Effect of adding different concentrations of nano silver to drinking water on some physiological and microbial traits of broilers ROSS 308.

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Abstract

This experiment was conducted in the poultry field of Al-Anwar Company in Babylon province for the period from 6/12/2021 to 9/1/ 2022.The aim of the study was to find out the effect of adding different concentrations of nano silver to broiler drinking water on some physiological and microbial traits. The 300 chicks were used "Ross 308 unsexed broiler chickens distributed randomly into 5 treatments with 3 replicates for each treatment and each replicate 15 chicks",The experiment treatments were as follows: (T1) the control treatment without addition, and the treatments T5, T4, T3, T2 adding silver nanoparticles at a concentration of (40, 30, 20, 10) ppm/liter, respectively. The study revealed the following: A highly significant increase (P≤ 0.01) in the concentration of ALT and AST enzymes, as well as malonaldehyde (MDA) in treatment T4 compared to the control treatment T1, and treatment T5 significantly (P≤ 0.01) in the concentration of glutathione peroxidase enzyme on all studied treatments. The concentration of low-efficiency lipoproteins (LDL) increased in treatment T5 and T3, but it was similar to that of the control treatment in this concentration. As for high-efficiency lipoproteins (HDL) its concentration increased by a significant level (P≤ 0.01) in treatment T4 on all treatments of the experiment. There was a significant decrease (p≤ 0.01) in the number of E.Coli in all studied treatments compared to "control treatment T1, while the number of Lactobacilli increased significantly (p≤ 0.01) in treatment T3 compared to control treatment T1.

Keywords: nano silver, microbial traits, broilers ROSS 308.
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Introduction

The science of nanoparticles is a newly emerging science that includes the manufacture and development of some materials into nanoparticles such as silver, gold and selenium. This science has quickly gained great importance in many areas, including health, nutrition, industry, medicine, and others (Naveen et al., 2010). The poultry industry has recently witnessed the use of nanotechnology in feeding and injecting hatching eggs (Jiang et al., 2008; Albanese et al., 2012; Al-Khafaji and AL-Jebory, 2019). The nanoparticles showed distinctive properties depending on their phenotypic shape, size, and chemical composition, Where their size ranged between 1-100 nanometers (Mansur et al., 1995), In particular, nanomaterials are characterized by occupying a very small area, but they have relatively large surface areas, and as a result, when massive materials are reduced to the nanoscale, they become more chemically affected surface and have variable physical properties that do not resemble the original materials but without changing their chemical properties (Albanese and others, 2012). Expanding the surface area-to-volume ratio allows nanoparticles to be more diverse and therefore

more used, as nanomaterials can be used alone or as carriers of other materials for the purpose of delivering them inside the body or coated with other materials (Sharma, 2012). Particles or nanoparticles are usually made using physical and chemical methods, but both methods are expensive and have negative environmental impacts, in addition to their need to provide special conditions of high pressure, thermal energy, and chemicals (Ravindran and Khan, 2013). Therefore, the biological method was resorted to, which is another way to manufacture nanoparticles, which is easier, inexpensive, safe, and does not have an environmental and health impact (Thakkar and Mhatre, 2010; Singhal et al., 2011). Recently, the trend has become to use the biological method, that is, to use biological sources such as microorganisms to produce nanoparticles (Rai et al., 2009).nano Silver are biologically synthesized compounds and are used because of their high efficacy as a broad-spectrum antibiotic for pathogenic bacteria that are resistant to antibiotics, as well as being one of the most well-known types of nanoparticles that are used in many applications (Rex et al., 2008). nano Silver have been used as alternative therapeutic solutions with high effectiveness and without side effects for antibiotics that have become useless in the treatment of some acute diseases due to bacterial resistance to those antibiotics (Arvizo, 2012).The reason is that the organism cannot easily develop resistance to silver nanoparticles because it inhibits the replication of the organism's DNA or causing damage to the plasma membrane of bacteria, thus losing the contents of the cytoplasm, or the silver nanoparticles inhibit the vital enzymes of bacteria (Zhou et al., 2012). Therefore, the current study aims to study the effect of different concentrations of nano silver added to Ross 308 broiler drinking water on productive performance and some physiological and microbial traits, and to determine the best level of addition.

