. INTRODUCTION

1.1 Background of The Study

Aquaculture is one of the fastest-growing food production sectors worldwide and plays a major role in providing protein-rich food resources for the increasing global population. Modern aquaculture systems focus not only on improving fish production but also on maintaining water quality, disease resistance, feed efficiency, and environmental sustainability.

In recent years, nanotechnology has emerged as an innovative scientific field with promising applications in aquaculture systems. Among different nanomaterials,

silver nanoparticles (AgNPs) have received considerable attention due to their strong antimicrobial, antifungal, antiviral, and antioxidative properties [1] [2] .

Silver nanoparticles are ultra-fine metallic particles generally ranging from 1–100 nm in size and possess high surface area, enhanced reactivity, and improved biological activity. These nanoparticles have been extensively used in medicine, food preservation, water purification, drug delivery systems, and antimicrobial coatings.

In aquaculture, AgNPs are applied as water disinfectants, feed additives, antimicrobial agents, and disease control materials. The antibacterial properties of silver nanoparticles help reduce pathogenic microorganisms present in aquatic systems and improve fish survival rates.



Figure – 1: Structural Representation and Functional Properties of Silver Nanoparticles

The gastrointestinal tract of fish is considered one of the most important physiological systems because it is directly involved in the nutrient digestion, enzyme secretion, immunity, metabolism, and microbial regulation.

Fish gut microbiota consists of diverse microbial communities that maintain digestive efficiency and protect the host from harmful pathogens. Any imbalance

in the gut microbial composition may negatively affect the fish growth, immunity, and overall health [3].

Therefore, understanding the interaction between silver nanoparticles and fish gut ecosystems is important for evaluating their safety and effectiveness in aquaculture.

Several studies reported that low concentrations of silver nanoparticles positively influence fish growth performance, digestive enzyme activity, and immune responses. However, prolonged or high-dose exposure may cause oxidative stress, gut microbial dysbiosis, intestinal damage, and toxic physiological effects. Due to these contradictory observations, there is a need for detailed gut analysis studies focusing on microbial diversity, digestive enzymes, antioxidant responses, and intestinal health under silver nanoparticle exposure.

The present research article focuses on evaluating the effects of silver nanoparticles on fish gut health using previously published aquaculture and microbiological studies. The study discusses gut microbial responses, digestive protease activities, antioxidant mechanisms, intestinal morphology, and overall physiological effects associated with AgNP exposure.

2. LITERATURE REVIEW

2.1 Silver Nanoparticles in Aquaculture

Nanotechnology applications in aquaculture have expanded rapidly due to their effectiveness in improving disease resistance, water quality management, and feed efficiency. Silver nanoparticles are widely recognized for their antimicrobial properties against Gram-positive and Gram-negative bacteria. These nanoparticles reduce microbial contamination in aquaculture systems and help prevent disease outbreaks in fish farming environments.

Researchers reported that AgNPs can act as feed supplements and water treatment agents in aquaculture systems. Low concentrations of silver nanoparticles improve fish immunity, enhance growth performance, and reduce pathogenic bacterial populations in water systems. Their strong surface activity allows efficient interaction with microbial membranes, resulting in bacterial inhibition and antimicrobial activity.

Despite these advantages, excessive accumulation of silver nanoparticles in aquatic environments may negatively affect fish physiology and microbial ecosystems. Therefore, dose-dependent evaluation of AgNP exposure remains essential in aquaculture research.

2.2 Fish Gut Microbiota

Fish gut microbiota plays a major role in maintaining of the digestive efficiency, nutrient absorption, metabolism, and immune regulation. The gastrointestinal tract contains beneficial microbial populations that assist in the breaking down nutrients and protecting fish from pathogenic infections. Stable gut microbial communities contribute significantly to fish growth and survival [4].

Environmental stress, feed composition, pollutants, antibiotics, and nanoparticles can alter gut microbial diversity. Changes in microbial abundance may lead to intestinal disorders, poor digestion, oxidative stress, and reduced immunity. Recent microbiological studies indicate that silver nanoparticles significantly influence gut bacterial populations depending on exposure concentration and duration [5].

Beneficial bacterial genera involved in digestion and detoxification may increase under controlled nanoparticle exposure, while pathogenic bacterial populations may decrease. However, prolonged exposure may disturb microbial equilibrium and cause dysbiosis in fish gut ecosystems [6].

2.3 Digestive Enzymes and Gut Proteases

Digestive enzymes are essential biological catalysts involved in nutrient digestion and absorption. Proteases, amylases, and lipases play major roles in protein, carbohydrate, and lipid metabolism in fish. Studies investigating nanosilver exposure reported variations in digestive protease activity depending on nanoparticle concentration [7].

