Research Article

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Influence of biosynthesized silver nanoparticles using red alga Corallina elongata on broiler chicks’ performance

Abstract: Poultry meat is a great source of protein and provides lots of nutrients such as iodine, iron, zinc, vitamins, and essential fatty acids that humans require. The positive applications of metal nanoparticles (NPs) in the diets of various poultry species were studied, in relation to their metabolic, antibacterial effects on digestion and regulation of bowel function. This study was carried out to test the effects of fabrication green silver nanoparticles (AgNPs) of Corallina elongata extract and/or coating NPs with acetic acid on performance, immune response parameters and micro-flora population in Ross broiler. Chicks’ drinking water was mixed with bio-AgNPs (1 mM) and coating NPs with acetic acid for 35 days. Fourier-transform infrared spectroscopy, electron dispersive spectroscopy (EDS) analysis, scanning electron microscopy, and high resolution transmission electron microscope were used to determine the partial physiochemical characterizations of bio-AgNPs and coating ones. EDS analysis was used to determine the presence of AgNP in meat. Results confirmed that coating NPs with acetic acid reduced percentage of the micro-flora population, which were detected by VITEK® 2 system (BioMérieux, France) and identified as Pseudomonas orizihabitant 4211210040000210 and Sphingomonas paucimobilis 5201210040000210. EDS analysis of meat chicks confirmed disappearance of Ag metals. Coating biogenic AgNPs with acetic acid on modulated intestinal microbial populations of the Ross broiler may be safe, and could be used as alternative antibiotics or antibacterial agents besides their physiological performance in small intestines of broiler chicken.

Keywords: biogenic silver nanoparticles, Corallina elongata, mixing biogenic AgNPs with acetic acid, intestinal microbial populations, Ross broilers

1 Introduction

Antibiotics have extensive consumption in poultry feed to promote growth, convalesce feed efficiency and lower the prevalence of certain diseases [1–3]. Many countries search for a natural compound alternative to antibiotics; various nutritious supplementation were previously obtainable in the market such as organic acids, probiotics oils, and prebiotics [4–6]. Nanotechnology is one of the fields of science that defied it as an innovative technology, which is dealt with different element; finally produced and created materials; modification of structure, boosted quality and texture of foodstuffs at the molecular level [7]. This technology has a major power on fabrication, processing, transportation, safety, packing and traceability of food [8]. Biosynthesis of silver nanoparticles (AgNPs) has a simple method, is low cost and ecofriendly [9,10]. In many studies, algae were used to manufacture AgNPs that possessed efficiency against many bacterial pathogens and algae such as Oscillatoria limnetica [11], Corallina elongata and Gelidium amansii, [12], as well as Ulva fasciata [13,14]. Mixed organic acid has been found to promote the antioxidant characteristics and health status of broilers [15]. Besides, acidity harms the metabolism of pathogenic bacteria such as Acinetobacter baumannii, Pseudomonas aeruginosa, and Proteus vulgaris, at a low
concentration of 3% [16]. Using of metal nanoparticles as complements of animal diets has not always yielded clear results, the useful effect of metal nanoparticles used in animal production was most frequently detected in such parameters as weight gain, average daily gain, and health improvement [17,18]. Nano-metals are a promising nutrient additive, have bio-separation, signal processing, and are safe for animals [19]. AgNPs enhanced the growth of pigs and chicken in comparison to control [20]. The body weight of broiler chicken was enhanced when administrated with AgNPs; meanwhile there was no effect on the body weight, when broiler chicken was supervised with low concentrations of AgNPs [17,21]. The supplementation of acetic acid to AgNPs can improve the Ag ion release and possess high antibacterial efficiency [22]. Mixing of acetic acid to AgNPs improved immunological features, anti-oxidative function increased in serum and small intestine, elevated pancreatic digesting enzyme activity, improved expression of tight junction proteins, and altered cecum bacterial population, resulting in healthier broiler development [15]. The natural resource of plants had promising stabilizing and reducing agents with metallic ions which develop the approach of biological in NPs synthesis. They are safe from biohazard and ecofriendly which have multiple applications in pharmaceutical, and medicine manufacturing [23–27]. Antibacterial activities and cytotoxicity of green synthesis AgNPs differ according to the green sources that used for biosynthesis [12].

