A Seminar Paper

On

Navigating the Benefits and Risk of Silver Nanoparticles in Poultry Production

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Navigating the Benefits and Risk of Silver Nanoparticles in Poultry Production[1]

By

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ABSTRACT

Nanotechnology is an area of rapid development that has emerged in response to various drug-resistant pathogens and the failure of conventional treatment methods. One of the most promising types of metal nanoparticles in this field is silver nanoparticles which have demonstrated remarkable effectiveness in combating various pathogens over time. AgNPs possess merits as well as demerits in poultry. AgNPs has displayed strong acaricidal activity against red mites of poultry at concentrations of 60 and 80 ppm. Moreover, administration of AgNPs has been demonstrated to lessen *Clostridium perfringens* colonization in the intestine and ceca. This can lead to reduced poultry diseases and mortality rates, as well as improved poultry production through improving overall poultry performance. As the emergence of antibiotic-resistant bacteria has become a significant public health concern, there is a need for new antibacterial approaches. AgNPs can be used as an alternative to some antibiotics against *Escherichia coli, Salmonella* sp etc. The higher the concentration of AgNPs used, the more its residues were found in the breast and thigh muscle. If the concentration of AgNPs applied exceeds 2.5mg, this may result in the transmission of AgNPs to consumers. It has cytotoxic effect to liver in a dose -dependent manner. It is important to carefully consider these factors before deciding to use silver nanoparticles in poultry production and hence, further research is needed. In summary, this paper outlines the advantages and disadvantages of silver nanoparticles in poultry production.

Keywords: Nanotechnology, silver nanoparticles, acaricidal activity, antimicrobial-resistant, silver residues, cytotoxic effect, dose, poultry production.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Since the 1980s, nanotechnology has attracted attention and developed quickly, and it has proven to be incredibly effective in battling a variety of infections and human diseases in several industries, including medical, pharmaceuticals, and the food industry (Huang *et al*., 2015; Ghidan *et al*., 2017). The creation of nanoparticles, including organic, inorganic, polymer-based, non-polymeric, and metallic nanoparticles, is the most widely used application of nanotechnology. Researchers have achieved success developed physical, chemical, and biological synthesis techniques to create metallic nanoparticles, the most preferred approach being biological synthesis because it is environmentally benign and comparatively inexpensive method to the physical and chemical processes that produce nanoparticles at the usage of numerous machines and chemicals at a high-cost rate, leading to ecological concern (Silva *et al*., 2021).

The key feature of these nanoparticles that makes them versatile and useful in many fields is their small size ranging from 1 to 100 nanometers and their shapes such as spheres, triangles, or rods. There are various types of nanomaterials, including metals like gold, silver, and copper, metal oxides such as zinc oxide, silicon dioxide, iron oxide, and titanium dioxide, as well as clay, organic and full carbon materials, and other nanomaterials such as nano-composites and nano-encapsulates (Amenta *et al*., 2015). Growing interest has been shown in using nanoparticles in animal feed for a variety of animals, including chickens, pigs, ruminants, and rabbits. These nanoparticles have been found to have multiple benefits, including promoting growth, stimulating the immune system, and acting as antimicrobial agents (Michalak *et al*., 2022). Among these, silver and gold nanoparticles are used as anti-inflammatory, antimicrobial agents, and nano biocides are widely used to enhance food safety by eliminating microbes using nano-sieves (Amenta *et al*., 2015).

Silver nanoparticles have drawn a lot of interest and have been employed in several industries, including optics, biology, water treatment, healthcare, and catalysis (Manzoor *et al*., 2021). According to (Sawosz *et al*., 2012), silver nanoparticles (AgNPs) have the potential to promote growth by increasing muscle mass and body weight. Additionally, (Pineda *et al*., 2012) suggest that AgNPs can serve as a means of carrying available oxygen (O2), which can enhance energy storage capacity and ultimately lead to improved growth in poultry. Poultry are those species of birds which rendered man an economic service and reproduce freely under his care. It includes chicken, duck, geese, pigeons etc. In a dose-dependent way, the silver residues in the breast and thigh muscles dramatically increased ($p < .05$). The health of the consumer of the chicken is proportional to the AgNPs retention in the poultry (Kumar *et al.*, 2019). Including silver nanoparticles (AgNPs) in broiler diets at levels exceeding 2.5 mg/kg had multiple detrimental effects, such as the accumulation of silver residue in broiler meat and the potential transmission of nanosilver to consumers (Sultan *et al*., 2022). In a different investigation, 50 ppm colloidal AgNPs were applied to chicken eggs. The results showed that there were no pathogenic infections or eggs that were spoiled, but pathogenic research showed that AgNPs had an adverse effect on hatchlings because of oxidative stress (Chmielowiec *et al*., 2015).

For safety reasons, it is now necessary to get the correct information on the dose of silver nanoparticles. In our country research of silver nanoparticles in poultry is still relatively limited, and more research is needed to fully understand their potential effects. It's worth noting that the exact mechanisms of how silver nanoparticles interact with cell membranes and cause cell death are not yet fully understood and is a debated topic. In hence further research is needed to elucidate the underlying processes involved (Silva *et al*., 2021).

Objectives:

1. To explore the potential benefits of silver nanoparticles in poultry production, including improved growth performance, disease resistance, reduced antibiotic resistance and decreased morality rate of chicken.

2. To evaluate the potential risks associated with the use of silver nanoparticles in poultry production, silver traces on poultry carcass, and potential toxicity to animals and humans.