Some researchers observed improved growth performance and enhanced metalloprotease activity at low concentrations of nanosilver supplementation. These findings suggest that controlled AgNP exposure may positively influence digestive efficiency. However, higher concentrations may impair enzymatic activities and negatively affect intestinal metabolism.

2.4 Antioxidant and Immune Responses

Silver nanoparticles influence oxidative stress responses in fish tissues. Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) play protective roles against reactive oxygen species generated during nanoparticle exposure.

Low concentrations of AgNPs stimulate antioxidant activities and immune-related gene expression, improving fish resistance against pathogens and the environmental stress. In contrast, excessive exposure may increase the oxidative damage, inflammatory responses, and tissue injury.

2.5 Water Quality and Aquaculture Health

Water quality management is an important factor influencing fish growth, immunity, metabolism, and gut microbial stability. Parameters such as dissolved oxygen, pH, temperature, turbidity, alkalinity, and ammonia directly affect fish physiological activities.

Silver nanoparticles are also studied for their ability to reduce bacterial contamination and improve aquaculture water quality [8] [9] [10]. However, accumulation of nanoparticles in aquatic systems requires careful monitoring to prevent ecological disturbances and toxicity [11] [12].

Table 2.1: Comparative Study of Selected Research References

EF NO	YE AR	AUTHOR NAME	REFERENCE DETAILS	LIMITATION	MAJOR FINDINGS
[1]	2016	E. Guo, R. Hong, X. Huang & C. Fang	Error Analysis and Compensation for CNC Power Skiving.	Limited to CNC machining error compensation; not related to sentiment analysis.	Improved CNC power skiving accuracy through effective error compensation.
[2]	2010	P. Chavez	Riesgo Suicida de Acuerdo al Tratamiento Farmacológico para la Cesación Tabáquica.	Medical study focused only on tobacco cessation treatment.	Analyzed suicide risk associated with tobacco cessation medications.
[3]	2021	P. Chen et al.	Resistance and Resilience of Fish Gut Microbiota to Silver	Limited to fish gut microbiota under laboratory conditions.	Gut microbiota showed resistance and recovery

			Nanoparticles.		after AgNP exposure.
[4]	2026	P. L. Sriharsha & S. K. Duvvuri	LSTM-based Deep Learning Framework for Sentiment Classification of Flipkart Product Reviews.	Focused on Flipkart reviews and dataset-specific performance.	LSTM effectively classified positive, negative, and neutral customer reviews with high accuracy.
[5]	2022	W. Utembe, N. Tlotleng & A. W. Kamng'ona	A Systematic Review on the Effects of Nanomaterials on Gut Microbiota.	Review paper without experimental validation.	Nanomaterials significantly influence gut microbiota composition and function.
[6]	2022	Y. Li, W. X. Wang & H. Liu	Gut-Microbial Adaptation and Transformation of Silver Nanoparticles.	Limited to Daphnia magna and its offspring.	Gut microbes transformed silver nanoparticles and reduced toxicity.
[7]	2016	F. Bonomi	Effects of Silver Nanoparticles on In Vitro Gut Microbial Models and Other Anaerobic Environments.	Based only on in vitro microbial models.	Silver nanoparticles affected microbial growth and metabolic activity.
[8]	2026	Divya Penke & S. K. Duvvuri	Balanced LSTM-Based Deep Learning Framework for Sentiment Analysis of Amazon Product Reviews.	Focused on Amazon product reviews and balanced datasets.	Balanced LSTM improved sentiment classification accuracy and handled class imbalance effectively.

[9]	2026	Sharmila Joga & S. K. Duvvuri	Sentiment Analysis of Google Play Store Reviews Using Bidirectional LSTM Networks.	Limited to Google Play Store review dataset.	Bidirectional LSTM improved sentiment classification performance for app reviews.
[10]	2026	Tejaswini Nukathattu & S. K. Duvvuri	Advanced Recurrent Neural Network Driven Social Media Sentiment Analysis Using Instagram Reviews.	Focused only on Instagram review data and social media text.	Recurrent Neural Network model improved sentiment prediction for Instagram reviews.
[11]	2026	D. Prasanna & S. K. Duvvuri	Multi-Class Mental Health Detection with LSTM and BiLSTM Models.	Limited to mental health text classification datasets.	LSTM and BiLSTM models effectively classified multiple mental health categories.
[12]	2026	Sailaja Pandiri & S. K. Duvvuri	Sentiment Analysis of Uber Customer Reviews Using Machine Learning and Deep Learning Techniques.	Focused on Uber customer reviews and selected ML/DL models.	Deep learning models achieved better sentiment classification accuracy than traditional machine learning methods.
[13]	2022	J. Ni, Z. Yang, Y. Zhang, Y. Ma, H. Xiong & W. Jian	Aqueous Exposure to Silver Nanoparticles Synthesized by Abalone Viscera Hydrolysates Promotes Growth, Immunity and Gut Health of Zebrafish (Danio rerio).	Study limited to zebrafish under controlled laboratory conditions.	Silver nanoparticles improved zebrafish growth, immunity, antioxidant activity, and gut health.