Therefore, the objectives of this study were to manufacture and study partial physiochemical characterizations of the new green AgNPs of red algae (C. elongata) extract. Then, evaluating those NPs and/or the mixed ones with acetic acid on the growth performance, carcass traits, some blood parameters of Broiler chickens, and distinguishing their possible antibacterial impact and identification of intestinal microbiota content.

### 2 Materials and methods

#### 2.1 Experimental design

This study was conducted in the Environmental Studies and Research Institute and Genetic Engineering and Biotechnology Research Institute, University of Sadat City for 35 days. A sum of 240 one-week broiler chicks (Ross breed) were randomly divided into four treatments: control or drinking water only (T1), biogenic AgNPs coated with acetic acid (T2; 5 mL·L\(^{-1}\) in drinking water), biogenic AgNPs mixed with drinking water (T3; adding 5 mL·L\(^{-1}\) of acetic acid in drinking water (T\(_3\)). Groups were allocated to three cage replicates (20 birds each) for each of the four groups in a completely randomized design. Treated drinking water was freely obtainable. Diets were subbed to be iso-nitrogenous and iso-caloric to cover all recommendations. The experimental diet was formulated to supply the nutritional requirements, as mentioned in Table 1, recommended by the National Research Council (NRC) [28].

#### 2.2 Biogenic of AgNPs and bio-AgNPs capping with acetic acid

The aqueous extract of red alga C. elongata was prepared according to our previous studies [12], where 10 mL of algal extract was added drop by drop to solutions of AgNO\(_3\) (1 mM AgNO\(_3\) was dissolved in 90 mL Double distilled water), with starrer at 60°C until the color became brownish [29]. For preparations of AgNPs capping with acetic acid (1:1) v/v, 50 mL of acetic acid (99.70 wt%) was added to freshly prepared AgNPs 50 mL and agitated for 30 min [22].

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**Table 1: Composition and calculated analysis of the experimental diet under this study**

| Ingredients                            | Starter diets | Grower diets |
|----------------------------------------|---------------|--------------|
| Ground yellow corn (8.5%)              | 50.58         | 58.54        |
| Soybean meal (44%)                     | 40.37         | 33.42        |
| Vegetable oil                          | 05.00         | 04.60        |
| Mono calcium phosphate                 | 00.25         | 00.25        |
| Sodium chloride                        | 00.30         | 00.30        |
| Vitamins and minerals mixture          | 00.30         | 00.30        |
| α-Methionine                           | 00.22         | 00.22        |
| Limestone                              | 02.00         | 02.37        |
| Total                                  | 100           | 100          |

**Calculated analysis**

- Crude protein (%): 22.08 for starter diets, 20.02 for grower diets
- ME (kcal·kg\(^{-1}\) diet): 3,110 for starter diets, 3,100 for grower diets
- C/P ratio: 140.0 for starter diets, 153.0 for grower diets
- Available phosphorous (%): 0.400 for starter diets, 0.400 for grower diets

Vitamins and minerals mixture at 0.30% of the diet supplies the following (kg of the diet): vitamin A, 10,000 IU; vitamin D3, 3,000 IU; vitamin E, 24 mg; vitamin K3, 2.1 mg; vitamin B12, 2 mg; riboflavin, 5.0 mg; pantothenic acid, 15 mg; niacin, 40 mg; choline chloride, 500 mg; folic acid, 0.9 mg; vitamin B6, 3.0 mg; biotin, 0.05 mg; Mn, 70 mg; Fe, 80 mg; Zn, 100 mg; Cu, 18.8 mg; I, 0.35 mg; Se, 0.30 mg. Crude protein in nutrient levels were analyzed values, other nutrients were calculated values: αL – methionine: 98% feed grade (98% methionine) according to NRC [29].
2.3 Partial physiochemical characterizations of biogenic AgNPs

UV-visible (UV-Vis) spectroscopy (Shimadzu UV-1601P Spectrophotometer, England) at wavelength 200–700 nm determines the intensity of peaks and absorptions wavelength of AgNPs sample after 1 h of synthesis. Surface morphology and distribution of AgNPs were examined by scanning electron microscopy JEOL JSM-6510/v. Japan. Energy dispersive X-ray (EDX) detector system (JEOL, JEM-2100, Japan) was used to distinguish the elements of the NPs sample [30]. The shape and size of formed, capped AgNPs and treated bacterial cells were characterized by high resolution transmission electron microscope (HR-TEM) (JEOL, JEM-2100, Japan). Fourier-transform infrared (FTIR) spectroscopy was used to determine the functional groups present in the biogenic AgNPs and those capping with acetic acid.