CHAPTER 2

MATERIALS AND METHODS

The seminar paper is completely a review paper. Therefore, all the information was collected from secondary sources with a view to prepare this paper. The key information was collected from various relevant articles, journals, thesis papers and internet. Good suggestions, valuable information and kind consideration from my honorable major professor, research supervisor, course instructors were taken to enrich this paper. After collecting necessary information, it has been compiled and arranged chronologically for better understanding and clarification.

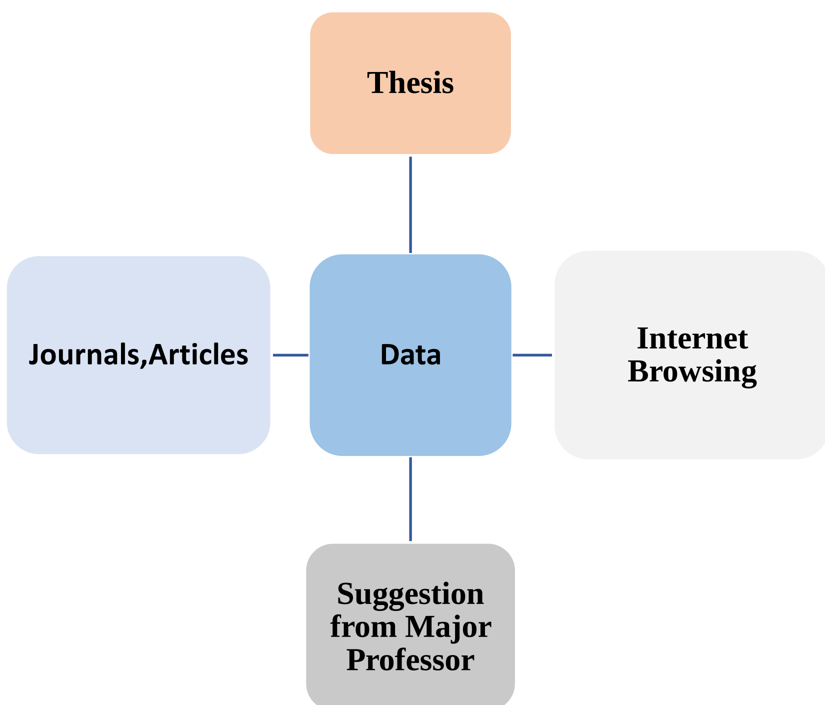


Figure 1. Sources of data and information used in present paper.

CHAPTER 3

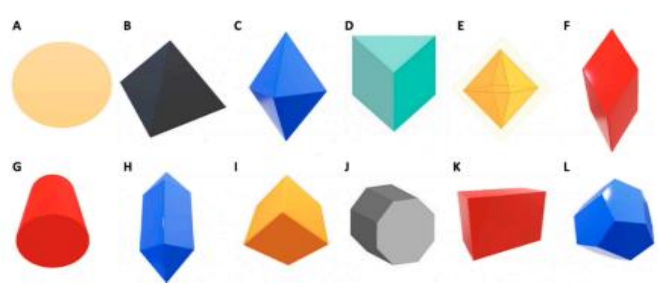
REVIEW OF FINDINGS

3.1 Silver Nanoparticles

AgNPs, which are silver nanoparticles with a size range of 1 to 100 nanometers, exhibit distinctive characteristics such as electrical, optical, and magnetic properties, making them highly versatile for various applications (Galatage *et al.*, 2021).

3.1.1 Size and Shape-Dependent Properties of AgNPs

Nanoparticles' small size (1-100 nm) and form (spherical, triangular, or rod) are what primarily drives their diverse functions (Huang *et al.*, 2015; Ghidan *et al*., 2017). AgNPs, mostly of smaller size spanning between 1 and 10 nm, adhered to the cell membrane of target bacteria and disrupted cell functions, especially bacterial respiration and penetration (Morones *et al*., 2005). It is also noted that the antimicrobial activity of silver increased with decreasing silver nuclei size (Nour *et al*., 2010). The relationship between a nanoparticle's form and size and their mechanism of antibacterial action has become an increasingly researched topic, as noted by (Silva *et al*., 2021). Silver nanoparticles (AgNPs) can be chemically produced in several shapes, including cubes, rods, platelets, pyramids, and bipyramids etc. (Xia *et al*., 2013, Helmlinger *et al*., 2015). It was found that the effectiveness of AgNPs varied depending on their shape. The findings indicated that silver nanoparticles (AgNPs) with pointed edges were more successful in entering bacterial cells compared to round AgNPs, which lack any edges (Dong *et al.*, 2012).

