# EFFECT OF SILVER NANOPARTICLES AS A WATER SUPPLEMENTATION ON WATER CONSUMPTION, INTESTINAL MICROFLORA AND SILVER RESIDUALS IN SOME EDIBLE PARTS OF BROILER CHICKS

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## SUMMARY

*The aim of this study was to investigate the effects of silver nanoparticles (AgNPs) as a water supplementation on water consumption, microorganism in small intestine, ceca and silver residuals in some edible parts of broilers form 0 – 35 days of age. A total number of 150 (Cobb 500) unsexed broilers were divided randomly into 5 treatments (30 chicks each), with 3 replicates with 10 chicks each. The experimental treatments were prepared by supplemented drinking water with different levels of silver nanoparticles (0.0, 2.5, 5.0, 7.5 and 10 ppm). At the end of the trial four birds per treatment wereslaughtered to estimate the count of beneficial and harmful Bacteria in small intestine , ceca and silver residuals in some edible parts of broilers.*
*The results showed that: Water consumption for broiler showed no significant effect related to different levels of AgNPs. 2- The count of Lactobacillus spp. and E.coliin both small intestine and ceca was affected significantly by different levels of AgNPs. 3- The estimation of residual of silver in some tissues showed that AgNPs wasn't accumulate in breast or thigh muscles, but it deposed in liver tissues and increase with increasing AgNPs level in broiler drinking water.*

*In addition, inclusions of different levels of AgNPs as water supplementation have significant effects on intestinal microflora and silver accumulation in liver tissue.*

***Keywords: broilers, silver, nanoparticles, microflora, residual***

## INTRODUCTION

Several applications of nanotechnology for food and agricultural production are being developed in research and development (R&D) settings. Key international challenges are associated with animal production, including environmental sustainability, health, disease control, and feed security. Nanotechnology holds promise for animal health, veterinary medicine, and other areas of animal production (Scott, 2005). Nanomaterials, which used in animal and poultry production for instance such as silicon dioxide, calcium, magnesium; and silver nanoparticles for water purification or antimicrobial packaging or feed storage, zinc as a feed colorant. Titanium dioxide, a feed colorant used as a ultraviolet (UV) protection barrier in the feed packaging industry is an approved inorganic nanoparticle because it becomes transparent and also loses its ability to act as a feed colorant in its nanoform (El Sabry *et al.,* 2018).

Nanosilver is one of the most commonly used nanomaterials because of its strong disinfectant properties (Chen *et al*., 2007). Silver is a noble metal that has been known since ancient time to control microbial proliferation even against antibiotic-resistant bacteria (Wadhera and Fung, 2005). The inhibitory effect of ionic silver is due to several biological events such as attachment to cell membranes, its adsorption to the negatively charged bacterial cell wall, changes of membrane permeability, generating reactive oxygen species (ROS) and de-activating cellular enzymes (Loghman *et al.,* 2012).

Recent studies on use of silver in nano-size as an alternative to antibiotics and its probiotic properties with increasing immunity have led to use of this nanoparticle largely, especially in veterinary and dependent sciences (Sawosz *et al.,* 2007). Nanosilver was toxic to mammalian liver cells, stem cells, and even brain cells (Hussain *et al.,* 2005 and 2006).

Sondi and Salopek-Sondi (2004) studied the antimicrobial activity of silver nanoparticles (AgNPs) against *E. coli* was as a model for Gram-negative bacteria. The results confirmed that *E. coli* cells treated with AgNPs were damaged, showing formation of "pits" in the cell wall of the bacteria, while the silver nanoparticles were found to accumulate in the bacterial membrane. A membrane with such morphology exhibits a significant increase in permeability, resulting in death of the cell.

Dong *et al.* (2012) studied the effect of silver nanoparticles as antimicrobial and found that the numbers of harmful *Salmonella, E. coli*, and *Streptococcus spp*. decreased by using silver nanoparticles.

Hassanabadi *et al.* (2012) found improvement in intestinal microflora in colonies where the highest numbers of lactobacillus ssp. colonies were seen in nano-silver treatments. Results of study also showed that *E. coli* decreased in treatments fed nano-silver with increased *lactobacillus ssp.* in intestinal content.

Duffy *et al.* (2018) investigated the antibacterial activity of silver nanoparticles against different strains of bacteria (*Salmonella* and *Campylobacter spp.*) isolated from poultry. The results indicatedthat there was a positive, selective, effect of silver nanoparticles on microflora in the digestive tract in poultry, mainly by their ability to inhibit the development of pathogenic bacteria.

