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Effect of Green Synthesized Silver Nanoparticles Supplementation on Broiler Growth, Carcass Traits, Blood Indices and Cecal Microbial Load

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Abstract: This study evaluated the effects of green-synthesized silver nanoparticles (AgNPs) derived from garlic and ginger extracts on broiler nutrition at 4, 8, and 12 mg/kg concentrations. Characterization showed spherical AgNPs with an average diameter of 240 nm, O–H and C–H functional groups, and an elemental composition of 22.03% silver along with traces of Cl, C, N, Na, and S. XRD analysis revealed a crystalline structure with prominent peaks at 2θ angles of 38.10°, 46.05°, 64.04°, and 76.36°, corresponding to specific planes. Growth performance results indicated that increasing AgNP concentrations led to a significant rise in body weight, with a final weight of 745.30 g at 12 mg/kg compared to 510.39 g in the control and a reduction in feed conversion ratio (FCR) from 2.78 to 1.59. Carcass evaluation showed the highest dressing and organ weights at 12 mg/kg, while kidney and gizzard weights peaked at lower concentrations. Liver function remained unaffected, while renal function tests showed increased creatinine, urea, globulin, total protein, and albumin values at specific concentrations. Antimicrobial analysis demonstrated reduced fungal and E. coli counts at higher AgNP doses, although aerobic bacterial growth increased. In conclusion, supplementing broiler diets with 12 mg/kg of AgNPs positively impacted growth, carcass traits, liver and renal health, and microbial balance in the intestine.

Keywords: Silver nanoparticles, Green synthesized AgNPs, Broiler, Microflora, Growth performance

Introduction

Antibiotics are extensively employed in poultry feed to promote growth and mitigate disease prevalence (El-Abd, Hamouda, Al-Shaikh, & Abdel-Hamid, 2022) particularly in broilers, where diseases significantly threaten the poultry industry's development in emerging economies. These diseases often stem from high levels of pathogenic bacteria, molds and yeasts adversely affecting flock health, meat quality and economic viability (Hamouda, Youssef, Saleh, El Sabry, & Nasr, 2023). Unfortunately, the efficacy of antibiotics is diminishing due to the global rise of antibiotic-resistant microbes. While new antibiotics are essential, their development is time-consuming and costly, meanwhile, the infections led by resistant strains keep increasing worldwide (Bruna, Maldonado-Bravo, Jara, & Caro, 2021). Eventually, the notion of nanotechnology has emerged as a promising solution for resistant microorganisms, involving the development, alteration and imaging of nanostructures, known as nanoparticles, with size ranges of 1–100 nm. With applications spanning various sectors including food packaging, veterinary, agriculture, electronics, medicine and healthcare offers diverse possibilities. Nanoparticles (NPs) are categorized into metallic and non-metallic forms (Alharbi, Alsubhi, & Felimban, 2022). Metal and metal oxide nanoparticles, distinctly Au, Ag and Se, being more capable and efficient than non-metal forms, have captured the attention of scientists (Abbasian & Jafarizadeh-Malmiri, 2020).

Silver (Ag) has a long history of use as an antimicrobial agent (Khan et al., 2022). Likewise, silver nanoparticles (AgNPs) have also been shown to possess significant antimicrobial properties. These are utilized in the poultry industry to prevent or abolish antibiotic-resistant strains, hence reducing mortality risks (Awaad et al., 2021). Astonishingly, green synthesized silver NPs are either less toxic or harmless to human beings (Abu-Dief et al., 2020), yet exhibit strong lethality against various prokaryotic cells like bacteria, fungi and viruses (Samuggam et al., 2021). The antimicrobial mechanism of silver ions involves the generation of reactive oxygen species (ROS) within microbial cells, leading to cascade of disruptive effects such as DNA and mRNA inhibition, cell membrane and cell wall destruction, protein synthesis inhibition, mitochondrial dysfunction and ETP (electron transport chain) inhibition (Jain, Pawar, Sarkar, Junnuthula, & Dyawanapelly, 2021). AgNPs possess unique chemical, electrical, optical magnetic and mechanical properties, that make them valuable in nanomedicine for antimicrobial, anti-plasmodial, antifungal and anti-cancerous, as well as for purposes like sensing, imaging, targeted drug delivery and wound healing (Alharbi et al., 2022). Taking into account their antimicrobial nature, the AgNPs have successfully been utilized in biolabeling, food packaging, electoral devices, wastewater treatment, paint and ceramics (Abbasian & Jafarizadeh-Malmiri, 2020). Additionally, these are deemed excellent alternatives to antibiotics because of their prevalent antimicrobial activities (Bruna et al., 2021).

The novelties in the synthesis of NPs have revolutionized various research domains. Traditionally, NPs are developed through three approaches encompassing physical, chemical and biological. However, physical and chemical methods are less favored because of their low yield and toxicity, respectively. Green synthesis, which involves the utilization of medicinal plants or microbes to develop NPs, is gaining prominence as a preferred mechanism due to its energy efficiency, low toxicity, high yield, availability and cost- and time-effectiveness (Alharbi et al., 2022). Plant-derived phytochemicals, renowned for their numerous antioxidant and antimicrobial properties, enhance the capabilities of nanoparticles (Shahzad Shirazi et al., 2022). Several plant parts including stems, leaves, fruits, flowers, bark and roots are commonly employed in the development of AgNPs (Alharbi et al., 2022). Garlic (*Allium sativum L.*) is globally recognized as a food, spice and traditional medicine, possessing anti-cardiovascular, anticarcinogenic and anti-aging attributes. Its extract, being an abundant source of bioactive compounds, plays an essential role in the reduction process of metal NP synthesis (Shafea & Mahmoud, 2021). Similarly, ginger extract, also employed in NP development, is valued for its antioxidant, anti-inflammatory and anticancer properties attributed to its phenolic compounds, gingerols and shogaols (Bakr, Abdelgayed, El-Tawil, & Bakeer, 2020).