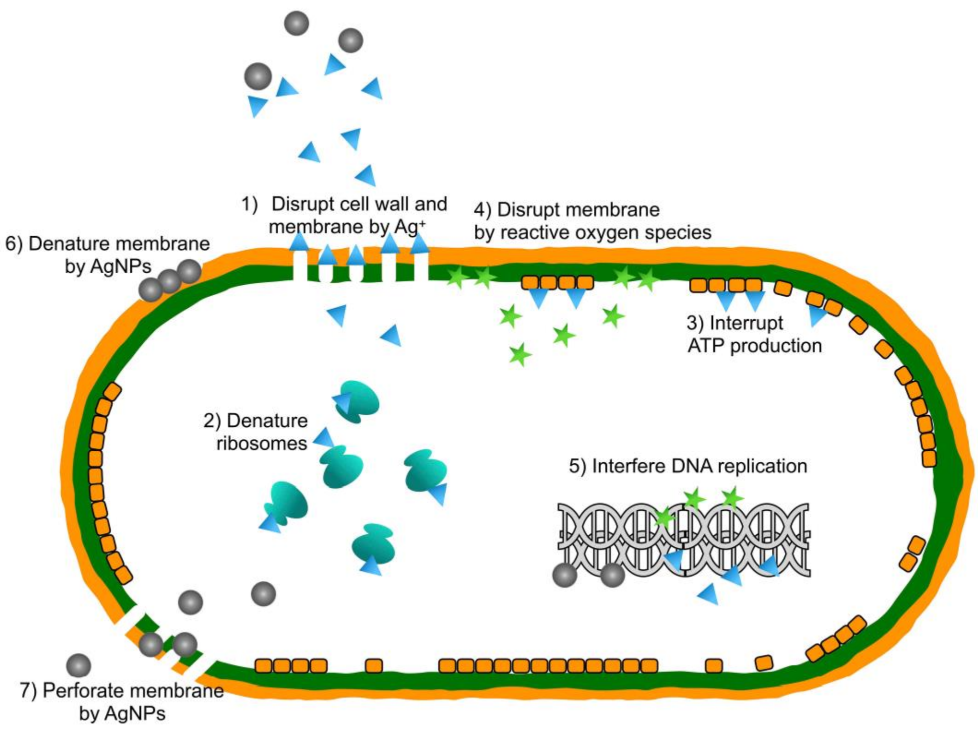


(Source: Silva *et al*., 2021)

Figure 2. The many configurations of created silver nanoparticles (AgNps).

3.1.2 Mechanism of Silver Nanoparticles

Silver nanoparticles are known to have the ability to interact with cell membranes and cause structural changes that can ultimately lead to cell death. (Lia *et al*., 2013). Additionally, silver nanoparticles can also generate reactive oxygen species (ROS) which can cause damage to cellular components such as protein synthesis and alteration, inhibition of enzymes, lipid synthesis as well as oxidation and damage of DNA and RNA of the bacterial cell (Silva *et al*., 2021). Although the actual mechanism underlying the antibacterial capabilities of silver nanoparticles is yet unknown, some antibacterial actions have been suggested in (Figure 3) (Silva *et al*., 2021).



(Source: Yin *et al*., 2020)

Figure 3. The antibacterial actions of silver nanoparticles (AgNPs). (1) Silver ions (Ag+) released by silver nanoparticles stick to or pass through cell walls and cytoplasmic membranes, causing disruption of both structures. (2) Ribosome denaturation: silver ions denature ribosomes and stop the production of protein. (3) Adenosine triphosphate (ATP) synthesis is disrupted because silver ions render the respiratory enzyme on the cytoplasmic membrane inactive. (4) Reactive oxygen species can rupture membranes. Reactive oxygen species are created when the electron transport chain breaks down. (5) DNA replication interference: Reactive oxygen species and silver attach to

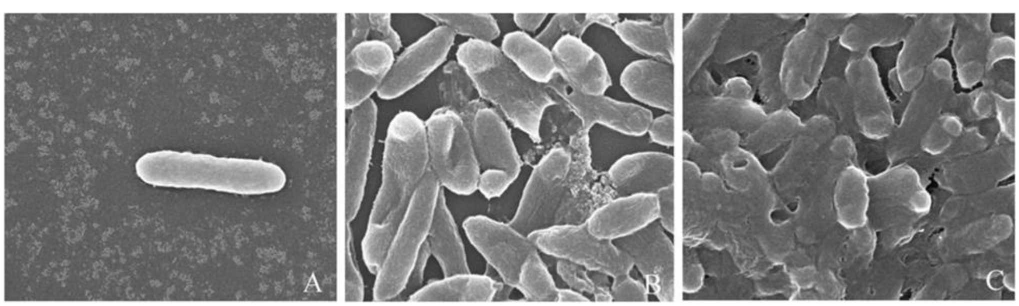
deoxyribonucleic acid to hinder its replication and cell division. (6) Membrane denaturation is brought on by the accumulation of silver nanoparticles in the pits of the cell wall. (7) Membrane perforation: silver nanoparticles directly cross the cytoplasmic membrane, allowing the cell's organelles to be released.

3.2 Benefits of Silver Nanoparticles in Poultry Production by Decreasing Diseases

The use of nanotechnology, specifically AgNPs, in poultry industries has demonstrated its ability to address the difficulties faced by these sectors. AgNPs provide a sustainable and efficient approach to managing diseases, resulting in improved poultry production while maintaining product quality (Silva *et al*., 2021).

3.2.1 Silver Nanoparticles Against Salmonellosis in Poultry

The bacterium *Salmonella* sp. is responsible for causing salmonellosis, an illness that affects both humans and animals. Public health considers salmonellosis to be a significant zoonotic disease. A significant source of bacterial agents is poultry. Poultry that is infected can spread diseases. (Wibisono *et al*., 2020). In this study, untreated cells *Salmonella enteritidis* showing intact cells in (Figure 4A). The use of Ag-NP particles with 50 ppm concentration on *S. enteritidis* bacterial cells for 3 hours resulted in visible morphological damage and damage to the cell wall (Figure 4B) and after 24 hours, complete bacterial lysis was observed, as well as the presence of Ag-NP particles (Figure 4C). This is supported by images captured through scanning electron microscopy (SEM) (Source: Torky *et al*., 2020).