Materials and methods

This experiment was conducted in the fields of Al-Anwar Company in Babylon province for 35 days, from 6/12/2021 to 9/1/2022. This is to show the response to oxidation indicators and microbial traits of broilers when adding nano-silver to drinking water. It used 300 unsexed Ross 308 broiler chicks, distributed randomly into 5 treatments, with 3 replicates for each treatment, and each replicate was 15 chicks. They were randomly distributed within pens with dimensions of 1 x 1.5 m.

Materials used in the experiment:

Sterile distilled water was used to dilute and prepare nano-silver, whereas liquid nano-silver was obtained from Nanosany Corporation in Iran and the size was (20 nm). Then it was prepared as follows:

1- 1 ml of nanosilver was mixed with 1000 ml of sterile distilled water to obtain a concentration of 1000 ppm.
2- Take 0.1 ml of the dilute solution per liter of water to get a concentration of 10 ppm.
3- Take 0.2 ml of the diluted solution per liter of water to obtain a concentration of 20 ppm.
4- Take 0.3 ml of the diluted solution per liter of water to obtain a concentration of 30 ppm.
5- Take 0.4 ml of the diluted solution per liter of water to obtain a concentration of 40 parts per million.

experiment treatments

a) The first treatment(T1) is a control treatment without an addition.
b) The second treatment (T2) is the addition of nano silver to drinking water at a concentration of 10 ppm/L.
c) The third treatment (T3) is the addition of nano silver to drinking water at a concentration of 20 ppm/L.
d) The fourth treatment (T4) was the addition of nano silver to drinking water at a concentration of 30 ppm/L.
e) The fifth treatment (T5) is the addition of nano silver to drinking water at a concentration of 40 ppm/L.

Chicks were fed on a starter diet (protein content 23% and energy quantity 3027 kilocalories/kg of feed) from one day of age until the third week of the birds' age ,After that, it was replaced with a growth diet(protein 20% and energy quantity 3195.3 kcal/kg feed) until the end of the fifth week and the feed and water were provided freely ad libitum and the diet was used, as shown in Table (1).

Table 1: shows the percentages of the components of the diets used in the experiment and their chemical composition

feed material	Starter diet 1-21 % day	Growth diet 235-35 days
yellow corn	30	40
wheat	28.25	24
Soybean meal (48% protein)	31.75	24.8
protein concentrate	5	5
sunflower oil	2.9	4.4
limestone	0.9	0.6
DCP . Dicalcium Phosphate	0.7	0.9
Mixture of vitamins and minerals	0.2	0.2
salt	0.3	0.1
Total	100	100
Crude protein (%)	23.04	20.06
The calculated representative energy (kilocalories / kg of feed)	3021.45	3194.92
Lysine %	1.27	1.07
methionine %	0.41	0.38
cysteine%	0.35	0.30
Methionine + cysteine %	0.82	0.78
% available phosphorous	0.41	0.43
c/p Energy Ratio : Protein %	131.14	159.77

* Brocon-5 Special W protein concentrate: of Chinese origin, each kg of it contains (40% raw protein, 3.5% fat, 1% fiber, 6% calcium, 3% available phosphorous, 3.25 % lysine, 3.90 % methionine + cysteine, 2.2% sodium, 2100 kcal/kg energy represented, 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 B3, 20 mg B6, 300 mg B12, 10 mg folic acid, 100 mcg 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)

** Chemical analysis of the diets was calculated according to the NRC (1994)

. protection program: Use the following health and preventive program, according to the company's program

Table 2: The protection program used in the experiment

age/day	The vaccine or vitamin used
1	Oil Vaccine (Newcastle + IB + Kimboro)
2-5	B-Complex vitamins + antibiotics
14	Newcastle + IB vaccine (eye instillation)

studied traits

1. Oxidation index measurements

These measurements include glutathione peroxidase, catalase, and malonaldehyde, as well as liver enzymes (AST and ALT).