The study seeks to elucidate the effects of green synthesized AgNPs on broiler chickens, specifically focusing on growth rate, carcass characteristics and microbial load. Due to the problems associated with antibiotic resistance and achieving sustainable poultry farming, this study explores the potential of AgNPs as growth promoters and antimicrobial agents. Utilizing green synthesis techniques, the present study aims to evaluate both the yield response and the health benefits of AgNPs derived from garlic and ginger in broiler chicken. A key objective is to determine the optimal concentration of AgNPs for maximizing growth performance while avoiding the negative impacts. Overall, this research contributes to the existing literature reviewing the application of nanotechnology in poultry farming and society particularly in the growth and feed of broiler chickens hence informing the development of effective strategies for the improvement of the poultry farming ecosystem.

Materials and Methods

Procurement of Ingredients, Chemicals and Reagents

All the chemicals including $AgNO_3$ (99.9% w/w) were analytical grades and utilized without further purification after being acquired from Future Chemicals, Faisalabad, Pakistan. Bulbs of garlic and ginger were collected from a local farm in Faisalabad, peeled and rinsed with deionized water to remove contaminants. A total of 32 chicks (with an average weight of 26g) were purchased from the bird market in Faisalabad, Pakistan.

Preparation of Garlic and Ginger Extracts

The extracts were prepared following the method of (Keshari, Srivastava, Singh, Yadav, & Nath, 2020). The ginger and garlic extracts were prepared by making 19 grams of dried plant powder dissolving it in 100mL of deionized (DI) water. The white powder was combined with distilled water in a 250 mL beaker and heated at 55°C on a hotplate for 15 min with constant stirring and afterward left to cool. For filtration, Whatman grade 1 filter paper was used, and the resultant filtration was kept in a sealed container and was also being kept in a refrigerator at 4 °C for further use.

Development of Garlic and Ginger Based AgNPs

Garlic and ginger extracts-based silver nanoparticles were prepared according to the guidelines of (Shafea & Mahmoud, 2021). The green silver NPs were developed by dropwise addition of mix extracts (20 mL) to 50 mL of 0.05 M silver nitrate ($AgNO_3$) while continuously stirring at 65 °C for 2 h. Formation of nanoparticles was indicated by the change in color from pale yellow to dark brown (Alzubaidi et al., 2023); (Baran et al., 2023); (Singh et al., 2023). The attained precipitate was washed out with methanol and deionized water to take out appropriate precipitation. In the end, calcination was done by placing the mixture in a furnace (350 °C) for 2 hours to remove extract residues. The received AgNPs were added in different proportions (T_1–T_3) to the chicks feed as shown in Table 1 and Table 2.

Table 1. Research designs howing treatment groups with their specific garlic and ginger extracts based AgNPs concentrations to be administered to chicks orally

Groups	Treatments
Control group (T_0)	No supplementation
Treatment group (T_1)	Green AgNPs supplementation (4 mg/kg)
Treatment group (T_2)	Green AgNPs supplementation (8 mg/kg)
Treatment group (T_3)	Green AgNPs supplementation (12 mg/kg)

Table 2. Percentages of feed ingredients fed to chicks at pre-starter (1–10 days), grower (11–21 days) and finisher (22–28 days) age stages

Ingredients	Pre-starter (1–10 days)	Grower (11–21 days)	Finisher 22–28 days
Corn	50.0	59.7	66.1
Wheat	5.3	5.2	5.2
Gluten	9.8	10.2	12.2
Soybean meal	27.2	17.3	10.14
Soybean oil	3.53	3.43	3.05
Lime stone	1.65	1.32	1.2
Dicalcium phosphate	1.92	1.81	1.85
Salt (NaCl)	0.38	0.38	0.38
Mineral premix	0.27	0.27	0.27
Vitamin premix	0.27	0.27	0.27
L-Lysine	0.54	0.59	0.56
DL-Methionine	0.27	0.05	0.05

Characterization Techniques

UV-Visible Spectroscopy

UV-vis spectroscopy analysis was done as described by Yassin et al. (2022). The green-synthesized silver NPs were diluted in distilled water (blank) and absorption of the blank solution was recorded in the 200–800 nm range using a UV-vis spectrophotometer.

FTIR Analysis

FTIR analysis was performed according to the description of Yassin et al. (2022) in order to evaluate the surface chemistry of green-silver nanoparticles. The silver nanoparticles were dispersed in a KBr matrix, later compacted to a transparent disc, pallet and used as a standard. The functional groups (stretches) were detected at resolution of 4 cm^{-1}.

XRD Analysis

A diffractometer mounted with a graphite monochromator was used to perform XRD patterns employing Cu-K radiation to analyze the crystalline form of biosynthesized silver nanoparticles as done by Yassin et al. (2022). To note XRD measurement on a film of the silver nanoparticles, step-scanning having 0. In the present study, the dose rate per step was 0.02 and the acquisition time per step was set at 5 sec. at 2-theta.

Scanning Electron Microscopy (SEM)

The technique was implemented to characterize samples. The electron beams scanned samples to generate magnified images in order to calculate nanoparticle size. The images were recorded at an applied potential of 5 kV via Nova NanoSEM.

EDX Analysis

The elemental composition analysis of biosynthesized silver nanoparticles was carried out through SEM coupled with an EDX analyzer following the method prescribed by Yassin et al. (2022).

Antimicrobial Activity of Silver NPs

Antimicrobial efficiency of green synthesized AgNPs was assessed against selected microbial strains (*Listeria monocytogens*, *Staphylococcus aurus*, *Bacilus* and *Salmonella*) using disk diffusion method as suggested by M1. The microbial cultures were uniformly plated on Muller Hinton Agar (MHA) media. Then, sterilized discs were aseptically transferred onto the MHA plates that have been inoculated beforehand. To the discs, 150 ppm strength of green silver nanoparticles in the solid state were used. The solvent and standard drugs like gentamycin and penicillin (20-30 µg), were utilized as negative and positive controls, respectively. Afterwards, MHA plates were incubated at 37 °C for the duration of 24 hours and inhibition zones were determined in mm.

Experimental Design

The research was conducted at the PARS campus, University of Agriculture, Faisalabad. A total of 32 chicks (with an average weight of 26g) were divided into 4 treatment groups with successive supplementation of AgNPs as shown in Table 1. Before the examination, all the chicks were adapted to the lab environment for one week. All the chicks were reared under the same conditions at an ambient temperature (25 °C) and humidity range of 50-70% with an appropriate supply of feed (Table 2) and water. Uninterrupted provision of light was made sure and the chicks were vaccinated on day 1 and 21.