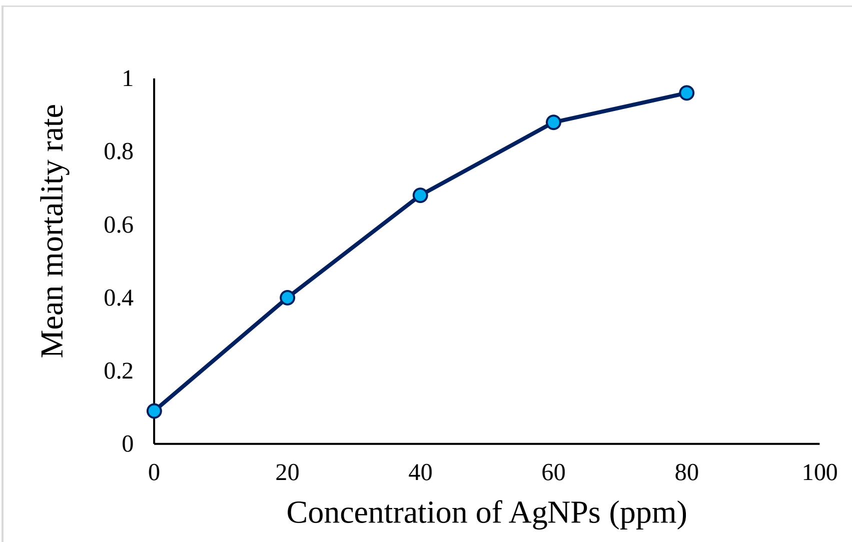
(Source: Torky *et al*., 2020)

Figure 4. AgNPs effect (50 ppm) on *S. enteritidis* cells revealed by SEM image after treatment for 3 h and 24 h.

3.2.2 Silver Nanoparticles Against Red mite of poultry (PRM) in Poultry

The most prevalent blood-sucking ectoparasite seen in laying hens, *Dermanyssus gallinae*, often known as the red mite of poultry (PRM), has evolved resistance to a wide range of acaricides. Silver nanoparticles (AgNPs) play a major role against PRM (Sioutas *et al*., 2023). The act of feeding on blood by *D. gallinae* (PRM) causes stress and irritation in laying hens, leading to behaviors such as feather-pecking and causing them to become anemic. In severe infestations, this can even cause hens death (Marangi *et al*., 2009; Koziatek *et al*., 2015).

In this study, strong acaricidal properties were demonstrated by nanoparticles. (Figure 5) shows the mean mortality rates of mites. At a significance threshold of 0.001, all treated groups had considerably higher mortality rates than the control group, with the mortality rates varying significantly between the groups [$F (4, 211) = 619.7, p < 0.001$] (Sioutas *et al*., 2023).



(Source: Sioutas *et al*., 2023)

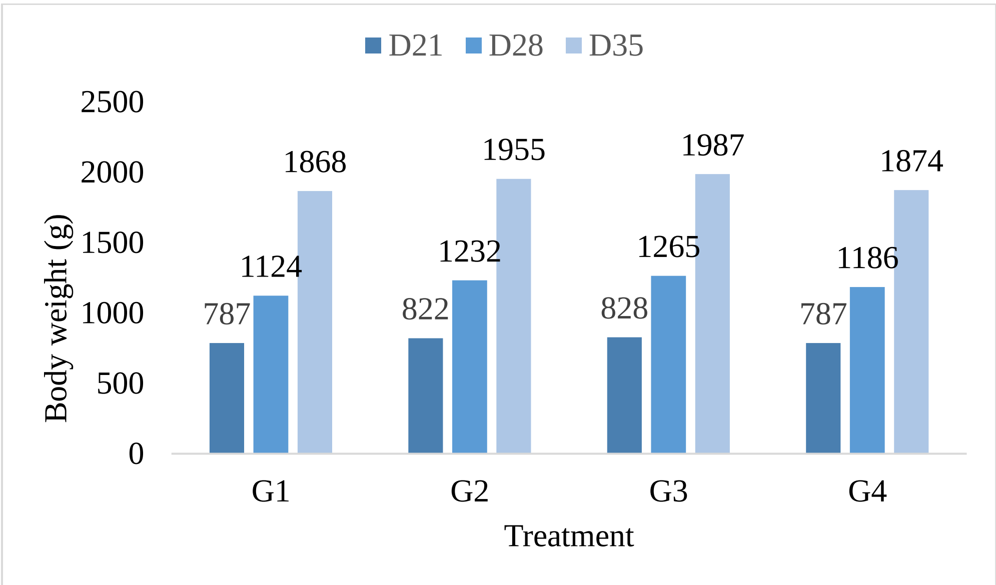
Figure 5. Mean mortality rates for the control and four treatment groups (20, 40, 60, & 80 ppm AgNPs).

3.2.3 Necrotic enteritis in broiler chickens is combated by silver nanoparticles

Production of broilers is negatively impacted by necrotic enteritis (NE) linked to *Clostridium perfringens*. The most common type A and type C of *C. perfringens* responsible for necrotic enteritis are both. (Salem *et al*., 2021). Acute NE kills 1% of broilers per day between the ages of 2 and 6 weeks, with a cumulative death rate of 10% to 40% (Cooper *et al*., 2010). Silver nanoparticles (AgNPs) shown potent antibacterial action against *C. perfringens* in broiler chicken (Salem *et al*., 2021).