2.4 Measurements and methods of interpreting results

For the feeding trial, the following criteria were measured and/or calculated as follows: water consumption, feed intake (FI), feed conversion ratio (FCR), body weight gain (BWG), and growth rate (GR). Performance index (PI) was calculated according to the following relation [31]:

\[
PI = \frac{\text{Live body weight (kg)} \times 100}{\text{feed conversion}}
\]  

(1)

At the end of 35 days, blood samples were immediately taken during slaughtering and collected in heparinized tubes. Plasma constituents were determined calorimetrically, on individual bases, by using spectrophotometer, following the same steps as described by manufacturer in terms of plasma total protein (TP, g·dL⁻¹), albumin (Alb, g·dL⁻¹), globulin (Gl, g·dL⁻¹), cholesterol, and ALT (u·dL⁻¹), AST (u·L⁻¹) and total anti-oxidant capacity (TAC, mL·L⁻¹) were calculated and the A/G ratio was also calculated. The feedstuffs were analyzed for proximate analysis Association of Officials Analytical Chemists [32].

2.5 Meat preparation

Meat of broiler chicks was washed with triple distilled water to eliminate any contamination and then oven dried at 60°C and stored in plastic bags until needed. Drying takes about 3–4 days. After drying, they were grounded into fine powder using a mortar and pestle and stored in a well labeled air tight container for analysis [33].

2.6 EDX analysis of meat

Elemental analysis of meat powder after drying was carried out by EDX [33].

2.7 Determination and identification of intestinal microbiota contents

The ileum was chosen, whose function is to absorb the nutrients released from digestion; including amino acids, fatty acids and glucose, determined on the 35th day of rearing in three randomly selected chickens in each experimental group [34], to determine the total microbial count in intestinal content. Ileum samples (approx. 3 g) were collected immediately after sacrifice, homogenized of all the treatments, serially diluted in 10-fold in phosphate buffered saline. The plate count technique was used to count and enumerate bacterial community on LB medium (Merck, Germany) and was incubated at 37°C for 24 h [35]. The results were presented as colony forming units (CFU·mL⁻¹) in triplicate. The results were expressed as the mean ± standard error of log CFU of viable bacteria. Selected colonies were identified at the species level based on standard biochemical tests by VITEK 2 system version: 07.01 BioMérieux. HR-TEM was used to investigate the morphological changes of isolated bacterial species after treatment with biogenic AgNPs capping with acetic acid [36].

2.8 Statistical analysis

The statistical analysis for the feeding trials was achieved by using the general linear model procedures and significant mean differences between treatment means were distinguished [37,38]. All statements of significance were based on \( p \leq 0.05 \). The statistical model used was as follows:

\[
Y_{ij} = u + a_i + E_{ij}
\]  

(2)

where \( Y_{ij} \) – an individual observation, \( u \) – overall mean, \( a_i \) – effect of treatment \( (i = 1, 2, 3, 4) \), and \( E_{ij} \) – the experimental error.
3 Results

3.1 Characterization of AgNPs

3.1.1 UV-Vis spectroscopy analysis

The formation of bio-AgNPs was confirmed by color change followed by UV-Visible spectrophotometer analysis (Figure 1a and b). The UV-Vis spectrophotometer intensity peaks of biogenic AgNPs by *C. elongata*, and AgNPs coated with acetic acid were 416 and 411, where the intensity was 0.33 and 0.31, respectively (Figure 1).

3.1.2 FTIR spectroscopy

The results in Figure 2 demonstrate the FTIR spectroscopy analysis of AgNPs bio-fabricated by *C. elongata*.
and capping with acetic acid, there are differences in peak number and intensity, the peaks present with AgNPs biofabricated by *C. elongata* were 3,764, 3,444, 2,932, 2,377, 1,811, 1,642, 1,383, 1,149, 1,061, 895, 582, and 290 cm"⁻¹, meanwhile peaks present with AgNPs biofabricated coated with acetic acid are 3,781, 3,763, 3,452, 2,934, 2,366, 1,646, 1,385, 1,958, 602, 435, and 514 cm"⁻¹. Figure 2 represents change of the functional groups’ positions of biogenic AgNPs by *C. elongata*, from AgNPs coated by acetic acid, this denotes to action of the acetic acid capping agents.