Growth Performance

The growth performance variables were calculated at the end of every week from days 7 to 28. Weight gain and FCR were computed using the following formulas:

Weight gain = Final wt. – Initial wt.
FCR = Feed intake/Wt. Gain
Feed intake = Food offered – Food reused

Carcass Yield

At the end of the research period, 3 chickens from each treatment group were randomly chosen and starved for 12 hours. The birds were then weighed individually and slaughtered. The percentage live body weight was calculated on the relative weights of liver, heart, pancreas, gizzard, kidney, spleen, stomach, intestine, breast, thigh and abdominal fat. The dressing percentage was estimated using the weight of the dressed carcass against the weight of the live birds and further presented in percentage following (Reda et al., 2020).

Microbial Analysis

The caeca and ileum contents collected from intestines of broilers were analyzed for microbial profile analysis involving coli form bacteria, enterobacteria, anaerobic bacteria, lactic acid bacteria and *C. perfringens* were recorded according to Sheiha et al. (2020).

Blood Chemistry

Blood specimens with anticoagulant were collected to determine the serum bilirubin, alkaline phosphatase, AST, ALT, serum protein, albumin, globulin, A/G ratio, blood urea, serum creatinine, cholesterol, triglyceride, HDL, LDL and VLDL of the experimental animals using Randox kit of USA. The hematological variables were estimated through the help of the biochemical analyzer machine (Bio-Rad) and those include; hemoglobin, total leukocyte count, total red blood cells, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, lymphocytes, neutrophils, monocytes and eosinophils.

Statistical Analysis

Measurement of the differences of the values was done using basic parametric statistical method; one way ANOVA via Statistix 8.1 software. Dunnet comparison test was employed to compare means and the results were depicted as mean ± SE (n=8).

Results and Discussion

Development of Silver Nanoparticles

The extracts were interfused with $AgNO_3$ solution, turning color from pale yellow to dark brown as an indication of the formation of silver NPs. Similar change in color was observed by (Keshari et al., 2020) during the production of *Cestrum nocturnum*-based AgNPs where color changed from light yellow to dark brown. Such a change in color is attributed to SPR (Hamouda et al., 2023).

Fourier Transform Infra-red Spectroscopy Analysis

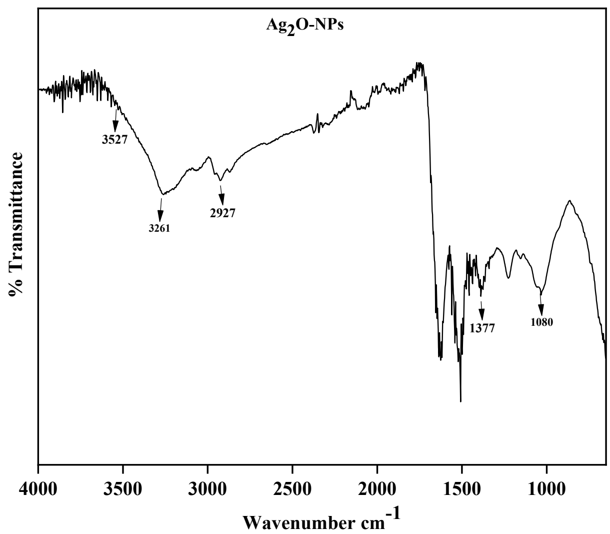

Figure 1. FTIR spectra of green synthesized AgNPs

FTIR analysis of garlic extract based green synthesized AgNPs exhibited distinct bands at 1153, 1377, 2927, 3261 and 3527/cm. The bands represent molecular vibrations: 1153 cm^{-1} is linked to O–H bonds in tertiary C–O–H groups, while 1377 cm^{-1} denotes C–H bond stretching. The stretching vibrations observed at 2927 and 3261 cm^{-1} are related to C–H and O–H bonds, respectively, with respect to garlic extract sugars. The bending vibration of C–N bond (3527 cm^{-1}) represents the presence of amines.(Keshari et al., 2020) analyzed peaks at 3477 cm^{-1} (O-H stretch), 3348 cm^{-1}(N-H stretch of amines and amides) and 2917 cm^{-1} (C-H stretch in methyl groups) in *Cestrum nocturnum* based green silver nanoparticles. The FTIR analysis (Fig.1) portrays the successful manufacture of silver nanoparticles. (Hassanen, Hussien, Mehanna, & Morsy, 2023) observed peaks in a chemo-/bio-synthesized silver granules as 3453, 2922 and 2864 cm^{-1} indicating O–H stretching, C–H stretching of alkanes and N–H bend of primary amines, respectively (Fig. 1).

Scanning Electron Microscopy Analysis

The images of AgNPs were taken at different magnifications i.e., 10,000, 25,000, 50,000 and 70,000X (Fig.2). The images taken below 50,000X lacked clarity and distinguishable identification of shapes, whereas images captured at 50,000X magnification demonstrated spherical shapes of particles. The SEM images at 70,000X depicted randomly arranged nanoparticles. The sizes of AgNPs ranged 100–350 nm with an average size of 240 nm (Fig.3). The findings are in line with the results of (Keshari et al., 2020) who observed spherical shape and random arrangement of silver nanoparticles at 80,000X in green synthesized silver nanoparticles developed using *Cestrum nocturnum*. Hamouda et al. (2023) observed a size range of biosynthesized AgNPs as 38.3–103 nm, while an average size of coffee extract based AgNPs was noted as 153 nm by (Abbasian & Jafarizadeh-Malmiri, 2020).