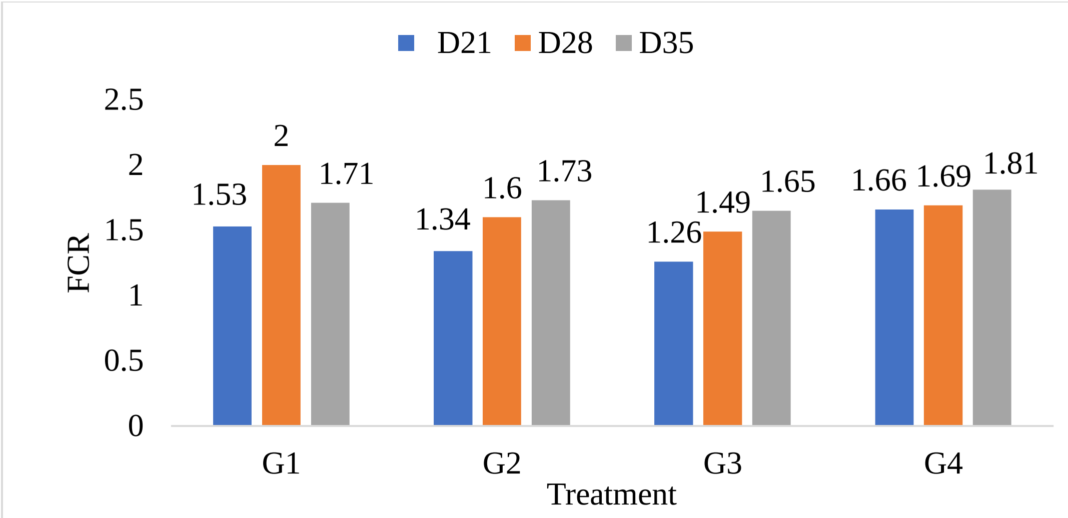
Here the data shows the performance parameters for growth in broiler chicken, and the results indicate that at 28 days old, the birds treated with AgNPs (G2 and G3) compared to the control group (G1 and G4), showed significantly higher body weight (P = 0.002) in (Figure 6). At days 14 to 28, the FCR of the birds that received AgNPs (G2 and G3) showed a significant improvement (P < 0.0001) in (Figure 7) (Salem *et al*., 2021).

Note: G1, chicks infected with *C. perfringens* but not treated with AgNPs; G2, chicks infected with *C. perfringens* and treated with AgNPs; G3, uninfected chicks treated with AgNPs; G4, chicks neither infected nor treated with AgNPs.



(Source: Salem *et al*., 2021)

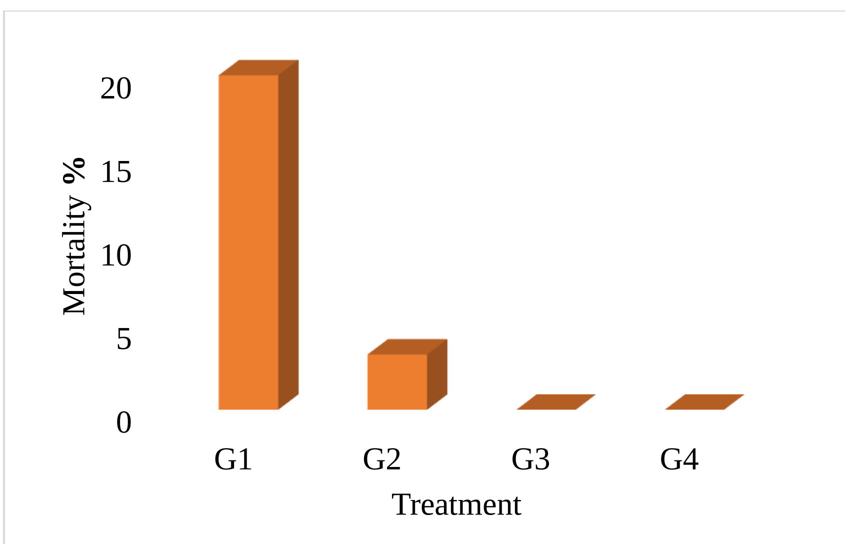
Figure 6. Impact of AgNPs on Body Weight of broiler chicken between control (G1& G4) and treated group (G2 & G3).



(Source: Salem *et al*., 2021)

Figure 7. Impact of AgNPs on FCR of broiler chicken between control (G1& G4) and treated group (G2 & G3).

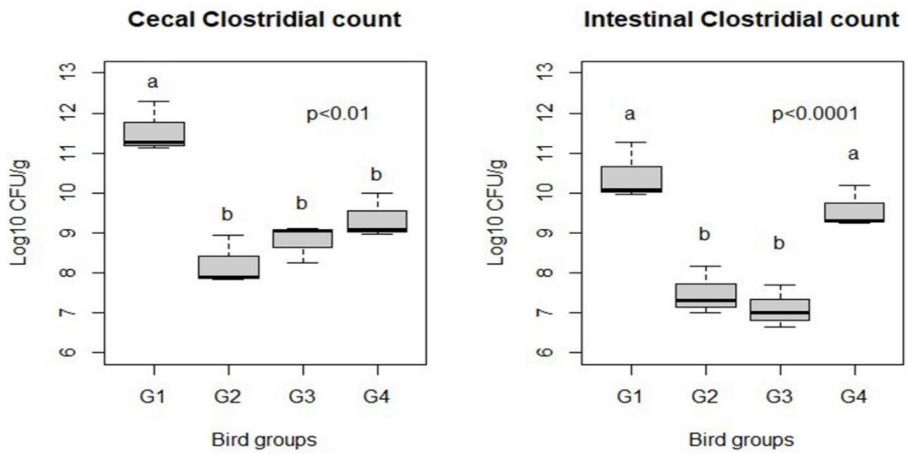
The mortality rates in G1 and G2 at 1 week Post Infection (PI) were 20% (6/30) and 3.3% (1/30), respectively, whereas the other two groups recorded no mortalities (Figure 8). Compared with the positive control group (G1), the reductions in mortality rates for the other groups were significant (P = 0.004). (Source: Salem *et al*., 2021)