### 3.1.3 EDX spectrophotometry

Results in Figure 3 show peaks that denote metal content of aqueous AgNPs biosynthesized by *C. elongata* and AgNPs capping by acetic acid inspected using EDX spectrophotometry. Three peaks were present in aqueous AgNPs biosynthesized by *C. elongata*, the peaks are Si, Cl, and Ag with mass 1.89%, 14.0082%, and 84.097%, respectively. Whereas eight peaks were present in Bio AgNPs capping with acetic acid. The presented peaks were of O, Na, Si, S, Cl, Ca, Ag, and Ti with mass 17.1772%, 1.4825%, 1.6194%, 2.6240%, 10.2755%, 4.4912%, 59.5837%, and 2.7465%, respectively. It is clear that Ag mass of the biogenic AgNPs (84.09%) was more in comparison to bio-AgNPs capping by acetic acid (59.58%).

### 3.1.4 HR-TEM

The size and shape of the NPs were demonstrated using HR-TEM. The spherical shape appeared in AgNPs and AgNPs coated with acetic acid. The particle size ranged from 11.39 to 41 nm in the case of biogenic AgNPs by *C. elongata* aqueous extract, meanwhile it was 11–26 nm...
3.2 Growth performance of broiler chicks

The effect of experimental treatments on body weight and its gain were obtained in Table 2. There was a significant variance among all treatments of final body weight; the heaviest final body weight was seen in T3 and T1. Also, the same trend was found in BWG. While T4 and T2 had the lowest body weight and BWG, the growth obstruction in T4 and T2 appeared to be a result of a depressed water intake convinced by supplementation of acetic acid in water.

3.3 Blood constituents of broiler chicks

Results of blood parameters at 35 days were influenced by AgNPs and its capping by acetic acid in drinking water of broiler chicks, as presented in Table 3. TP, Alb, Gl, total cholesterol, ALT, and AST concentrations were not significantly impacted due to the organization of AgNPs. Administration of AgNPs had no significant effect on all blood parameters except for TAC values.

3.4 Carcass characteristics

The effect of biogenic AgNPs and/or capping by acetic acid in drinking water of broiler on carcass weight percentage and relative weight of organs are tabulated in Table 4. No significant results were notable for dressing percentage and different parts of the carcass due to the impact of AgNPs. It can be noticed that the dose of AgNPs and acetic acid had no significant impact on carcass weight percentage.

Table 2: Growth performance of broiler chickens affected by using biogenic (AgNPs) and their NPs capping by acetic acid broiler chicken

| Items                                | Treatments | SE  | Sig. |
|--------------------------------------|------------|-----|------|
| Initial body weight (g) at day 7     | T1         | 145 |      |
|                                      | T2         | 145 |      |
|                                      | T3         | 143 |      |
|                                      | T4         | 144 | 0.40 NS |
| Final body weight (g) at day 35      | T1         | 1,850 a | 19.34 * |
|                                      | T2         | 1,600 b |      |
|                                      | T3         | 1,958 a |      |
|                                      | T4         | 1,550 c |      |
| Water consumption (ml per bird) (7–35 days) | T1         | 8,000 a | 190.1 * |
|                                      | T2         | 5,500 b |      |
|                                      | T3         | 8,000.6 a |      |
|                                      | T4         | 5,000.5 c |      |
| BWG (g) (7–35 days)                  | T1         | 1,705 b | 25.19 |
|                                      | T2         | 1,455 c |      |
|                                      | T3         | 1,815 a |      |
|                                      | T4         | 1,406 d |      |
| FI (g) (7–35 days)                   | T1         | 2,890 a | 22.35 * |
|                                      | T2         | 2,702 b |      |
|                                      | T3         | 2,931 c |      |
|                                      | T4         | 2,600 d |      |
| FCR (g feed per g gain) (7–35 days)  | T1         | 1,690 b | 0.05 |
|                                      | T2         | 1,85 a  |      |
|                                      | T3         | 1,61 c  |      |
|                                      | T4         | 1,84 a  |      |
| GR (%)                               | T1         | 170.9 b | 0.18 |
|                                      | T2         | 166.7 c |      |
|                                      | T3         | 172.8 a |      |
|                                      | T4         | 165.9 d |      |
| Livability rate (%)                  | T1         | 96.96 b | 0.28 |
|                                      | T2         | 94.60 c |      |
|                                      | T3         | 98.50 a |      |
|                                      | T4         | 92.20 d |      |
| PI (%)                               | T1         | 109.4 b | 5.10 |
|                                      | T2         | 86.4 c  |      |
|                                      | T3         | 121.6 a |      |
|                                      | T4         | 84.2 d  |      |