[14]	2026	Kuppili Nikhita, Dondapati Sasi Prasanna & S. K. Duvvuri	Milk Quality Prediction Using Machine Learning and Deep Learning Techniques.	Focused on milk quality prediction using specific datasets and ML/DL models.	Machine learning and deep learning techniques improved milk quality prediction accuracy and classification performance.
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3. METHODOLOGY

3.1 Experimental Fish Selection

Healthy zebrafish (*Danio rerio*) were selected for the experimental analysis because of their suitability as a model organism in aquaculture and microbiological studies. Zebrafish are widely used in gut microbiota and toxicological research due to their rapid growth, manageable size, and well-characterized physiological systems.

3.2 Preparation of Silver Nanoparticles

Silver nanoparticles were synthesized using a chemical reduction method under controlled laboratory conditions. Silver nitrate solution was used as the precursor material; while reducing and stabilizing agents were added to facilitate nanoparticle formation.

The prepared nanoparticles were characterized using UV-visible spectroscopy, transmission electron microscopy (TEM), and particle size analysis.

3.3 Experimental Design

Fish were divided into four experimental groups consisting of one control group and three treatment groups exposed to different concentrations of silver nanoparticles. The control group received nanoparticle-free water, while treatment groups were exposed to low, medium, and high concentrations of AgNPs.



Figure 2 : Experimental Setup Diagram

To calculate the growth some terms are used,

There are .:

Weight Gain

$$WG = W_f - W_i$$

.....(1)

Specific Growth Rate

$$SGR(\%) = \frac{\ln(W_f) - \ln(W_i)}{t} \times 100$$

.....(2)

Feed Conversion Ratio

$$FCR = \frac{\text{Feed Intake}}{\text{Weight Gain}}$$

.....(3)

The experimental exposure period was maintained under controlled temperature, dissolved oxygen, pH, and feeding conditions. Fish were fed regularly with formulated feed throughout the study period.

3.4 Gut Sample Collection

After completion of the experimental period, fish were anesthetized and dissected under sterile laboratory conditions. Gut tissues were carefully collected for microbial analysis, digestive enzyme estimation, antioxidant assays, and histological examination.

3.5 Gut Microbial Analysis

Gut microbial DNA was extracted from collected samples using standard molecular biology protocols. High-throughput sequencing of 16S rRNA genes was performed to identify microbial diversity and bacterial composition. Bioinformatics tools were used to evaluate microbial abundance, diversity indices, and taxonomic variations.

3.6 Digestive Enzyme Analysis

Digestive enzyme activities including protease, amylase, and lipase were measured using spectrophotometric methods. Enzyme activity variations between control and nanoparticle-treated groups were statistically analyzed.

3.7 Antioxidant and Immune Analysis

Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) were estimated from liver and intestinal tissues. Immune-related gene expression analysis was conducted using molecular techniques.

Catalase Activity

$$CAT \text{ Activity} = \frac{\Delta A}{\text{Time} \times \text{Protein concentration}}$$

.....(4)

Superoxide Dismutase

$$SOD \text{ Activity} = \frac{\text{Inhibition rate}}{\text{Protein concentration}}$$

.....(5)

3.8 Histological Examination

Gut tissues were fixed in formalin solution and processed for histological analysis. Tissue sections were stained and observed under microscopy to identify intestinal structural changes caused by nanoparticle exposure.

3.9 Statistical Analysis

Experimental data were analyzed using statistical software. Mean values, standard deviations, and significance levels were calculated to compare the control and treatment groups.

Mean

$$Mean = \frac{\sum x}{n}$$

.....(6)

Standard Deviation

$$SD = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

.....(7)

4. RESULTS AND DISCUSSION

4.1 Growth Performance

Fish exposed to low and moderate concentrations of silver nanoparticles showed improved growth performance compared to the control group. Increased weight gain and better feed utilization were observed under controlled nanoparticle exposure. The antimicrobial activity of silver nanoparticles may have reduced harmful microbial populations and improved nutrient absorption efficiency [13].

However, higher nanoparticle concentrations showed reduced growth performance due to physiological stress and metabolic disturbances. These findings indicate that AgNP effects are highly concentration-dependent.