1-1 .. Determination of ALT enzyme activity Alanine Amino Transferase ,This measurement was made using a kit supplied by the French company Orphee, according to the method of Reitman and Frankel (1957). The examination was conducted based on measuring the activity of the enzyme by colorimetric methods by measuring the pyruvic acid from alanine, as the pyruvic acid is reacted with the compound DNPH to form a complex of red color measured at a wavelength of 546 nm and it

was estimated according to the international unit/liter.

2-1.. Estimation of the enzyme activity (AST) Aspartate Amino Transferase for this measurement, a kit supplied by the French company 0rphee was used, according to the method of Rrtiman and Frankel (1957). This method is based on the enzyme's ability to convert aspartic acid to oxaloacetic acid, which spontaneously converts to pyruvic acid, which in turn reacts with 2,4-dinitrophenyl hydrazine (DNPH) to form a red complex measured at a wavelength of 546 nm.

3-1. Determination of glutathione peroxidase (GSH-PX) enzyme activity

Glutathione was measured using the method of Sedlak and Lindsay (1968), which is based on the use of a precipitation solution containing metaphosphoric acid (Na2EDTA). Sodium chloride (NaCl) was added and the solution was placed in a centrifuge at 4500 rpm for 10 minutes. The value of glutathione was estimated as the difference in the absorbance values of the samples in the presence or absence of DTNB and at a wavelength of 340 nm.

4-1. Determination of the activity of the enzyme catalase (CAT)

it used a kit supplied by the French company Orphee and based on Hadwan and Abed (2016) and the method that supports spectrophotometry to estimate the activity of catalase and that method depends on measuring the amount of hydrogen peroxide destroyed by the catalase enzyme and using Redox pigment. There was a change in the intensity of the color at a wavelength of 570 nm or fluorescence at a wavelength of 530/545 nm, which indicates the activity of the enzyme catalase in the sample.

5-1.. Determination of Malondialdehyde (MDA) level

Its concentration was measured using a kit from the French company Orphee, based on (Aust and Buege, 1978). This method determines the amount of lipid peroxides by measuring aldehyde, which is a product of the breakdown of lipid peroxide, and it takes place by reacting one molecule of Malondialdehyde and two molecules of thiobarbituric acid to form a red-colored MDA-TBA compound that can be measured at a wavelength of 535 nm.

2. Measurement of lipoproteins

This measurement includes both high-density lipoprotein (HDL) and low-density lipoprotein (HDL) proteins

1-2 . Measurement of the concentration of high-density lipoproteins (HDL)

A ready-made estimation kit was used based on the precipitation of LDL-c, VLDL-c and Chylomicron by Phosphotungesticacid and Mg+2 and magnesium ions. HDL-c in the upper filtrate which can be estimated using a cholesterol estimation kit,The reading was carried out at a wavelength of 546 nm and the concentration of HDL-c in the blood serum was calculated by using a kit equipped with the French company (BIOLO) and the examination was conducted based on the steps indicated by the manufacturer in the attached guide. The samples were read using a scale Optical spectrum, based on Burstein et al. (1970).

2-2.. Measurement of Low-Density Lipoprotein Concentration:

It was calculated according to the Friedewald formula (Assmann, 1993), as

LDL-c = cholesterol - (HDL + VLDL

3. Counting the numbers of microorganisms in the ileum

at the age of 35 days, 2 birds were taken from each duplicate. After the birds were slaughtered, they were dissected and the small intestine extracted. Then, the contents of the ileal region were collected separately, where approximately 2 gm was placed in a tube measuring 5 mm and for each replicate, it was kept at a temperature of 20°C in the freezer and then The number of microorganisms were estimated as follows:

1-3. Calculation of the numbers of Lactobacilli and E.Coli bacteria in the ileum.