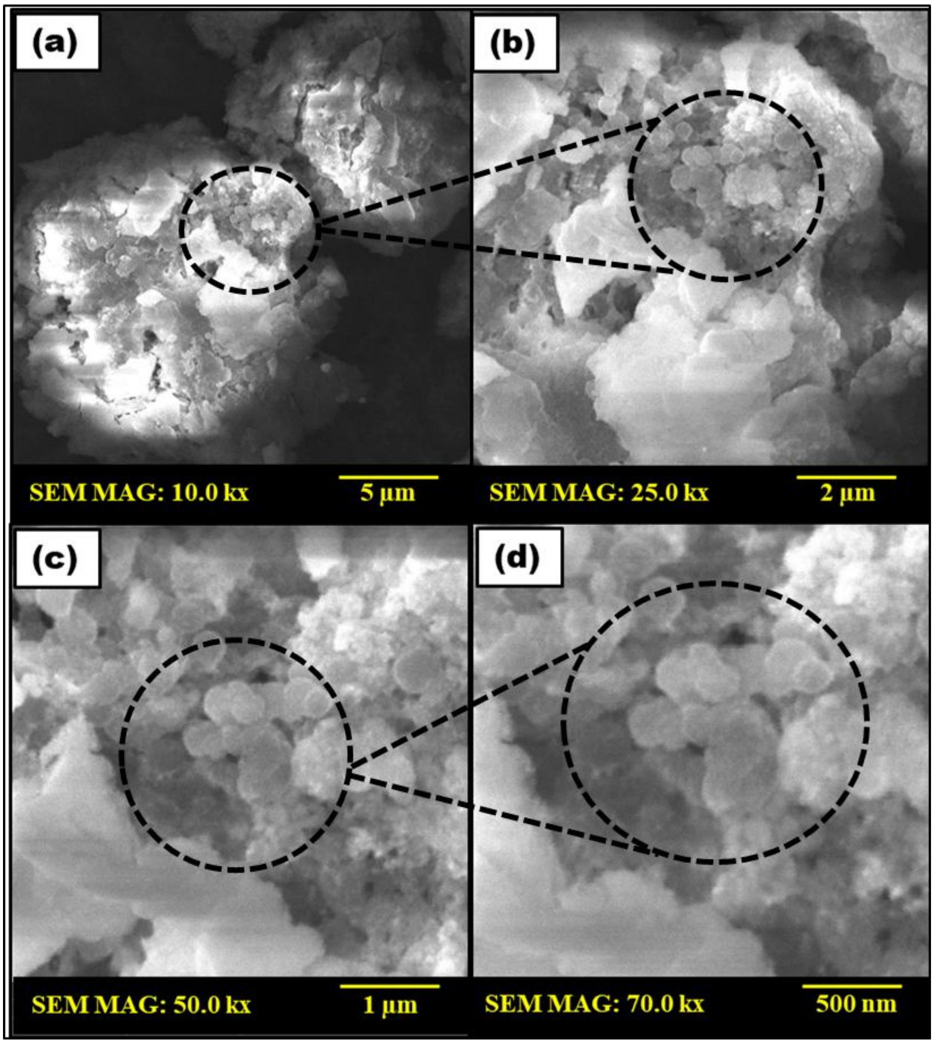

Figure 2. SEM images of AgNPs (a) at 10,000X magnification, (b) magnified at 25000X, (c) spherical shape Ag nanoparticles (50,000X) and (d) uneven surface and randomly arranged AgNPs (70,000X).

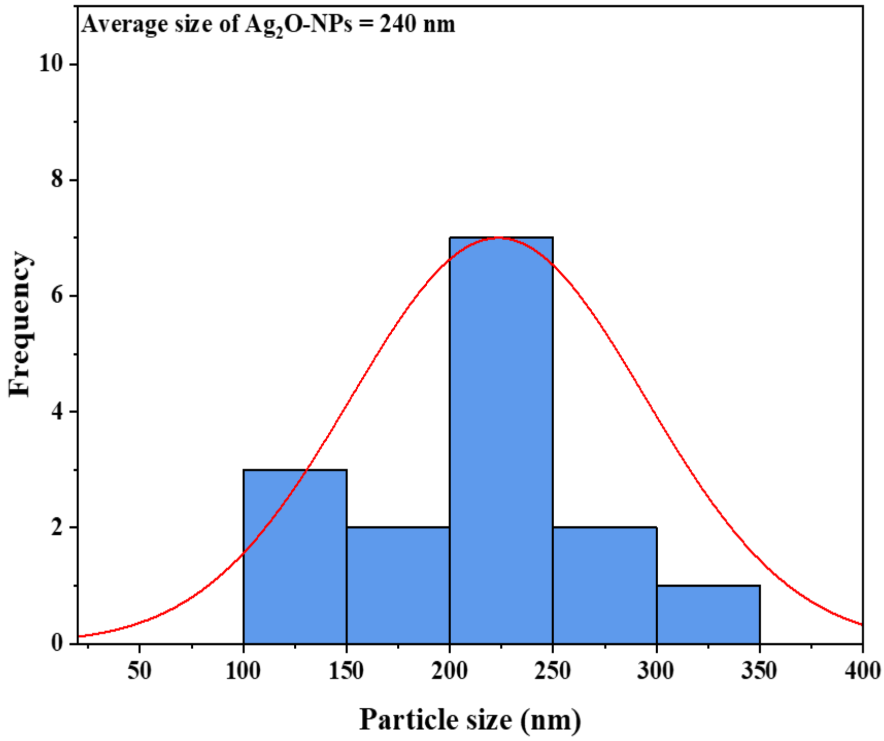

Figure 3. Distribution curve of frequency and particles size of spherical green AgNPs

EDX Analysis of AgNPs

EDX analysis depicts peaks stemming from characteristics X-ray emissions and elemental composition, exhibiting primary constituents of silver and oxygen, as well as certain impurities (Fig.4). The metallic silver showed 3.57 and 22.03% atomic and weight percentages, respectively. In a study by (Amin, 2020), *Ulva lactuca* based green Ag nanoparticlesillustrated the absorption peak with 51.72%. The significant peak detected at 2.98 keV indicates L_α transition (M–L shell, n=2 to n=3) of Ag, while a minor peak observed at 0.525 keV portrays K_α transition (L–K shell, n=2 to n=1) of oxygen.

Mohamed et al. (2021) observed strong absorption peaks at 2.4-4 keV for AgNPs prepared using garlic extract. The rest of the peaks signify the presence of impurities like Cl, C, N, Na and S elements. A detailed summary of the EDX spectra for the samples is presented in Table 3. EDX analysis of *Origanum majorana* leaf extract-based AgNPs performed by Yassin et al. (2022) revealed the presence of silver, oxygen, chlorine and carbon as 71.37, 3.69, 17.89 and 2.93%, respectively.