On the other hand, Kulak *et al.* (2018) studied whether silver nanoparticles (AgNPs) administered to chickens as a hydrocolloid as a dose of 2.87 or 12.25 mg per bird will affect the accumulation of this element in tissues. The data of theexperiment showed that the accumulation of AgNPs increased in the intestine and liver with the increase AgNPs dose.

This study aimed to investigate the effect of using different levels of silver nanoparticles in broiler drinking water on water consumption, microorganisms in digestive tract and silver residual in different tissues.

## MATERIALS AND METHODS

The present study was conducted in Poultry Nutrition Farm, Faculty of Agriculture, Ain Shams University, Shalakan, Kaliobia Governorate, Egypt. 150 one-day unsexed broiler chicks (Cobb-500) were used in this experiment. Chicks were weighted and randomly allotted into five treatment groups, 3 replicates of 10 chicks per each group. Treatments groups were as follows: Control (drinking water without supplementation), T1-4 (drinking water supplemented with AgNPs at 4 levels (2.5 – 5.0 – 7.5 – 10.0 ppm) to get 5 treatments. Diets were formulated to meet requirements basal on manual guide of Cobb 500. The composition of basal diets waspresented in Table (1)

The remainder and scattered water as well as the consumed water was periodically estimatedfor each replicate and thereafter, the average periodically water intake per bird was calculated by dividing water consumed by their chick number each in replicate and treatment.

At the end of theexperiment (35 days of age), four birds from each group were randomly selected for microbiology measurements of digestive tract content (Ileum and cecum) and residual of silver in tissues).

Content of small intestinal tract and cecum were collected to determine the microbiological flora in (Microbiological Laboratory, Faculty of Science, South Valley University) for enumeration of total bacteria, *E. coli* and *lactobacillusspp.* Samples were taken in falcon tubes and cooled until incubation.

**Table 1. Composition and calculated analysis of starter grower and finisher diets**

| | Diets | | |
|---|---|---|---|
| **Ingredients** | **Starter*** | **Grower*** | **Finisher*** |
| Yellow corn | 55.76 | 59. 70 | 63.70 |
| Soybean meal 48% | 37.84 | 33.10 | 28.22 |
| Soy oil | 2.44 | 3.40 | 4.42 |
| Bone meal | 2.91 | 2.60 | 2.26 |
| Limestone | 0.24 | 0.35 | 0.50 |
| HCL Lysine | 0.00 | 0.04 | 0.08 |
| DL Methionine (99%) | 0.21 | 0.21 | 0.22 |
| Salt | 0.30 | 0.30 | 0.30 |
| Premix**(Vit+Min) | 0.30 | 0.30 | 0.30 |
| **Total** | **100.00** | **100.00** | **100.00** |
| **Calculated chemical analysis*** ** | | | |
| Crude protein (%) | 23.01 | 21.04 | 18.99 |
| M E (kcal / kg) | 3003 | 3102 | 3204 |
| C \P ratio | 130 | 147 | 168 |
| Calcium (%) | 1.00 | 0.95 | 0.90 |
| Available phosphorus (%) | 0.50 | 0.45 | 0.40 |
| Methionine (%) | 0.63 | 0.60 | 0.58 |
| Methionine + Cysteine (%) | 0.95 | 0.90 | 0.85 |
| Lysine (%) | 1.35 | 1.25 | 1.15 |

* Starter (1-14 day old), Grower (15-28 days- old) and finisher (29-35 day old),

** Each 3 kg contains: Vit A 12 000 000 IU, Vit $D_3$ 2 000 000 IU, Vit E 1g, Vit $K_3$ 2 g, Vit $B_1$ 1 g, Vit $B_2$ 5 g, Vit $B_6$ 1.5 g, Vit $B_{12}$ 10 mg, Nicotinic acid 30 g, Pantothenic acid 10 g, Folic acid 1 g, Biotin 50 mg Choline chloride 250 g, Iron 30 g, Copper 10 g, Zinc 50 g, Manganese 60 g, Iodine1 g, Selenium 0.1 g, Cobalt 0.1 g and carrier (CaCO3) to 3 kg,

*** Calculated analysis according to NRC (1994).

Residuals of silver estimated in breast and thigh muscles and liver tissue by the method of National Institute of Standards and Technology (National Institute of Standards and Technology, 2008).

Data were analyzed by using general linear model (GLM) of SAS (SAS, 2004) using one-way analysis of variance. Duncan's Multiple range test (Dancan, 1955) was used to check the significance among the means.