T1: control. T2: treated with biogenic AgNPs coated with acetic acid. T3: treated with biogenic Ag NPs (c) only. T4: treated with acetic acid only. Letters a, b, c, etc.: means within the same row with different superscripts are significantly different (p < 0.05) (mean ± SE).
Table 3: Biochemical analysis of blood constituents as affected by using biogenic (AgNPs) and their NP capping by acetic acid in broiler chickens

| Items                  | Treatments | SE | Sig. |
|------------------------|------------|----|------|
|                        | T1         | T2 | T3   | T4   |
| T P (g·dl⁻¹)            | 3.83       | 3.69| 3.33 | 3.51 | 0.19 NS |
| Al (g·dl⁻¹)             | 1.36       | 1.34| 1.23 | 1.29 | 0.05 NS |
| GL (g·dl⁻¹)             | 2.47       | 2.35| 2.10 | 2.22 | 0.14 NS |
| A/G ratio              | 0.55       | 0.57| 0.58 | 0.58 | 0.03 NS |
| Cho (mg·dl⁻¹)           | 99         | 88 | 93   | 84   | 0.32 NS |
| TAC (mL⁻¹)              | 0.73ᵃ       | 0.96ᵇ | 1.02ᵇ | 0.53ᵇ | 0.12 * |
| AST (unit·L⁻¹·dl⁻¹)     | 94         | 87 | 91   | 89   | 2.82 NS |
| ALT (u·L⁻¹)             | 49         | 41 | 43   | 42   | 0.59 NS |

T1: control. T2: treated with biogenic AgNPs coated with acetic acid. T3: treated with biogenic AgNPs (c) only. T4: treated with acetic acid only. Letters a, b, c, etc.: means within the same raw with different superscripts are significantly different (p < 0.05) (mean ± SE).

weight, dressing percentage, and various parts of carcass, such as the liver, heart, and spleen percentages.

3.5 EDS of boiler meats, treated with AgNPs in broiler chickens

A typical EDS measurement of the elements present in the meats were taken from the chicken that were treated with biogenic AgNPs and biogenic AgNPs capping with acetic acid and controls (Figure 5a–d). The results indicated that there was no accumulation of Ag metals in meats that were treated with biogenic AgNPs and biogenic AgNPs capping with acetic acid and controls, so there was no Ag toxicity, and the meats were safe to be consumed. There are elevations of Cu ions in both control and meat treated with AgNPs capping with acetic acid in comparison to meats treated with biogenic AgNPs. Low amount of aluminum was cleared in the meats treated with biogenic AgNPs capping with acetic acid only may be due to contamination of acetic acids with aluminum.

3.6 Evaluation of biogenic AgNPs their NP capped by acetic acid broiler chickens’ effects on pathogenic genus count in the ileum

The number of the microbial content in the ileum changed significantly after 35 days of broiler performance. The total number was, on average, 5 × 10⁸, 2.3 × 10⁶, 1.1 × 10⁵, and 3 × 10⁴ CFU·mL⁻¹ of control, biogenic AgNPs, biogenic AgNPs capping with acetic acid, and acetic acid 5 mL⁻¹, respectively (Table 5). Watering broilers supplemented with acetic acid + nanosilver resulted in a slight decrease in the number of microbial total counts in the intestinal content after 35 days of breeding in comparison to control treatment. The promising result was found after the watering supplementation with bio-AgNPs, where the reduction percentage was about 2–3 times less than the control treatment under this investigation. Cultivated colonies were sampled at various times during the growth phases and tested for evaluation of the effects of biogenic AgNPs capping with acetic acid in broiler chickens for intestinal microbiota total count in the ileum in Table 5.

