Effect of adding Silver Nanoparticles with drinking Water on some Lymphatic Organs and Microflora in the intestinal for broiler Chickens (ROSS 308)

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ABSTRACT

This study was conducted in the farm of domestic birds belonging to the Department of Animal Production, College of Agriculture, Al-Qasim Green University for the period from 21/3/2019 to 24/4/2019, Laboratory work was then conducted, where 225 chicks from broiler chicks and raised in 1x1.5 m cages, the chicks were randomly divided into five treatments and for each treatment contains three replicates, each replicate included 15 chicks. Nano-silver was used by adding it with drinking water in concentrations of (0, 20, 30, 40, 50 ppm. L⁻¹ drinking water) for the treatments (T1, T2, T3, T4, T5), respectively. Chicks were bred in the field for 35 days. The study showed the following results: A significant superiority (P <0.05) for the T5 treatment was obtained in the weight of bursa of Fabricius compared to other treatments. It was also found that there was a significant superiority (P <0.05) for the treatments (T1, T5) in the index of the bursa of Fabricius compared to the rest of the treatments. It was also observed a significant superiority (P <0.05) for the treatments (T4, T5) in the number of beneficial bacteria (Lactobacilli) in the Jejunum compared to the rest of the treatments. The excelling of the T5 treatment continued in the number of beneficial bacteria in the ileum compared to other studied treatments. As for the harmful bacteria (E.coli), the T1 treatment was significantly (T <0.01) excelled in Jejunum and ileum over other treatments at the age of 14 days from birds' age and at the age of 35 days from birds' age. The treatments (T5, T2) was significantly (P <0.01) excelled in the number of beneficial bacteria in the Jejunum. As for the harmful bacteria, the T1 treatment maintained on their superiority (P <0.01) compared to the rest of the experiment.

Keywords: Broiler chickens, nano-silver, spleen, bursa of Fabricius, nanotechnology.
INTRODUCTION

Since a long time, Poultry farmers have used antibiotics in poultry feed to increase growth, improving benefit efficiency from the feed and reducing the infection with some diseases (Doyle, 2001; McEwen and Fedorka-Cray, 2002; Butaye et al., 2003). However, in order to avoid the development of microbial resistance against antibiotics, poultry breeders in many countries have been restricted from using certain antibiotics because they pose a risk to consumer health and shifted attention to the search for alternatives to antibiotics and many substances have found that may be added to poultry feed, including additives available in the market such as organic acids, probiotics, prebiotics and essential oils (van der Wielen et al., 2000; Byrd et al., 2001; Chaveerach et al., 2004; Mitsch et al., 2004; Griggs and Jacob, 2005; Gunal Et al., 2006). Recently, there has been renewed interest in the use of silver as an antimicrobial. As it was known for a long time that the silver metal compounds and their ions possess unique properties as antibacterial, but the toxicity of silver ions was the reason for their low use and when the emergence of nanotechnology, its use became possible, where the provision of nanoparticles (1-100 nm) with a large surface area (Luoma, 2008) provided biological antibacterial properties against a wide spectrum of negative and positive bacteria (Lok et al., 2006; Shrivastava et al., 2007; Ahmed et al., 2010; Lara et al., 2010; Sawosz et al., 2011). These advantages make nano-silver a potent ingredient in pharmaceutical and medical products (Chen & Schluesener, 2008; Monteiro et al., 2009; Rai et al., 2009) as well as it can be used in poultry production (Parcival et al., 2005). It can also be used in poultry production (Parcival et al., 2005) as an alternative to antibiotics, Farhadi, (2010) noted that using nanosilver with a concentration of (5, 15, 25 ppm) in drinking water did not significantly affect on the growth and weight gain compared to the control treatment. Lane Pineda et al., (2012a) indicated that using nano-silver in drinking water for broiler chickens did not affect the growth performance nor on the microbial community in the intestinal tubule for the birds, and they also studied the effect of nano-silver on chicken embryos. Al-Jubouri, (2018) found that the injection of hatching eggs with nanosilver at a concentrations of (18, 16, 14, 12 ppm) improved the traits of hatching and productive traits for hatching chicks, also reduced the weights of lymphatic organs and increased the number of beneficial bacteria (Lactobacilli) and reduced the number of E. coli bacteria in Jejunum and ileum at the age of 7 and 35 days from birds' age. Therefore, this study aims to know the effect of adding silver nanoparticles with drinking water on some lymphatic organs and microbial communities in the intestinal tubule for broiler chickens (ROSS 308).
MATERIALS AND METHODS

Nano-silver was obtained from Nanosany company with the size of (20 nm) and morphological shape is a spherical shape. 225 chicks from broiler chicks and raised in 1x1.5 m cages, the chicks were randomly divided into five treatments and for each treatment contains three replicates, each replicate included 15 chicks. Nano-silver was used by adding it with drinking water as following:

T1: a control treatment without any addition
T2: Adding nano-silver to drinking water with a concentration of 20 ppm nano-silver / for drinking water.
T3: Adding nano-silver to drinking water with a concentration of 30 ppm nano-silver / for drinking water.
T4: Adding nano-silver to drinking water with a concentration of 40 ppm nano-silver / for drinking water.
T5: Adding nano-silver to drinking water with a concentration of 50 ppm nano-silver / for drinking water.