(Source: Salem *et al*., 2021)

Figure 8. Mortality rates between control (G1& G4) and treatment group (G2 & G3).

In this case, (Figure 9) shows the cecal and intestinal burdens of *C. perfringens*. At 7 days post-infection, G2, G3, and G4 showed significantly lower cecal *C. perfringens* numbers than G1 (P = 0.001). Moreover, compared to G1 and G4, both G2 and G3 had considerably reduced intestine *C. perfringens* numbers (P < 0.0001). (Salem *et al*., 2021).



(Source: Salem *et al*., 2021)

Figure 9. Effect of Ag NPs on broiler chicken cecal and intestinal *C. perfringens* counts (log10 CFU/g). a, different superscripts indicate significant difference (Tukey's test; P < 0.05).

3.2.4 ***Escherichia coli (E. coli)*** Defense in Poultry using Silver Nanoparticles

According to (Amaral *et al*., 2004), the principal signs of bacterial infections in chicken included diarrhea, respiratory distress, weight loss, poor feed conversion, and high mortality. These symptoms were either brought on or facilitated by the presence of *E. coli*, which is found in surface water or shallow water in poultry farm. Using such bacterially contaminated drinking water is one of common effective ways to spread poultry diseases and is also one of the main causes of rising mortality (Kumar *et al.,* 2019).

In this study, the (Table. 1) and (Table. 2), among many other things, shows that the highest mortality in the case group was 5.22% while the highest mortality in the control group was 16.88%. The case group mortality is considered highly acceptable in the farmers' circle. The average final weight of the chickens/birds in the case group was 1.92 kg, compared that of 1.77 kg in the control group. (Kumar *et al*., 2019).

Table 1. The growth performance details of the case study (*n* = 2)

Sl.no	Week	Slot I			Slot II		
		Total no of chicks= 1765			Total no of chicks=1781		
		% Mortality	FI (kg)	BW (kg)	% mortality	FI (kg)	BW (kg)
1	1st	1.25	274.87	331.17	0.78	278.65	335.73
2	2nd	1.81	906.38	797.18	1.57	916.84	806.38
3	3rd	2.44	1975.48	1360.38	2.75	1986.95	1368.28
4	4th	3.29	4045.59	2560.5	3.65	4066.92	2574
5	5th	5.16	5881.76	2560.5	5.22	5930.95	3240.96

(Source: Kumar *et al*., 2018)

Table 2. The growth performance details of the control study (*n* = 2)

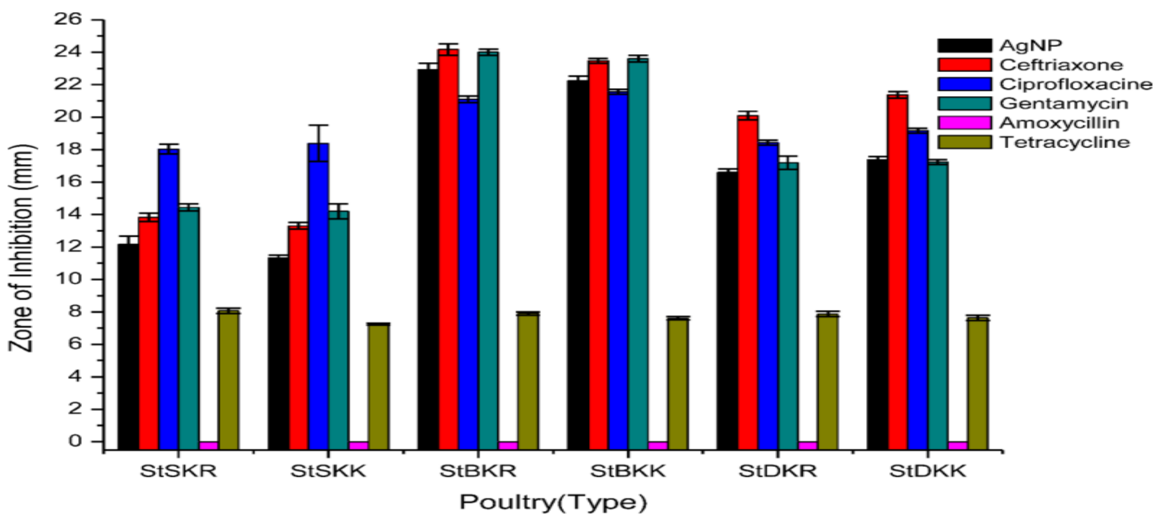
Sl. no.	Week	Slot I			Slot II		
		Total no. of chicks=1717			Total no. of chicks=1811		
		mortality%	FI (kg)	BW (kg)	mortality%	FI (kg)	BW (kg)
1	1st	1.86	249.211	286.45	1.87	262.81	302.09
2	2nd	3.08	874.2	615.68	2.97	923.05	650.09
3	3rd	8.73	1589	1018.55	6.24	1721.83	1103.7
4	4th	10.54	2709.5	1612.8	9.83	2880.6	1714.65
5	5th	16.88	4622.19	2525.79	15.45	4959.05	2709.87