The following statistical model was applied

$$Y_{ij} = \mu + T_i + e_{ij}$$

- $Y_{ij}$= is the effect of the observation, μ = overall mean, $T_i$ = the effect of $i^{th}$ levels of nanoparticles, $e_{ijk}$ = random error.

## RESULTS AND DISCUSSION

***Water consumption (WaC, ml):***

Data in Table (2) indicate that (WaC, ml) per chicks increased by supplementing 2.5 ppm AgNPs, T1 in drinking water compared with treatments T2-4. The corresponding figures were 6654.33 Vs. 6538.67, 6577.66, 6580.33 and 6461.00, respectively, during the overall experimental period (1-35 days). On the other hand, supplementing 10.0 ppm (AgNPs), T4 reflected the lowest water consumption compared with the other treatments. The differences weren't statistically significant. These results agree with those of Vadalasetty *et al.* (2018) who found that daily water consumption hasn't affected by treatment at 30 days old.

*Microbiology measurements of digestive tract content|:*

Table (3) shows the effect of (AgNPs) supplementation in drinking water on microbiology measurements of broiler chicks at the end of 35 days of age. Experimental treatments with (AgNPs), at high levels (7.5 ppm, $T_3$ and 10.0 ppm, $T_4$) had significant decrease in *lactobacillusspp.* and *E. coli* count in the small intestine and ceca compared with control group (0.0 AgNPs). The corresponding count of *lactobacillusspp.* ranged between ($0.76 \times 10^4$ and $0.06 \times 10^4$), while *E. coli* ranged between ($0.22 \times 10^4$ and $0.00 \times 10^4$) in the small intestine. In the same order, *lactobacillusspp.* count ranged between ($0.78 \times 10^4$ and $0.01 \times 10^4$), and *E. coli* ranged between ($0.35 \times 10^4$ and $0.06 \times 10^4$) in ceca. the differences between treatments were significant.

**Table 2. Effect of silver nanoparticles supplemented in broiler drinking water on water consumption**

| Items | Treatments | | | | | SEM | Sig. |
|---|---|---|---|---|---|---|---|
| | 0.0 control | 2.5 | 5 | 7.5 | 10 | | |
| **WI 1-14 day** | 1576.67 | 1615.00 | 1581.66 | 1588.33 | 1520.00 | **68.52** | **NS** |
| **WI 15-28 day** | 2811.67 | 2858.33 | 2815.00 | 2798.33 | 2760.00 | **60.16** | **NS** |
| **WI 29- 35 day** | 2150.48 | 2180.95 | 2180.95 | 2193.80 | 2180.95 | **41.50** | **NS** |
| **WI 1- 35 day** | 6538.67 | 6654.33 | 6577.66 | 6580.33 | 6461.00 | **131.96** | **NS** |

SEM : Standard error NS: Non-significant,WI = water intake

In general, the lowest figures of *E. coli* and *lactobacillusspp.* were noticed when (AgNPs) was supplemented in drinking water by 10.0 ppm. These results are in similarity to those of Sondi and Salopek- Sondi (2004), Kout Elkloub *et al.* (2015) and Duffy *et al.* (2018), who showed that there were significant effects of (AgNPs) levels on *E. coli* and *lactobacillusspp.* bacteria. On the other hand, Pineda *et al.* (2012) recorded that the different levels of AgNPs didn't affect microorganisms in ileum or cecum of broiler chicks.

**Table 3 Effect of silver nanoparticles supplemented in broiler drinking water on microorganism in the small intestine and ceca**

| Items | Treatments | | | | | SEM | Sig. |
|---|---|---|---|---|---|---|---|
| | 0.0 control | 2.5 | 5 | 7.5 | 10 | | |
| **Small intestine** | | | | | | | |
| ***Lactobacillus spp.*** | $0.76 X10^{4a}$ | $0.36 X10^{4b}$ | $0.31 X10^{4b}$ | $0.08 X10^{4c}$ | $0.06 X10^{4c}$ | $0.02 X10^4$ | ** |
| ***E.coli*** | $0.18 X10^{4b}$ | $0.22 X10^{4a}$ | $0.20 X10^{4b}$ | $0.007 X10^{4c}$ | $0.009 X10^{4c}$ | $0.05X10^4$ | ** |
| **Ceca** | | | | | | | |
| ***Lactobacillus ssp.*** | $0.58 X10^{4b}$ | $0.78X10^{4a}$ | $0.01X10^{4d}$ | $0.17X10^{4c}$ | $0.01X10^{4d}$ | $0.10 X10^4$ | ** |
| ***E.coli*** | $0.18 X10^{4b}$ | $0.32 X10^{4a}$ | $0.17 X10^{4b}$ | $0.15 X10^{4b}$ | $0.06 X10^{4c}$ | $0.02 X10^4$ | ** |

a,b: Means in the same row with the same letter are not significantly different.
SEM: Mean standard error,significant **: at (P≤ 0.01)