1 g of ileum contents and all replicates were taken under sterile conditions and decimal dilutions were made from them up to (10)-10 dilution using sterile peptone water by Micropipette.According to Harrigan and Mc Cance (1976), by transferring 1 mL of each decimal diluent into two Duplicate Petri dishes and immediately adding 15 mL of instantly prepared MRS Agar in a 46° water bath to each dish. M .After the occlusal hardening, it was placed in a gar and a candle was

placed with it to consume oxygen. Then, after covering it, it was placed in the incubator at a temperature of 37 ° C for 48 hours, and then the number of developing colonies was calculated by multiplying the number of colonies by the inverted dilution

statistical analysis

The statistical program Statistical Analysis System -SAS (2012) was used in data analysis to study the effect of different treatments on the studied traits according to a complete random design (CRD), and significant differences between the means were compared with Duncan (1955) multinomial test.

Results and discussion

oxidation indicators

Table (3) shows the effect of adding different concentrations of nano silver to broiler drinking water on the concentration of alanine aminotransferase (ALT), aspartat amino transferase (AST), Glotathon peroxidase (GPX), catalase (CAT) and malondehyde (MDA),Where the results showed a highly significant increase (P ≤ 0.01 in the concentration of ALT enzyme for treatment T4 on all treatments followed by treatment T3 in terms of the increase in concentration on treatments T5 and T2, but it was similar to the control treatment in the concentration of this enzyme . Also, we note that treatment T5 was significantly similar in ALT enzyme concentration with treatment T3 on the one hand and with treatment T2 on the other hand and that treatment T2 recorded the lowest values in ALT concentration. As for the AST enzyme, we notice a highly significant (P≤ 0.01) increase in its concentration in favor of treatment T4 on all treatments, followed by treatment T2 in terms of increase Where it outperformed treatments T5, T3, T1, followed by treatment T3, which excelled treatment T5, where , for the control treatment, it was identical with both T5 and T3 in the concentration of AST enzyme. The glutathione peroxidase enzyme GPX, the T5 treatment with a level (P≤ 0.01) was superior to all the studied treatments in its concentration. The treatments T4 , T3 and T2 were highly significant (P ≤ 0.01) compared to the control treatment, but they were similar among themselves in the concentration of this enzyme. From the table, we also note that "there are no differences in the concentration of CAT enzyme among the studied treatments. Finally, "we note a highly significant increase in malondehyde in favor of treatment T4 on all treatments, and there were no significant differences between treatments T2, T3, T5, and control treatment T1 in MDA concentration. The reason for the increase in AST enzyme activity in T4 treatment, as well as ALT enzyme in T4 treatment, maybe due to the decrease in thyroid activity. Whereas between Kaplan and Larsen, (1985) there is a relationship between thyroid hormones and liver enzyme activity when a decrease in thyroid activity is accompanied by an increase in AST activity and thus a decrease in protein synthesis. It is worth noting that the enzymes ALT and AST are found in body tissues, but they are transferred to the blood serum after tissue lysis, and they are found in high concentrations in the liver and kidneys (Al-Daraji et al., 2008). Farhad, (2011) referred to the effect of nano silver in increasing the activity of ALT and AST enzymes, while Ognik et al. (2016) indicated that nano silver increase ALT activity, but reduces AST activity. This is inconsistent with Ahmadi et al., 2012); Andi et al., (2011) reported that silver nanoparticles had no effect on liver enzymes. As for the significant increase in the concentration of glutathione peroxidase in treatment T5.The reason may be due to the effect of nano silver on the increase of free radicals and oxidation, and the high concentrations of them work to increase the phagocytic cells and increase the connective tissue, and thus a case of focal necrosis occurs in the liver, which leads to an increase in glutathione and the expansion of veins (Kim, 2008).

Table 3: The effect of adding different concentrations of silver nanoparticles to the drinking water of Rose 308 broilers on the concentration of alanine aminotransferase (ALT), aspartate aminotransferase (AST), Glotathon peroxidase (GPX), catalase (CAT) and malondehyde (MDA) in blood plasma.