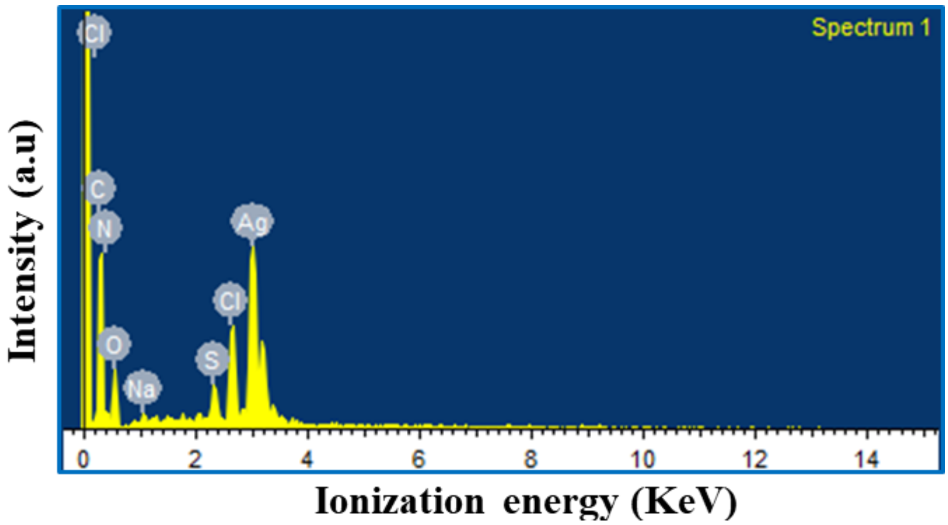

Figure 4. EDX pattern of Ag_2O nanoparticles

Table 3. EDX summary of Ag_2O NPs

Element	K ratio	Intensity corn.	Wt.%	Atomic%
Cl	0.02269	0.8654	3.95	1.94
C	0.07305	0.809	29.62	43.05
N	0.01737	0.1047	25.16	31.34
Na	0.00128	0.7476	0.58	0.44
S	0.00733	0.9974	1.27	0.69
O	0.01248	0.3032	17.39	18.97
Ag	0.12093	0.832	22.03	3.57
Total			100	

XRD Analysis of AgNPs

The XRD analysis, using Bruker D8 X-ray powder diffractometer, intended to analyze structure and crystalline nature of silver NPs, presented four significant peaks in the whole spectrum of 2θ, encompassing values as 38.10°, 46.05°, 64.04° and 76.36°, corresponding to planes at 111, 200, 202 and 311. The values are close to the findings of (Keshari et al., 2020) in *Cestrum nocturnum* based green AgNPs at 2θ, who observed peaks values as 38.06°, 44.23° and 67.43° at 111, 200 and 200 planes, respectively. The sample exhibited diffraction peaks underscoring standard silver and its face-centered cubic structure, confirming AgNPs formation. Moreover, additional peaks were also observed due to bioorganic phase of green extracts on the surface of NPs (Fig.5). The crystalline structure of Ag-NPs was noted. Sharpness of peaks is the reason behind highly crystalline structure of green AgNPs (Shaik et al., 2019). Keshari et al. (2020) also detected crystalline structure in silver nanoparticles developed using *Cestrum nocturnum*.

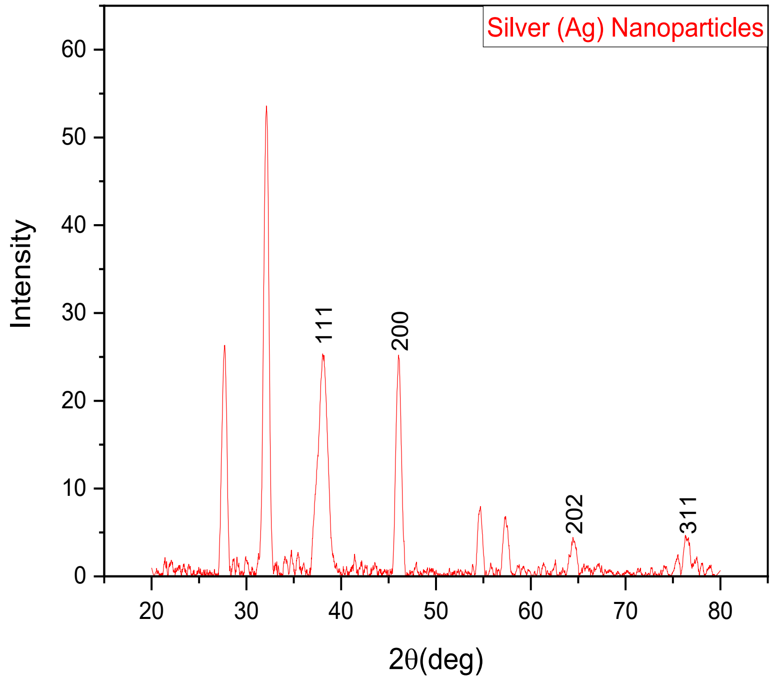

Figure 5. X-ray powder diffractometer

Growth Performance

The data (Fig.6) underscores that weight of broilers improved with the time and increasing concentrations of AgNPs. At day 28, the lowest weight was observed in control as 510.3g, followed by the groups fed 4 mg/kg, 8 mg/kg and 12 mg/kg green synthesized AgNPs, demonstrating body weights as 610.3, 675.7 and 745.30g, respectively. At the 28th day, the values for net body weight gain for control, 4 mg/kg, 8 mg/kg and 12 mg/kg groups were recorded as 262, 299, 361 and 440 g, respectively. The comparable effect of various dose levels of AgNPs (T_0-T_3) on body weight gain of chicks is presented in Fig.7. The results on FCR of broilers (Fig.8) elucidated that FCR decreased with increasing concentrations of green silver nanoparticles. Notably, control represented the highest FCR value as 2.78, while the littlest FCR scores were exhibited by 12mg/kg group as 1.59.