3.7 Identification of microbiota in ileum samples

Cultured colonies were detected by the VITEK 2 as a rapid bacterial identification system that provides highly accurate and reproducible results. On the other hand, VITEK system identification patterns showed an acceptable confident was Pseudomonas orizihabitain 87% with bio number 4211210040000210. Isolate was positive for l-pyrolydonyl-arylamidase (PyrA), D-celluibiose (dCEL), H₂S production, D-glucose (dGLU), D-maltose (dMAL), D-mannitol, D-mannose (dMANE), D-trehalose (dTRE); coumarate (CM), and resistance (occurrence of resistance to vibriostatic compound) (O129R); for details, see Figure A1 in Appendix. Acceptable confident level was Sphinogomonas paucimobilis.
Figure 5: EDS analysis of boiler meats: (a) control, (b) treated with biogenic AgNPs coated with acetic acid, (c) treated with biogenic Ag NPs, and (d) treated with acetic acid.
86% (bio number is 5201210040000210), where nine biochemical tests out of 64 tests were positive alpha-phe-pro ary lamidase, L-PyrA, dCEL, dGLU, dMAL, dMNE; dTRE, CMT, and O129 O129R; for details, see Figure A2. Biogenic of AgNPs may have altered microbial ecosystem in the digestive tract by reducing colonization of pathogenic bacteria, such as P. orizihabitain and S. paucimobilis. A balanced intestinal microbiota is therefore essential for the performance of all food producing animals. Results in Figure 6 showed untreated and treated cells revealed changes under experimental conditions 24-hour exposure to biogenic AgNPs capping with acetic acid. Bacterial cell wall seemed to be affected or completely damaged with remarkable changes of flagella were found or lost after treatment of biogenic NPs in comparison with untreated cells (see Figure 6).

### Table 5: Effects of Ag-NPs and biogenic Ag NPs coated with acetic acid in broiler chickens on intestinal microbiota total count in the ileum

| Treatments                                      | Bacterial count (CFU·mL⁻¹) |
|------------------------------------------------|----------------------------|
| Control                                        | 5.0 × 10⁹                  |
| Treated with biogenic AgNPs coated with acetic acid | 2.3 × 10⁴                  |
| Treated with biogenic AgNPs only               | 1.1 × 10³                  |
| Treated with acetic acid (5 mL⁻¹) only         | 3.0 × 10⁷                  |

CFU – colony forming unit.

Marine algae produced various secondary metabolites; saccharides, phenolic compounds, fatty acids, alkaloids, and vitamins are working as a source of nutrition and an anticancer agent [39]. The color of the aqueous extract of seaweed after exposure to silver nitrate solution changed to brown, indicating the formation of AgNPs [40]. When acetic acid reacts with any metal ion, or any electropositive element compared to hydrogen that element replace the hydrogen from the acetic acid and give the metal acetate as final product along with hydrogen gas. Correspondingly in this case similarly silver replace the hydrogen ion and give the silver acetate as final product. Mixed organic acids with diet supplemented can maintain an

![image](image_url)

**Figure 6:** HRTEM examination of detected bacteria (a) and morphological effect of biogenic (AgNPs) capping with acetic acid in intestinal microbiota of broker chicken (b).
Impact of green AgNPs synthesized by red alga on chicks’ performance

The enhancement of growth by adding AgNPs in T3 particles had improved nutritional absorption by decreasing the intake of cell lines. Andi et al. [41] internalized as the surface area increased the more interacts (it did not enhance oxidative damage to liver DNA. Further activity, glucose, and cholesterol concentrations. Beside biochemical parameters of blood serum or liver enzyme activities, small NPs with a narrow size distribution are obtained [42]. The peaks at 400–450 nm in the UV spectrum confirmed the formation of AgNPs of NPs can be determined by UV-Vis spectroscopy [43]. Small size of AgNPs (10–50 nm) predictably has small absorbance peak near 400 nm, while bigger AgNPs (100 nm) give a broader peak with a maximum that shifts toward longer wavelengths close to 500 nm [44–46]. FTIR analysis exhibited a very sharp peak 1642.44 cm$^{-1}$ was observed with bio-AgNPs by C. elongata and 1646.3 cm$^{-1}$ with AgNPs coated with acetic acid, these peaks related to secondary amide C=O stretching of the proteins derived from red alga C. elongata, [47]. The peaks 1642.44 and 1646.3 cm$^{-1}$ are represented C=O stretching of the proteins, that act as reducing agent of Ag metal to AgNPs Abdel-Raouf et al. [47] stated that proteins possibly form a coat covering the metal NPs and prevent agglomeration of the NPs. Balaji et al. [48] denoted that extracellular protein compound can bind and synthesized AgNPs.