Treatments	mean ± standard error				
	MDA U/L	CAT U/L	GPX U/L	AST U/L	ALT U/L

T1	0.38± 4.90b	2.33± 47.17	18.29 0.87±c	13400 3.05±cd	2.99± 35.26 b
T2	0.61± 4.93b	2.18± 43.33	24.02 0.81±b	205.33 .7±44b	2.63± 16.03 d
T3	0.26± 5.28b	2.17± 45.66	23.42 1.45±b	149.67 4.33±c	3.50± 32.73 bc
T4	0.43± 7.75a	5.42± 37.45	24.69 1.46±b	727.00 4.72±a	4.07± 49.20 a
T5	0.39± 5.74b	1.42± 42.30	29.62 1.16±a	118.46 5.50±d	3.12± 22.86 cd
significant level	**	NS	**	**	**

** Different letters within the same column indicate a significant difference at the level (P<0.01).
Treatments: T1 as the control treatment (without addition), T2 adding nano silver at a concentration of 10 ppm /L, T3 adding nano silver at a concentration of 20 ppm /L, T4 adding nano silver at a concentration of 30 ppm / L, T5 adding nano silver at a concentration of 40 ppm /L.

2. Low density lipoprotein (LDL) and High density lipoprotein (HDL) concentrations.

The results in Table (4) show the effect of adding different concentrations of nanosilver to broiler drinking water on the levels of low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Where there was a significant increase (P≤ 0.01) in the LDL level for treatment T3, T5, but it was identical with the control treatment in this trait, followed by treatment T2, whose plasma LDL level was significantly higher than treatment T4.As for the high-density lipoproteins (HDL), there was a significant increase at a level (P≤ 0.01) in favor of treatment T4 on all treatments, and treatments T5, T3, T2, T1 and T1 were significantly similar in this trait. The significant increase in LDL and HDL levels in nanosilver addition treatments. It may be due to its high antibacterial property and thus works to change the environment of the intestines and cause an imbalance in the digestive processes of fats and thus changes in the process of excretion of steroids and reducing bile salts (Peter and Katbleen, 2003)or, the reason may be due to the increased oxidation of the LDL molecule and its aggregation in high concentration as a result of its loss of functional activity and change of shape, and then a decrease in the ability of receptors in liver cells to be recognized and taken, thus leading to an increase in cholesterol (Lewingtin et al., 2007).The LDL molecule is the main carrier of cholesterol, as it is taken from the various cells of the body for the purpose of using it in cellular construction and the manufacture of products that include fat in its composition and construction (Nelson and Cox, 2004).

Table 4: The effect of adding different concentrations of silver nanoparticles to Rose 308 broiler drinking water on Low-density lipoprotein (LDL) and High-density lipoprotein (HDL) concentrations.

Treatments	mean ± standard error	
	HDL	LDL
T1	1.84± 24.20b	1.66± 96.80a
T2	3.00± 24.50b	4.33± 69.20b

T3	0.25± 30.00b	3.60± 96.90a
T4	4.55± 44.06a	4.24± 50.73c
T5	0.90± 29.36b	4.84± 85.66a
Significant level	**	**

** Different letters within the same column indicate a significant difference at the level (P<0.01).
Treatments: T1 as the control treatment (without addition), T2 adding nano silver at a concentration of 10 ppm /L, T3 adding nano silver at a concentration of 20 ppm /L, T4 adding nano silver at a concentration of 30 ppm / L, T5 adding nano silver at a concentration of 40 ppm /L.