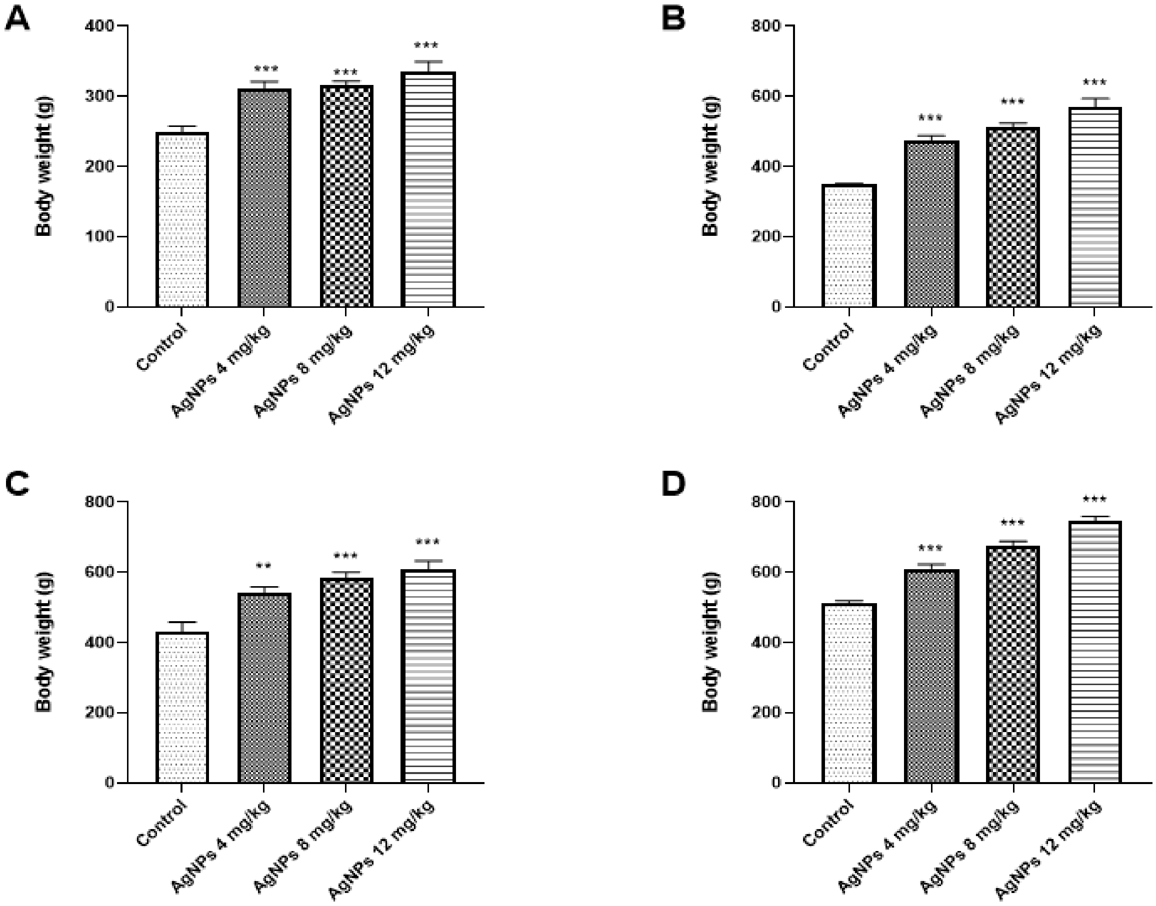

Figure 6. Graphical representation of weekly body weight of chicks
(A: is referred as 1st week, B: is the weight of chicks in the 2nd week of treatment, C: is the weight of 3rd week and D: is the weight of 4th week During all this period Chicks were treated with green synthesized AgNPs at the dose of 4 mg/kg, 8 mg/kg and 12 mg/kg).

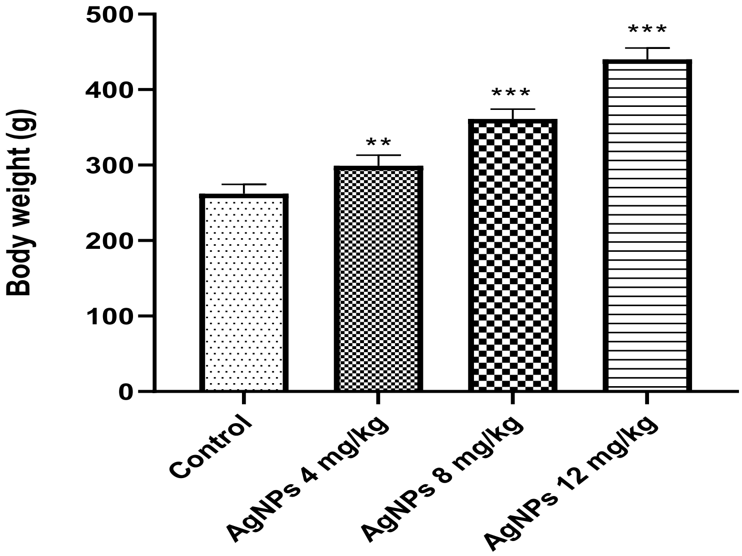

Figure 7. Graphical presentation of body weight gain of chicks with the different doses of green synthesized AgNPs

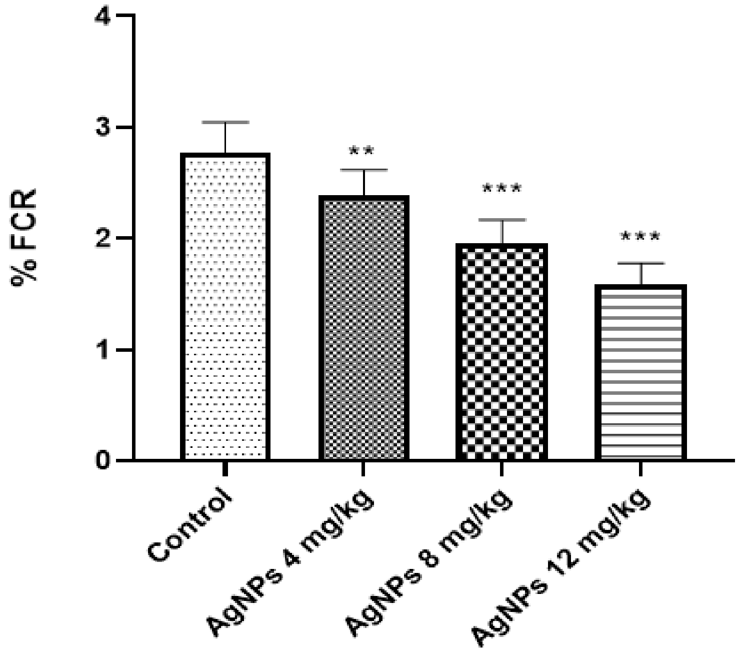

Figure 8. Graphical presentation of FCR of broiler chicks on treatment with different doses of green synthesized AgNPs