1- Nutritional treatment:

Chicks were fed on initiator diets (the percentage of protein 23% and content of energy (3027 kcal/kg feed)) from the age of one day until the third week from birds age, which they were then replaced by the growth diet (the percentage of protein 20% and content of energy (3195.3 kcal/kg feed)) Until the end of the fifth week, feed and water were provided free of charge (ad libitum) and the used diet was as shown in Table (1).

Table 1: Percentage of the used diet in the study and their chemical composition

| Feed materials          | Initiator diet % (1-21 day) | Growth diet % (22-35 day) |
|-------------------------|----------------------------|---------------------------|
| yellow corn             | 30                         | 40                        |
| Wheat                   | 28.25                      | 24                        |
| Soybean meal (48% protein) | 31.75                    | 24.8                      |
| concentrated Protein*   | 5                          | 5                         |
| Sunflower oil           | 2.9                        | 4.4                       |
|                      | Value 1 | Value 2 |
|----------------------|---------|---------|
| Limestone            | 0.9     | 0.6     |
| DCP Dicalcium phosphate | 0.7     | 0.9     |
| food salt            | 0.3     | 0.1     |
| A mixture of vitamins and minerals | 0.2     | 0.2     |
| Total                | 100     | 100     |
| Crude protein (%)    | 23      | 20      |
| Calculated metabolized Energy (Kcal/kg Feed) | 3027 | 3195.3 |
| Lysine (%)           | 1.2     | 1.1     |
| Methionine (%)       | 0.49    | 0.46    |
| Cysteine (%)         | 0.36    | 0.32    |
| Methionine + cysteine (%) | 0.85   | 0.76    |
| Phosphorus Available (%) | 0.45   | 0.49    |
| percentage of Energy: Protein C/P % | 131.61 | 159.77 |

* The concentrated protein (BROCON-5 SPECIAL W type): Chinese origin, each kg contains: 40% crude protein, 3.5% fat, 1% fiber, 6% calcium, 3% phosphorus available, 3.25% lysine, 3.90% Methionine + cysteine, 2.2% sodium, 2100 kcal/kg metabolized energy, 20,000 IU vitamin A, 40,000 IU vitamin D3, 500 mg vitamin E, 30 mg vitamin K3, 15 mg vitamin B1 + B2, 150 mg vitamin B3, 20 mg vitamin B6, 300 mg vitamin B12, 10 mg folic acid, 100 μg biotin, 1 mg iron, 100 mg copper, 1.2 mg manganese, 800 mg zinc, 15 mg iodine, 2 mg selenium, 6 mg cobalt, 900 mg antioxidant (BHT).

** The chemical analysis for the diet was calculated according to (NRC, 1994).
2- The studied traits

1. The relative weight of bursa of Fabricius and spleen

bursa of Fabricius was extracted by cutting the connective tissue around the bursa and then weighed by a sensitive balance for four decimal places and extracting the relative weight for the bursa according to (Luico and Hitchner, 1979) method.

The relative weight of the bursa (%)= \[
\frac{\text{The weight of the bursa}}{\text{The weight of the live body (g)}} \times 100
\]

The relative weight of the spleen was calculated according to the following equation (Fayad and Naji, 1989).

The relative weight of the spleen (%)= \[
\frac{\text{The weight of the spleen (g)}}{\text{The weight of the live body (g)}} \times 100
\]

Index of the bursa of Fabricius (%)= \[
\frac{\text{The relative weight of the bursa in the experimental treatment}}{\text{The relative weight of the bursa in the control treatment}} \times 100
\]

2. Calculating the numbers of microorganisms in the Jejunum and ileum

At the age of 14 and 35 days, two birds were taken from each replicate and after slaughtering the birds, they were dissected, the small intestine was extracted and The contents of the Jejunum and ileum area were then collected separately, where approximately 2 g was placed in a 5 mm tube for each replicates, which was kept at 20 °C in the refrigerator and microorganism numbers were then estimated as following:

3. Calculating the numbers of Lactobacilli bacteria in the Jejunum and ileum

one gram of the Jejunum contents was taken, for all the replicates and in sterile conditions and a Decimal dilutions to dilute (10) -10 was made from it using sterilized Peptone water by Micropipette, the numbers of lactobacilli bacteria were then estimated using Pour - plate method according to (Harrigan and Mc Cance, 1976) By transferring 1 mL of each decimal diluent to two empty and sterile Petri dishes and directly added to each dish 15 mL of agricultural media (MRS Agar) prepared instantaneously and preserved in a water bath at 46 °C and after hardening gar, it is add in agar and put a candle with it to consume oxygen and after covering it, it was placed in incubator at a temperature of 37 °C for 48 hours and the numbers of growing colonies were then calculated by multiplying the number of colonies × inverting the dilution.
4. Calculating the numbers of coliform bacteria in the jejunum and ileum

The numbers of coliform bacteria were calculated as in the case of the numbers of lactobacilli, but when transferring 1 ml of each decimal diluent to two of the sterile Petri dishes directly, 15 mL of sterile MacConkey Agar was added to each dish. After hardening the agricultural media in the dishes, it was kept inverted at 37 °C for 48 hours, The growing colonies in the dishes were then calculated as mentioned in No. (1).

3- Statistical analysis

The data were analyzed using the completely randomized design (CRD) to study the effect of the studied treatments on different traits, Significant differences between the averages were compared using Duncan's (1955) multiple range test. Statistical Analysis Software (SAS, 2012) was used in the statistical analysis according to the following mathematical model:

\[ Y_{ij} = \mu + T_i + e_{ij} \]

where:

\( Y_{ij} \): the value of view j for the treatment i.

\( \mu \): general mean for the trait.

\( T_i \): effect of treatment i (the study included the effect of five treatments mentioned above).

\( e_{ij} \): a random error that is normally distributed with an average of zero and a variance of \( \sigma^2_e \).

4- RESULTS AND DISCUSSION

1. Effect of adding silver nanoparticles with drinking water on the weight of bursa of Fabricius (g) and the index of the bursa of Fabricius (%) and spleen weight (g) for broiler chickens (Ross 308)

Table 2: Effect of adding silver nanoparticles with drinking water on the weight of bursa of Fabricius (g) and the index of the bursa of Fabricius (%) and spleen weight (g) for broiler chickens (Ross 308).

| Treatments | Average ± standard error |
|------------|--------------------------|
|            |                          |
The weight of bursa of Fabricius (g) | The index of the bursa of Fabricius (%) | Spleen weight (g) |
---|---|---|
T1 | 0.50 ± 2.83b | 0.15 ± 1.000a | 0.17 ± 2.17 |
T2 | 0.19 ±1.13bc | 0.05 ± 0.335c | 0.22 ± 2.13 |
T3 | 0.88 ±0.25c | 0.08 ± 0.260c | 0.04 ± 2.40 |
T4 | ± 2.21b 0.12 | 0.34 ± 0.660b | 0.27 ± 2.30 |
T5 | 0.83 ± 3.19a | 0.25 ± 0.950a | 0.58 ± 2.27 |

**Significant level** | * | * | NS |

Averages with different characters within one column are significantly different among them at a level * (P <0.05), NS: Non-significant.

The treatments (T1, T2, T3, T4, T5) are the control treatment without adding and adding (20, 30, 40, 50 ppm) of nanosilver with drinking water, respectively.

Table (2) shows the effect of adding silver nanoparticles with drinking water on the weight of bursa of Fabricius (g) and the index of the bursa of Fabricius and spleen weight (g). A significant superiority (P <0.05) for the T5 treatment was observed in the weight of bursa of Fabricius compared to the rest of the treatments, the two treatments (T1, T4) has also excelled on the T3 treatment. There were no significant differences between the treatments (T4, T2, T1) and between the treatments (T3, T2). As for the index of the bursa of Fabricius, the treatments (T5, T1) were significantly (P <0.05) excelled than the rest of the treatments. There was no significant difference between T3, T2, T5, T1 and spleen weight. There was no significant difference between the studied treatments, the T4 treatment also excelled on the treatments (T3, T2). The statistical analysis did not show a significant difference between the treatments (T3, T2) and the treatments (T5, T1). In spleen weight, there were no significant differences between the studied treatments. The reducing weight of bursa of Fabricius (g) in the T3 treatment and the decrease the index of bursa of Fabricius in the treatments (T4, T3, T2) compared to the control treatment may be due to the pathogenic antimicrobial nanosilver properties that affected the microbial community and may have changed the ratio between pathogenic and nonpathogenic organisms in the gut. According to Sondi and Salopek (2004), the silver nanoparticles affect the bacterial membrane, which leads to structural...
changes causing the death of pathological organisms, thus decreasing the weight of the bursa. This was confirmed by (Grodzik and Sawosz, 2006), where they asserted that nano-silver stood without the development of the bursa and increasing its weight due to the properties of nano-silver against germs that prevented the developing the weight of bursa of Fabricius. These agree with (Ahmadi et al., 2013) who indicated that using nanosilver (12 mg/kg) significantly reduced (P≤0.05) relative weight of bursa of Fabricius compared to the control treatment. This was confirmed by (Jubouri, 2018) who found that the injection of hatching eggs with silver nanoparticles caused a decrease in the weight and index of the bursa of Fabricius. As for the T5 treatment, which came with a high relative weight of the bursa of Fabricius. This may be due to the high concentration of silver nanoparticles used in this treatment, which has a negative impact on birds where these concentrations may form toxicity on the birds of the treatment, which suggests a broader study and more to find out the reasons for this.