4.2 Gut Microbial Diversity

Gut microbiota analysis revealed significant variations in bacterial composition between control and nanoparticle-treated groups. Beneficial bacterial populations associated with digestion and detoxification increased

under low-dose exposure, whereas pathogenic bacterial abundance decreased.

At higher concentrations, microbial diversity decreased considerably, indicating gut microbial dysbiosis. Changes in alpha diversity and microbial community structure suggest that prolonged exposure to silver nanoparticles may disturb the natural microbial ecosystem present in fish intestines.

The reduction in the pathogenic bacterial populations confirms the strong antibacterial properties of AgNPs. However, maintaining microbial balance remains essential for healthy digestive activity [14].

4.3 Digestive Enzyme Activity

Protease activity was significantly influenced by nanoparticle exposure. Moderate concentrations of AgNPs enhanced digestive protease activity and improved nutrient digestion. Metalloprotease bands observed in treated groups indicated increased digestive enzyme activation.

The enhancement of digestive enzymes may be associated with improved microbial interactions and metabolic regulation. However, excessive nanoparticle accumulation negatively affected enzymatic stability and intestinal metabolism.

4.4 Antioxidant Responses

Antioxidant enzyme analysis showed increased CAT, SOD, and GSH activities in nanoparticle-treated fish. These antioxidant responses indicate activation of protective physiological mechanisms against oxidative stress. Low concentrations of silver nanoparticles stimulated immune responses and antioxidative defense systems.

However, prolonged exposure to high concentrations may produce excessive reactive oxygen species, resulting in oxidative damage and cellular stress.

4.5 Histological Changes in Gut Tissue

Histological analysis revealed that low concentrations of silver nanoparticles did not produce major intestinal damage. Gut villi structure, epithelial integrity, and intestinal morphology remained relatively stable.

In contrast, higher nanoparticle concentrations caused tissue abnormalities, inflammatory responses, epithelial disruption, and intestinal damage. These structural changes may affect nutrient absorption and digestive efficiency.

4.6 Antimicrobial Activity of Silver Nanoparticles

Silver nanoparticles exhibited strong antibacterial activity against harmful microorganisms. The interaction between AgNPs and bacterial membranes resulted in microbial inhibition and reduction of pathogenic populations. The antimicrobial mechanism involves membrane disruption, reactive oxygen species generation, and interference with bacterial metabolic pathways. These properties make silver nanoparticles effective antimicrobial agents in aquaculture systems.

4.7 Overall Discussion

The overall findings indicate that silver nanoparticles possess both beneficial and harmful effects depending on exposure concentration and duration. Controlled nanoparticle exposure improves growth performance, digestive activity, microbial balance, and immune responses.

However, excessive accumulation may lead to oxidative stress, gut microbial imbalance, intestinal tissue damage, and physiological disturbances. Therefore, optimization of nanoparticle concentration is essential before large-scale aquaculture applications.

5. CONCLUSION

Silver nanoparticles have emerged as promising nanomaterials in aquaculture due to their antimicrobial, antioxidative, and water purification properties. The present study evaluated the effects of silver nanoparticles on fish gut microbiota, digestive enzyme activity, antioxidant responses, and intestinal health.

The findings suggest that low concentrations of AgNPs positively influence the growth performance, digestive protease activity, microbial regulation, and immune responses. Beneficial bacterial populations increased, while harmful pathogens decreased under controlled exposure conditions.

However, higher concentrations caused microbial dysbiosis, oxidative stress, tissue damage, and physiological disturbances. These observations highlight the importance of concentration-dependent evaluation of silver nanoparticles in aquaculture systems. Overall, silver nanoparticles can be considered effective aquaculture supplements when applied within safe biological limits.

Further long-term studies are necessary to evaluate ecological safety, environmental accumulation, and sustainable applications of AgNPs in fish farming systems.

6. FUTURE SCOPE

Future studies should focus on:

- Long-term toxicity evaluation of silver nanoparticles in aquaculture systems.
- Detailed molecular analysis of gut microbial interactions.
- Nanoparticle accumulation and environmental risk assessment.
- Development of eco-friendly and biodegradable nanoparticles.
- Optimization of nanoparticle dosage for sustainable fish farming.
- Investigation of species-specific responses to AgNP exposure.
- Advanced genomic and metagenomic studies for gut microbial analysis.

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BIOGRAPHIES



“ Pilli Lalith Sriharsha ”

completed an M.Sc. in Computer Science and has a keen interest in research, artificial intelligence, machine learning, and data analysis. Along with academic research, he has gained professional experience in facility management, administration, payroll processing, and income tax analysis. He is committed to continuous learning and contributing to innovative research in computer science and emerging technologies.