Similar trends were observed by (Keshari et al., 2020) while assessing impact of AgNPs dietary supplementation on growth and many other aspects of broilers in two phases (8–28 and 28–42 days). The findings suggested that AgNPs supplementations at 2.5 mg/kg demonstrated the highest body weight and weight gain as 2600 and 2480 g, respectively after 42 days. However, higher NP concentrations i.e., 20 mg/kg, notably suppressed these parameters. Additionally, the least FCR value (1.89) was noted at 2.5 mg/kg supplementation of silver nanoparticles. (Ahmadi et al., 2013) noted boosted growth rate in the chicks and reduced the feed conversion rate upon feeding broiler chicks AgNPs at 300, 600 and 900 ppm (Youssef, El-Banna, Elzorba, & Galal, 2019). Likewise, (Al-Sultan et al., 2022) observed that dietary incorporation of AgNPs enhances the final body weight of broiler chickens. Another study by Kumar et al. (2020) suggests that different concentrations of silver nanoparticles mixed with drinking water made growth performance of the broiler chickens much better than control group. The results of the current study are however different from those of Dosoky et al. (2021), who showed that silica-silver nanoparticles have no effect on the growth rate of the birds and the hematological, biochemical and oxidative stress parameters.

Effect of Silver NPs on Carcass Characteristics of Broilers

Carcass characteristics encompassing dressing, liver, heart, kidney, gizzard and thigh weights of broilers were calculated from each treatment groups (control, 4mg/kg, 8mg/kg and 12 mg/kg) on the final day of experiment. The findings portrayed in Table 4 emphasize that the broilers consuming 12mg/kg treatment group supplementation of AgNPs had the highest dressing weight (325g), liver weight (10.55g), heart weight (2.86g) and thigh weight (82.66g). The highest values for kidney weight (0.91g) and gizzard weight (14.58g) were noted for broilers feeding on 4 mg/kg and 8 mg/kg AgNPs treatment groups, respectively. (Awaad et al., 2021) observed that AgNPs had a significant impact on the dressing weight and the thigh weight but failed to exert substantial changes on heart or gizzard weight in broiler chickens.

Ahmad et al. (2022) referred that the nanoparticles @ 4, 8 and 12 ppm enhanced small intestine and abdominal fat weights in broiler chickens and were minimal on liver and gizzard percent weight. Further, in a study conducted by (Saleh & El-Magd, 2018) on broiler nutrition, an elevation in small intestine and liver's relative weights was delineated, while the gizzard, proventriculus & pancreas organs didn't show any variation due to the intake of various concentrations of AgNPs and silver nitrate. The improved and excellent broiler performance rate, carcass and relative dimensions of vital organs were analyzed in the group of broiler chickens with fed 2.5 ppm AgNPs as compared to the control group(Al-Sultan et al., 2022). In contrast, Al-Saeedi et al. (2021) reported that dressing % and the relative weight of heart and spleen in broiler chickens were not significantly influenced by the different levels of AgNPs interfused with drinking water.

Table 4. Effect of varied doses of green-synthesized AgNPs on carcass characteristics of broilers

Treatment Groups	Dressing weight	Liver weight	Kidney weight	Heart weight	Gizzard weight	Thigh weight
Control	256.66 ±4.05	9.07±0.01	0.50±0.02	2.50±0.28	13.35±0.23	44.66±3.17
4 mg/kg AgNPs	288.66 ±4.05**	10.45±0.35*	0.91±0.04***	2.45±0.27^{ns}	13.75±0.38^{ns}	54±1.70*
8 mg/kg AgNPs	307.33±2.60***	10.33±0.31*	0.86±0.04***	2.61±0.24^{ns}	14.58±0.712^{ns}	67.66±2.02***
12 mg/kg AgNPs	325±2.33***	10.55±0.33*	0.85±0.02***	2.86±0.26^{ns}	13.67±0.33^{ns}	82.66±1.45***

*Values are presented as Means ± SE (n=8). Significant differences are indicated by ** (p<0.01) and *** (p<0.001) when compared to the control group

Blood Chemistry

Liver functioning analysis was carried in order to estimate the potential toxic impact of green-synthesized silver nanoparticles' different doses on broiler chickens. The results (Table 5) suggest that ALT, AST, ALP, GGT and total bilirubin were enhanced with increasing doses in the green-AgNPs treated groups as compared to control, recording the topmost values at 12 mg/kg of green-AgNPs. At a 12 mg/kg of AgNPsdose, ALT, AST, ALP, GGT and total bilirubin presented the utmost values as 49.33 U/L (control: 15), 257.33 U/L (control: 124), 1025 U/L (control: 349), 59 U/L (control: 16.9) and 0.90 mg/dL (control: 0.48), respectively. Decreased serum AST activity was notedin subjected animal treated with 8 and 12 mg/L AgNPs, however there was no alteration in serum ALT (Pourgholam, Khara, Safari, Sadati, & Aramli, 2017).