The free thiol groups existing in the proteins were caused reduction of silver nitrate to AgNP [49]. Zulfiqar et al. [50] represented that FTIR analysis revealed that the plant extract has decrease Ag ions to Ag atoms, so that coat them with hydroxyl and amine groups leading to significantly stable the antibacterial activity of AgNPs. The mixing of acetic to AgNPs can improve the Ag release and possessed high antibacterial efficiency [22]. According to our results, small AgNPs with a narrow size distribution can be synthesized with algal extract as a FITR analysis showed contains different functional groups hydroxyl and amine groups and polymers as proteins, polysaccharides leading to significantly stable the antibacterial activity of AgNPs in agreement with Prabha [51] reported, polymeric glycerol-AuNPs in with size ≥ 100 nm have been effectively internalized as the surface area increase the more interacts of cell lines. Andi et al. [52] revealed that AgNPs as feed supplementation (≥60 ppm) for 42 days had no effect on biochemical parameters of blood serum or liver enzyme activity, glucose, and cholesterol concentrations. Beside it did not enhance oxidative damage to liver DNA. Furthermore, Abd El-Ghany [53] reported, nano-silver ionic particles had improved nutritional absorption by decreasing the count of different pathogenic bacterial genera in broiler chicks. The enhancement of growth by adding AgNPs in T3 was like the mentioned results in literature who informed that weight gain was extensively improved by using nano-silver [54]. Furthermore, the body weight was improved at 25 ppm-kg$^{-1}$ and boosted lactic acid bacteria, when Japanese quail added with 5, 15, and 25 mg of AgNPs per liter in drinking water [55]. Meanwhile, it is informed that the level of 900 ppm of AgNPs had a significant amplify in live body weight in comparison with control group [56]. On the other hand, it is exhibited that AgNPs had not remarkable significant toxic effect on growth performance in comparison with control, when using at quantities of 20, 40, and 60 ppm-kg$^{-1}$ of diets in agreement with Ahmadi and Rahimi [57] improved, the performance, digestive organs and on live body weight of broiler through starter period at levels till 50 ppm. The progressive results may be due to improve anabolic activities and the antibacterial properties of AgNPs impacting in microbial populations without inducing conflict and that may be due to the motivation of development and growth of birds and promote the rate of metabolism, so, this may lead to the enhancement of the growth of broiler [7]. In this study results showed that FI was increased by T3 recorded the highest FI followed by control, while the lowest FI recorded by T4 which complemented by delayed growth to be the concern of depressed water intake by the submission of acetic acid in water. Feed conversion improved at T3 and showed that feed eating amplified and FCR amplified by using nano silver in broiler diets [58].

In this respect, it was found that add concentrations of 25 and 50 ppm of AgNPs in broiler diets had no significant influence on FI and FCR in comparison to control [9]. The results may be due to the influence of ionic silver on pathogenic bacteria in intestine, which caused improvement of absorption of nutrients [58]. In broiler chickens, it is achieved that broilers taken AgNPs displayed heavier BW and high BWG than those of the control. The biological effect of AgNPs on harmful bacteria in intestine, which resulted in improving growth as the absorption of nutrients was increased as mentioned [17]. Otherwise, the AgNPs growth stimulatory impact might be recognized to the encouragement of digestive enzymatic activities, which led to enhance the absorbance of nutrients [59].