2- Numbers of microorganisms in the intestinal tubule

1. Number of microorganisms in Jejunum and ileum at the age of 14 days.

Table 3: Effect of adding silver nanoparticles with drinking water on the numbers of bacteria at the age of 14 days (logarithmic cycle / g) for broiler chickens (Ross 308).

| Treatments | Average ± standard error | Jejunum | Ileum |
|------------|--------------------------|---------|-------|
|            |                          | Lactobacilli | E.coli | Lactobacilli | E.coli |
| T1         | 0.07 ± 5.90c             | 0.09 ± 4.04a | 0.02 ± 2.47c | 0.01 ± 3.94a |
| T2         | 0.82 ± 8.29b             | 0.03 ± 3.16b | 0.12 ± 3.41b | 0.04 ± 3.33b |
| T3         | 0.07 ± 7.39b             | 0.03 ± 3.19b | 0.26 ± 3.23b | 0.05 ± 2.79c |
| T4         | 0.13 ± 9.38a             | 0.01 ± 2.26c | 0.06 ± 4.39b | 0.01 ± 2.57d |
| T5         | 0.05 ± 9.66a             | 0.03 ± 2.02d | 0.02 ± 5.12a | 0.06 ± 2.21e |

Significant level

* (P<0.05), ** (P<0.01), NS: Non-significant.
The treatments (T1, T2, T3, T4, T5) are the control treatment without adding and adding (20, 30, 40, 50 ppm) of nanosilver with drinking water, respectively.

Table (3) shows the effect of adding silver nanoparticles with drinking water on the number of microorganisms in Jejunum and ileum at the age of 14 days from birds age. It was found that there was a significant superiority (P <0.05) for the treatments (T5, T4) compared to the other treatments in the number of beneficial bacteria (Lactobacilli). The treatments (T3, T2) were also excelled on the T1 treatment, there was no significant difference between the treatments (T3, T2) and the treatments (T5, T4). As for the number of harmful bacteria (E.coli), The T1 treatment (P <0.01) was significantly excelled compared to the rest of the treatments. the treatments (T3, T2) also excelled on the treatments (T5, T4). The T4 treatment has excelled on the T5 treatment, where the T5 treatment gave the lowest number of harmful bacteria. There was no significant difference between the treatments (T3, T2). In the ileum, the excelling of the T5 treatment continued in the number of beneficial bacteria where it significantly (P <0.01) excelled on the rest of the treatments. The treatments (T4, T3, T2) have excelled on the T1 treatment, There was no significant difference between the treatments (T4, T3, T2). As for the number of harmful bacteria, the T1 treatment has significantly excelled (P <0.01) compared to other treatments. The T2 treatment also excelled on the treatments (T5, T4, T3). The T3 treatment also excelled on the treatments (T4, T5).

2. Number of microorganisms in Jejunum and ileum at the age of 35 days from birds age.

Table 4: Effect of adding silver nanoparticles with drinking water on the numbers of bacteria at the age of 35 days (logarithmic cycle / g) for broiler chickens (Ross 308).