(Source: Kumar *et al*., 2018)

3.3 Effects of Commercially Available Antibiotics and AgNPs on the Isolated bacteria

The use of antibiotics in the production of chicken has substantially grown, not only for therapeutic but also for preventative and growth-promoting purposes. The overuse of antibiotics in poultry production is a major factor in the emergence of bacterial resistance. Therefore, it is essential to explore alternative methods to promote growth and limit infections in poultry farms, particularly in developing countries where economic factors are significant (Kousar *et al*., 2021).

As shown in (Figure 10) *Staphylococcus aureus* showed a significant ($p < 0.05$) level of sensitivity with an inhibitory zone against AgNPs (17.11 mm), Ceftriaxone (19.37 mm), Ciprofloxacin (19.45 mm), and Gentamycin (18.44 mm), but not against amoxycillin, and a non-significant ZOI was measured in the case of Tetracycline (Source: Roy *et al*., 2020).



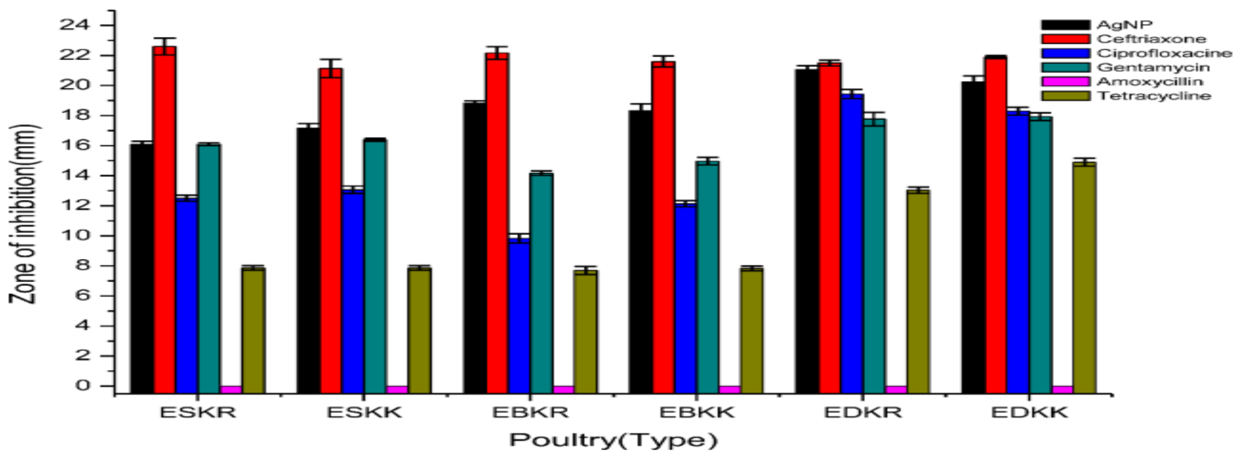
(Source: Roy *et al*., 2020)

Figure 10. Graphically representation of sensitivity test of *S. aureus* against AgNPs and different antibiotic through ZOI.

Note: ES=*E. coli* from sonali, EB=*E. coli* from broiler, ED=*E. coli* from domestic chicken; KR= KR market samples, KK=Kewatkhali market samples.

In this case, *E. coli* demonstrated a significant ($p < 0.05$) level of sensitivity with an inhibitory zone against AgNPs (18.62 mm), Ceftriaxone (21.83 mm), Ciprofloxacin (14.21 mm), and

Gentamycin (16.22 mm), whereas such a ZOI was absent against amoxycillin, and a non-significant ZOI was measured in case of Tetracycline as shown in (Figure 11).



(Source: Roy *et al*., 2020)

Figure 11. Graphically representation of sensitivity test of *E. coli* against AgNPs and different antibiotic through ZOI.

Note: ES=*E. coli* from sonali, EB=*E. coli* from broiler, ED=*E. coli* from domestic chicken; KR= KR market samples, KK=Kewatkhali market samples.

3.4 Potential Risk of Silver Nanoparticles in poultry Production

Researchers discovered that during a 22-day period, chickens fed with silver nanoparticles (AgNPs) accumulated silver in their livers and yolks but not in their muscles, kidneys, or albumens. Within the control group, there was no silver found. The study suggests that feeding hens with AgNPs could lead to consumer exposure to these nanoparticles (Gallocchio *et al*., 2017).

3.4.1 Silver Nanoparticles impact on silver traces in broiler meat

Numerous researchers employed various AgNPs concentrations in the broiler chickens' drinking water and found residues at all concentrations in the edible sections of the muscle (Kulak *et al*., 2018; Salem *et al*., 2021).

Silver residues were present in all samples, including the control group, at amounts that could be detected in parts per million (ppm). According to (Table. 3) at a level of statistical significance of

($p < 0.05$), the average amount of silver residues in the breast and thigh muscles significantly increased with increasing dosages of silver nanoparticles (AgNPs) (Sultan *et al*., 2022).