Table 5. Effect of varied doses of green-synthesized AgNPs on liver functioning parameters of broilers

Parameters	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	Total Bilirubin (mg/dL)
Control Group	15±0.57	124±2.30	349.33±1.45	16.9±0.37	0.48±0.01
4 mg Ag NPs	18±0.57ns	138.66±1.45**	708.66±2.33***	24±1.15**	0.37±0.01**
8 mg Ag NPs	29±1.15***	218.33±2.02***	923±2.30***	39±1.15***	0.69±0.01***
12 mg Ag NPs	49.33±2.02***	257.33±2.33***	1025±2.30***	59±1.15***	0.90±0.01**

*Values are presented as Means ± SE (n=8). Significant differences are indicated by ** (p<0.01) and *** (p<0.001) when compared to the control group

Renal Function Analysis

Renal function analysis aimed at assessing key parameters such as creatinine, albumin, globulin, total protein and urea. The calculations (Table 6) indicate that creatinineand urea demonstrated the highest values in the broilers fed12 mg/kg green-AgNPs treatment group as 1.37 mg/dL (control: 0.91) and 23 mg/dL (control: 12), respectively, while albumin had the highest levels at AgNPs dosage of 4 mg/kg as 3.15 g/dL (control: 3.4). Globulin and total protein presented topmost values at a dosage of 8 mg/kg as 2.26 g/dL (control: 1.37) and 5.40 g/dL (control: 4.44).

The founded results of a study by Al-Sultan et al. (2022) showed that the total protein (mg/dL), total cholesterol, urea, creatinine and phosphorus of the broilers chickens that were treated with nanoparticles were significantly lower than those of the control group ($p < 0.05$). In research conducted by Ahmadi and Branch (2012), it was depicted that AgNPs-supplemented feed (20–40 ppm/kg) negatively affected the broilers blood lipid profile for the higher level of cholesterol, LDL and triglycerides.

Table 6. Effect of varied doses of green-synthesized AgNPs on renal function parameters

Parameters	Creatinine (mg/dL)	Albumin (g/dL)	Globulin (g/dL)	Total Protein (g/dL)	Urea (mg/dL)
Control Group	0.91±0.03	3.4±0.15	1.37±0.008	4.44±0.02	12±0.57
4 mg AgNPs	0.63±0.01 ns	3.15±0.02 ns	2.24±0.01 ***	5.14±0.08***	13±0.57ns
8 mg Ag NPs	0.40±0.29 ns	2.86±0.08 **	2.26±0.02 ***	5.40±0.11 ***	18±0.57**
12 mg AgNPs	1.37±0.01 **	2.2±0.05 ***	1.57±0.01 ns	4.67±0.03 ns	23±1.15***

*Values are presented as Means ± SE (n=8). Significant differences are indicated by ** (p<0.01) and *** (p<0.001) when compared to the control group

Microbial Profile Analysis

The antimicrobial activity of varied concentrations of green-AgNPs was evaluated against a number of bacterial strains, encompassing *E. coli, P. aeruginosa, C. diphtheria, B. subtilis, S. aureus, B. cereus, S. typhimurium and Serratia marcescens* using standard methods. The results (Table 7) disclosed significant differences (p<0.001) in the broilers fed 12 mg/kg green silver nanoparticles compared to control group. With respect to fungal content, control group showed value as 4.1×10^{1} CFU/g, while values at 4, 8 and 12 mg/kg were recorded as 2.6×10^{1}, 2.2×10^{1} and 2.0×101 CFU/g, respectively. Total aerobic bacteria were enhanced from 2.2×10^{5} (control) to 3.1×10^{6} CFU/g (12 mg/kg AgNPs dose) with increasing concentrations of AgNPs. As for coli group bacteria, the colony forming units decreased significantly (p<0.001) from 3.6×10^{5} (control) to 1.1×10^{5} CFU/g (12 mg/kg AgNPs dose). The outcome of the current study on antimicrobial activity revealed significant effect against different strains of bacteria. These findings were in-line with the previous studies on the antimicrobial activity (Alavi & Hamblin, 2023; Hayat et al., 2023; Wasilewska et al., 2023).

Table 7. Effect of varied doses of green-synthesized AgNPs on microbiological analysis of the contents of the jejunum and ceca of broiler (CFU/g)

Parameters	Total number of fungi	Total number of aerobic bacteria	Total number of E.coli group bacteria
Control	4.1×101	2.2×105	3.6×105
4mg/kgAgNPs	2.6×101 ***	2.5×106 **	2.2×105 **
8 mg/kgAgNPs	2.2×101 ***	2.8×106 **	1.5×105 ***
12 mg/kgAgNPs	2.0×101 ***	3.1×106 **	1.1×105 ***

*Values are presented as Means ± SE (n=8). Significant differences are indicated by ** (p<0.01) and *** (p<0.001) when compared to the control group

The study demonstrates that green synthesized AgNPs from garlic and ginger extract had an overall positive impact on broiler growth rate and carcass characteristics, particularly at 12 mg/kg of AgNPs, whereas higher kidney and gizzard weights were observed at low concentrations. Liver function remained stable across treatments, whereas renal function exhibited dose-dependent changes, underlining the need for dosage control. It was observed that green-synthesized AgNPs possessed the antimicrobial potential in the given context and significantly depicted the reduction in the fungal and coliform bacteria but a slight increment in the aerobic bacteria pointed towards further research. In conclusion, feeding broilers 12 mg/kg of AgNPs opens up a beneficial potential to enhance poultry production but since this field is relatively new, it becomes important to conduct more research to determine safety measures and gain the most benefits.

Scientific Ethics Declaratıon

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

*All animal experiments performed in this study complied with the guidelines of the Institutional Review Board (IRB), University of Agriculture Faisalabad. Each of the abovementioned methods was done in a manner that complied with the relevant guidelines and regulations. All surgical operations were performed following the guidelines of the Institute of Laboratory Animal Resources, Commission on Life Sciences University, National Research Council (1996).

Conflict of Interest

* The authors declare they have no conflict of interest.

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Authors' Contribution Statement

* Arifa Mehreen & Adnan Afzal: Conceptualization, Supervision, Muhammad Saleem & Muhammad Rizwan Javed: Data curation, Investigation, Methodology, Hafiz Aamir Ali kharl: Writing –original draft, Data curation, Investigation, R&D.

Data Avaılabılıty

* The data supporting the findings of this study are available upon reasonable request from the corresponding author. Access to the data will be provided contingent on compliance with any relevant ethical and legal requirements. For any inquiries regarding data availability, please contact the corresponding author directly.

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