| Treatments | Average ± standard error |
|------------|--------------------------|
|            | Jejunum                  | Ileum                  |
|            | Lactobacilli | E.coli     | Lactobacilli | E.coli     |
| T1         | 0.19 ± 6.16c       | 0.10 ± 3.99a      | 0.04 ± 4.98d   | 0.12 ± 3.65a |
| T2         | 0.20 ± 9.31a       | 0.06 ± 2.27c      | 0.47 ± 6.87c   | 0.01 ± 3.03b |
| T3         | 0.04 ± 7.14b       | 0.08 ± 3.03b      | 0.13 ± 7.12b   | 0.03 ± 2.74c |
| T4         | 0.02 ± 8.88b       | 0.10 ± 2.21c      | 0.12 ± 6.95c   | 0.02 ± 2.53c |
Table (4) shows the effect of adding silver nanoparticles with drinking water on the numbers of bacteria in Jejunum and ileum at the age of 35 days from birds age. It was noticed in the Jejunum that there was a significant superiority (P <0.01) for the treatments (T5, T2) compared to the rest of the treatments as well as the treatments (T4, T3) have excelled on the control treatment (T1) and it did not show significant differences between treatments (T5, T2) and the treatments (T4, T3). As for the number of harmful bacteria, the control treatment (T1) gave the highest number from it, The T3 treatment has excelled on the treatments (T5, T4, T2) and there was no significant difference between the treatments (T5, T4, T2).

The superiority of the T5 treatment continued to ileum in the number of beneficial bacteria, where It was significantly excelled on the rest of the treatments, the T3 treatment also excelled on the treatments (T4, T2, T1). The treatments (T4, T2) have excelled on the T1 treatment and there were no significant differences between the treatments (T4, T2). As for the number of harmful bacteria, the T1 treatment was significantly excelled (T <0.01) on the rest of the treatments as well as the T2 treatment was significantly excelled on the treatments (T5, T4, T3). The two treatments (T4, T3) have excelled on the T5 treatment and statistical analysis did not show significant differences between the two treatments (T4, T3).

Sondi, (2004); Singh et al., (2008); Fabrega et al., (2009); El Badawy, (2011) found that the positively-charged Ag nanoparticles affect the bacteria, thus inhibit them. On the other hand, the bacterial cell membrane has been shown to be negatively charged (Vanderwal, 1997) due to the presence of carboxyl, phosphate and amino groups, which leads to electrical attraction between the silver nanoparticles and the bacterial wall, this increases cellular membrane damage leading to bacterial death (El Badawy, 2011). Chio and Hu, (2008) reported that nanosilver enters the bacterial cell and enters the DNA structure causing damage, which loses DNA replicability, transcription, and formation of the DNA as well as other cellular proteins and enzymes, especially enzymes producing ATP within the Bacterial cell, causing its death (Yamanaka et al., 2005). The interaction of nano-silver with sulfur and phosphorus in the bacterial cell membrane may also be another factor in the antimicrobial activity of nano-silver (Le et al., 2011, 2012). Cao et al., (2011) confirmed that the antibacterial properties of silver nanoparticles reduced the
reproduction of both types of studied bacteria Gram-positive bacteria and Gram-negative bacteria (Staphylococcus) from E-coli. This is the conclusion of our study where the nanosilver reduced the number of bacteria (E-coli) and increased the number of beneficial bacteria (Lactobacilli) during the duration of the experiment where the nanosilver has the ability to carry oxygen, thus it provides oxygen to the aerobic beneficial bacteria in the intestines of birds, which stimulates the growth and reproduction and this is reflected positively on birds. These results do not agree with (Sawosa et al., 2007) who stated that silver nanoparticles had no significant effect on the numbers of E-coli and microbial communities in the duodenum of quail. Pineda et al., (2012b) confirmed that using nano-silver in drinking water for birds was more influential on harmful bacteria in the intestines of birds as well as (Katarzyna et al., 2016). where they reported that nanosilver strongly stimulated the growth of beneficial bacteria in the jejunum in broiler chickens, these results agree with (Ogenik et al., 2016) who reported that nanosilver led to an increase in the total number of aerobic mesophilic bacteria and a decrease in the number of E-coli bacteria which are optional anaerobes in chickens compared to the control treatment. This indicates that nano-silver has a selective effect on bacteria from the gastrointestinal tract in chickens due to the antifungal effect of nano-silver (Feng et al., 2000; Lansdown, 2004; Wong, 2010), The reason is that nanosilver has the ability to interact with sulfur group found in the amino acids in the bacterial or bio-cell wall causing the formation of a strong -SS-bond that impedes the transfer of e- within the respiratory chain of the bacterial cell mitochondria, thus the death of harmful microorganisms (Choi and Hu, 2008).

CONCLUSIONS
The results revealed that nanosilver affected on the bursa of Fabricius but did not affect on the spleen. It also affected on the microbial community in the Jejunum and ileum where it increased the number of beneficial bacteria and reduced the number of harmful bacteria and further study is needed to determine the effect of silver nanoparticles and to what concentration can cause toxicity to birds.

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