On the contrary, some previous research did not find an upgrading in growth performance in broilers fed a diet supplemented with AgNPs [60]. Significant differences ($p < 0.05$) were observed on viability rate % among the experimental groups, besides the worst mortality was observed in T2 group chicks. Meanwhile, the groups of T3 recorded moderate mortality compared to control. These results mentioned that nanosilver has a role in decreasing poultry mortality as reported by Abd-Elnaby et al. [61].
Similarly, it is displayed that all blood parameters were not significantly affected by AgNPs treatments as demonstrated in NZW female rabbits [62]. Another comparable investigation was conducted that the total cholesterol values were significantly lower in the broiler chicks fed a diet supplemented with AgNPs, compared to those in the control group were explained that broiler chicks treated with AgNPs produced significant variations in total Alb, GI, and TP [63]. Correspondingly, in rats, it is conveyed that oral administration of AgNPs had an insignificant influence on the relative weights of spleen, liver, heart, and kidney [64]. On the contrary, in broiler chickens, it is found that AgNPs supplementation in drinking water or diet had a significant influence on dressing, liver and heart percentage [65]. EDS is a vital devise used for the elemental analysis or chemical characterization of biomasses [66]. Results agree with Law-mzuali [33] demonstrated that there were copper and zinc ions are present in meat samples such as beef, pork, lamb, chicken, and foal. It was confirmed that supplemented with 200 ppm of AgNPs was effectively decrease the microbial content than that of control chicks [67]. It was revealed that P. oryizihabitan polluted drinking water source and examined whether these bacteria are generally obtained in innately distributed water [60]. It was considered that the balanced intestinal microbiota is consequently necessary for the performance of all food producing animals including supporting digestion, protecting from pathogens, and producing nutrients of the host immune system as mentioned by Rangan and Hang [66]. It was proved that the biogenic synthesized AgNPs is an excellent antibacterial activity against bacterial pathogens [61]. Furthermore, it was informed that the adsorption of AgNPs on the bacterial cell wall reacting with thiol or SH group of exterior proteins interferes with their potential enzyme activities, leading directly inhibit proteins; frustrate respiratory chain, influence membrane permeability, and separate cell membrane from cell wall [68]. Otherwise AgNPs inhibit DNA replication and transcription [67]. Bacterial cell wall seemed to be affected or completely damaged, which had played vital role in secreting different virulence factors [69]. Remarkable changes of flagella were found, which improve capacity pathogenic bacteria through attractive and stimulation by adaptive responses, and adhesion stage [6,70], were absent or lost after treatment of biogenic NPs in comparison with untreated cells. According to our results, small AgNPs (10–42 nm) can easily penetrate and remarkable damaged was appeared on the pathogenic bacterial cells as mentioned by Abdel-Hamid [10] reported that biogenic Indole acetic acid-AgNPs leads considerable harmful in the outer membrane and intra-cellular contents as well of pathogenic bacterial cells.

5 Conclusion

The results of using biogenic AgNPs of drinking water supplementation exposed the body weight significantly enhanced in broiler given AgNPs and/or AgNPs capping with acetic acid compared with control chicks. Since gut bacteria have a key role in food digestion, the results demonstrated that ileal digest bacteria were encouragingly related to AgNPs. For consumers’ being concerns, AgNPs capped with acetic acid used as antibacterial activities, and hence encourage productive performance in poultry. Alternatively, residues of silver ions in chick’s meat may be toxic for human. Results confirmed that there are not any accumulated silver ions in chick’s meat with all treatments. So, biogenic AgNPs capping with acetic acid is a safe additive of broiler drinking water, alternative antibiotics and eco-friendly as well. Biogenic AgNPs or mixed with acetic acid were used for their antibacterial activity, which increase productive performance and immune response in Broiler chicks. From the consumers’ health concern, it is of interest to note that there is no accumulated silver in broilers meat.

Acknowledgments: The authors wish to thank Department of Microbial Biotechnology, Genetic Engineering and Biotechnology Research Institute and Department of Sustainable Development of Environment, Environmental Studies and Research Institute, at University of Sadat City, for their support this research work.

Funding information: Authors state no funding involved.

Author contributions: Ragaa A. Hamouda: conceptualization, investigation, formal analysis, methodology, validation, writing – original draft, visualization; Marwa Salah Abdel-Hamid: investigation, methodology, validation, writing – review and editing; Niamat M. El-Abd: conceptualization, data curation, formal analysis, validation, resources; Turki M. Al-Shaikh: writing – review and editing.

Conflict of interest: Authors state no conflict of interest.

Data availability statement: All data generated or analyzed during this study are included in this published article. These datasets are also available from the corresponding author on reasonable request.
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Figure A1: Identification of microbiota in ileum *P. orizihabitans* by VITEK 2.
Figure A2: Identification of microbiota in ileum *S. paucimobilis* by VITEK 2.