***Residual of silver in some tissues (ppm):***

Data in Table 4 showed the effect of nanoparticles on the silver residuals in breast, thigh, and liver tissue. Results showed that there weren't silver residual in breast and thigh muscles in all treatments, however, there were residual in liver tissues in treated groups compared with the control group. These results might be related to that silver is aheavy metal so, it deposited in liver tissue.

**Table 4 Effect of silver nanoparticles supplemented in broiler drinking water on residual of silver in some tissues of broiler (ppm)**

| Item | Treatments | | | | | SEM | Sig. |
|---|---|---|---|---|---|---|---|
| | 0 | 2.5 | 5 | 7.5 | 10 | | |
| *Breast muscles* | 4.65 | 4.62 | 4.47 | 4.12 | 4.72 | 0.51 | NS |
| *Thigh muscle* | 2.82 | 2.97 | 3.00 | 3.45 | 3.37 | 0.49 | NS |
| *Liver tissue* | 5.85d | 5.95d | 7.60c | 9.70b | 10.57a | 0.32 | ** |

Means within the same row with different superscripts are significantly different. Sig=Significance** (P≤0.01),. NS=Non-significant.

Similar observations have been reported by Gallocchio *et al*. (2017) in layer hens, they concluded that silver accumulates in liver and yolks but not in muscles, kidneys and albumen belonging to hens of the treated group. However, Ahmedi and Rahimi (2011) found that there was an accumulation of silver significantly in some edible parts such as breast, thigh muscles and liver and this accumulation in organs increased with increasing the levels of silver nanoparticles in drinking water.

## CONCLUSION

In conclusion, the best level of silver nanoparticles can be used as water supplementation is 2.50 ppm of (AgNPs), but there was a residual of silver in liver tissue.

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# تأثير إضافة جسيمات الفضه النانويه لماء الشرب على إستهلاك المياه والكائنات الحية الدقيقة فى القناة الهضمية والمتبقيات من الفضة في بعض الأجزاء الصالحة للأكل من كتاكيت التسمين

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الهدف من هذه الدراسة هو معرفة تأثير جزيئات الفضه النانويه (AgNPs) كاضافة لماء الشرب على استهلاك المياه ، والكائنات الحية الدقيقة في الأمعاء الدقيقة و الاعور والمتبقيات من الفضة في بعض الأجزاء الصالحة للأكل من دجاج التسمين عند ٣٥ يومًا من العمر. في هذه التجربة تم استخدام عدد ١٥٠ كتكوت تسمين غير مجنس (كب ٥٠٠) قسمت عشوائياً إلى ٥ معاملات (٣٠ كتكوت لكل منها) ، بواقع ٣ مكررات كل منها ١٠ كتكوت. تم اضافة جزيئات النانو فضة في المعاملات التجريبية بمياه الشرب بمستويات مختلفة من (٠.٠ ، ٢.٥ ، ٥.٠ ، ٧.٥ ، ١٠ جزء في المليون). في نهاية التجربة تم ذبح أربعة طيور لكل معاملة لتقدير عدد البكتيريا النافعه والضارة في الأمعاء الدقيقة والاعور وبقايا الفضة في بعض الأجزاء الصالحة للأكل من دجاج التسمين.

أظهرت النتائج الأتى:

١- لم يظهر استهلاك الماء لدجاج التسمين أي تأثير معنوي مرتبط بمستويات مختلفة من اضافةAgNPs.

٢- تأثر عدد *Lactobacillus spp*. و *E.coli* في كل من الأمعاء الدقيقة والاعور بشكل كبير بالمستويات المختلفة من AgNPs.

٣- أظهر تقدير الفضة المتبقية في بعض الأنسجة أن استخدامAgNPs لم يحدث تراكم للفضة في لحم الصدر أو الفخذ ، ولكنه ترسب في أنسجة الكبد وتزداد نسبة التراكم مع زيادة مستوى AgNPs في ماء الشرب.

بالإضافة إلى ذلك ، فإن اضافة مستويات مختلفة من AgNPs لماء الشرب لدجاج التسمين لها تأثيرات كبيرة على البكتيريا في الامعاء الدقيقة والاعور وتراكم الفضة في أنسجة الكبد.