Table 3. The effect of dietary AgNPs level on residues of silver (mg/kg) in meat of broilers

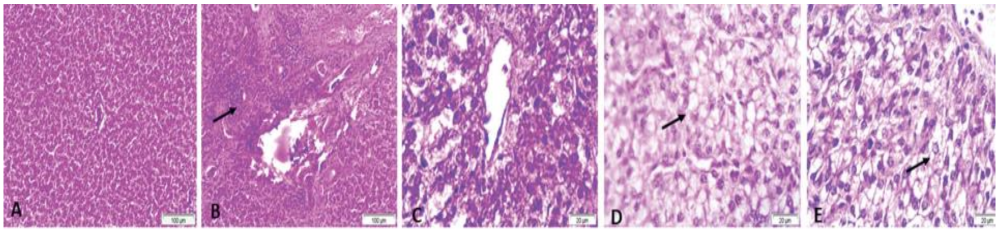
	AgNPs levels (mg/kg diet)					
Item	**C**	**NS2.5**	**NS5**	**NS10**	**NS20**	**Sig.**
Breast muscle	0.01^{b}	0.04^{b}	0.06^{b}	0.07^{ab}	0.11^{a}	0.02
Thigh muscle	0.02^{c}	0.10^{c}	0.15^{b}	0.17^{b}	0.29^{a}	0.001

(Source: Sultan *et al*., 2022)

Note: a,b Means with different superscript letters in the same row are significantly different ($p < 0.05$). C (Control, basal diet); NS2.5, NS5, NS10, NS20 (Basal diet with 2.5, 5, 10, 20 ppm AgNPs), respectively. Sig= Significant.

3.4.2 Impact of Silver Nanoparticles on Broiler Chicken (Liver Cytotoxicity)

AgNPs had a cytotoxic effect on liver (Figure 12) cells in a dose-dependent manner in broilers and greater than 2.5mg/kg of silver nanoparticles might be harmful to chicken and human health (Sultan *et al*., 2022).



(Source: Sultan *et al*., 2022)

Figure 12. Micrographs for liver of broiler chickens treated with various levels AgNPs for 42 days. (A) Liver from control group showing normal hepatic architecture. (B) Liver from 2.5 mg group showing focal mononuclear infiltration (arrow). (C) Liver from 5 mg group showing congestion and vacuolar degeneration. (D) Liver from 10 mg group showing hepatocellular vacuolation (arrow). (E) Liver from 20 mg group showing hepatocellular vacuolation (arrow).

3.4.3 Silver nanoparticles (AgNPs) impact on carcass characteristics and water retaining capacity

The information in (Table 4.) demonstrated a strong relationship between worm carcass weight, dressing %, and relative organ weights and AgNPs concentration. The relative weights of the liver, gizzard, kidney, and spleen were all dramatically reduced by the addition of 20 mg AgNPs/kg food. With 10 and 20 mg AgNPs/kg diet, the proportions of WHC in both the thigh and breast muscles significantly decreased (Sultan *et al*., 2022).

Table 4. Effect of different dietary treatment on carcass traits and relative organ weight and WHC in broiler chicken

	AgNPs levels (mg/kg diet)				
Item	**C**	**NS5**	**NS10**	**NS20**	**Sig.**
Worm Carcass Wt.	1610^{c}	1723.33^{ab}	1686.68^{bc}	1634.79^{c}	0.005
Dressing %	64.00^{b}	66.53^{ab}	65.54^{b}	65.39^{b}	0.01
Liver %	1.73^{b}	1.97^{a}	1.87^{ab}	1.72^{b}	0.006
Gizzard %	1.72^{c}	1.92^{ab}	1.83^{b}	1.69^{c}	0.001
Kidney %	0.61^{a}	0.63^{a}	0.64^{a}	0.51^{b}	0.014
Spleen %	0.13^{b}	0.17^{a}	0.15^{ab}	0.14^{b}	0.017
Breast muscle (WHC%)	70.55^{a}	71.41^{a}	65.58^{b}	63.87^{b}	0.003
Thigh muscle (WHC)%	70.27^{ab}	70.95^{a}	70.10^{ab}	67.10^{b}	0.02

(Source: Sultan *et al*., 2022)

Note: NS5, NS10, and NS20 (Basal diet with 5, 10, and 20 ppm AgNPs, respectively) are variations of the control diet (C). a,b Means in the same row with various superscript letters differ considerably ($p < 0.05$). Water-holding capacity (WHC).

CHAPTER 4
CONCLUSION

The poultry sector suffers from a great loss of production due to pathogenic diseases brought on by contaminated water sources used in poultry farms as well as incorrect handling, which in turn results in a fall in growth rate and an increase in mortality. Silver nanoparticles have been found to have antimicrobial properties that successfully fought pathogenic illnesses and decreased poultry mortality rates**.** Silver nanoparticles can improve the growth performance of poultry by increasing feed intake, body weight gain, feed conversion ratio and decreasing mortality rate. This can reduce the need for antibiotics, which can lead to antibiotic resistance and other negative health effects.

Although silver nanoparticles are generally considered as safe but there is still some concern about their potential toxicity to animal and human. Some studies have suggested that they may have toxic effects on liver function and can accumulate in meat and organs over time. This can potentially pose a risk to human health if the meat is consumed because different broiler meat portions accumulate AgNPs in a dose-dependent way, and their usage and marketing as feed additives or growth promoters must be controlled and regulated due to the risks associated with the transfer of nanosilver to human.

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