3. Microbial examinations

Number of microorganisms in the intestinal tubule (ileum) at 35 days of age

Table (5) shows the effect of adding different concentrations of silver nanoparticles on the preparation of E.Coli and Lactobacili microorganisms in the ileum at 35 days of age. Where it is noticed a "significant" decrease (P≤ 0.01) in the numbers of E.Coli bacteria in all the studied treatments compared to the "control" treatment. Then comes treatment T2, where the number of E.Coli bacteria in this treatment increased significantly on treatments T5, T4, T3 and also "the number of E.Coli bacteria increased significantly in treatment T3 on T5 and T4, while the lowest number was calculated for E.Coli bacteria It was in treatment T5 compared to treatment T4.As for Lactobacili, a highly significant (P≤ 0.01) was observed for the T3-treated birds in their numbers compared to the rest of the treatments.As for the treatments T5, T4, and T2, the number of Lactobacili bacteria was low compared to the control treatment, and the results also showed that the T4 treatment recorded a significant decrease (P≤ 0.01) in the numbers of the same bacteria, meaning that no numbers appeared in the intestinal tubule of these. Bacteria compared to T5 and T2 treatment. The results of our experiment indicate a decrease in the number of E.Coli bacteria with silver nanoparticle addition treatments compared to the control treatment. The reason may be that the positively charged nanosilver affects the bacteria and thus work to inhibit them through the attraction that occurs between the positively charged nanosilver and the negatively charged bacterial cell membrane due to the presence of the carboxyl group, the phosphate group, and the amino group, leading to increased cell membrane damage. And thus the death of bacteria (El Badawy, 2011).Or, the reason may be the ability of nano silver to enter the bacterial cell and become part of the DNA synthesis, causing “damage” so that the DNA loses the ability to replicate, copy and form DNA in addition to cellular proteins and enzymes, especially the enzymes producing ATP (adenosine triphosphate) inside the bacterial cell, which It causes her death (Chio and Hu, 2008).The researcher Le et al., (2011) found that nanosilver interacts with sulfur and phosphorous in the bacterial cell membrane, and this is what gives it antibacterial activity. Or, the reason may be that the silver nanoparticles have the ability to interact with the SH group (sulfur group) present in the amino acids in the bacterial cell wall. Or viral causes the formation of a strong -SS- bond that hinders the transfer of the e- electron within the respiratory chain in the mitochondria of the bacterial cell and thus the death of harmful microorganisms (Choi and Hu, 2008).The antibacterial properties of silver nanoparticles reduced both types of bacteria studied, i.e. Gram-positive and gram-negative staphylococci of E.Coli and this is what was found by the results of our current study, as silver nanoparticles reduced the numbers of E.Coli bacteria in the studied treatments compared to the control treatment, this is what was agreed On it Al-Saeedi et al. (2021).As for the increase in the numbers of the beneficial bacteria Lactobacilli in the T3 treatment, the reason may be due to the ability of the silver nanoparticles to carry oxygen, and thus it provides oxygen to the aerobic beneficial bacteria inside the

bird's intestines.Which stimulates the growth and reproduction of these bacteria and thus this is reflected positively on birds. This does not agree with Sawosa et al., (2007) who stated that silver nanoparticles had no significant effect on the numbers of microorganisms and the microbial community in the intestinal tube of quail.. As for other studies, the role of silver nanoparticles in stimulating and strongly stimulating the growth of beneficial bacteria in broiler broilers has also confirmed its role in increasing the total number of aerobic bacteria and decreasing the number of E.Coli bacteria, which are facultative anaerobes in chickens (Katarzyna, 2016; Ogenik et al., 2016).

Table 5: The effect of adding different concentrations of silver nanoparticles on the numbers of microorganisms in the intestinal tubule (ileum) at the age of 35 days for broilers (logarithmic cycle / g).

Treatments	mean ± standard error	
	Lactobacilli	E. Coli
T1	115.47±10000.00b	57.73± 8000.00a
T2	288.67± 6000.00c	173.20± 2600.00b
T3	288.67± 18000.00a	57.73± 1900.00c
T4	0.00± 0.00e	17.32± 1100.00d
T5	230.94± 5000.00d	11.54± 700.00e
Significant level	**	**

** Different letters within the same column indicate a significant difference at the level (P<0.01).
Treatments: T1 as the control treatment (without addition), T2 adding nano silver at a concentration of 10 ppm /L, T3 adding nano silver at a concentration of 20 ppm /L, T4 adding nano silver at a concentration of 30 ppm / L, T5 adding nano silver at a concentration of 40 ppm /L.

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