

Research Article





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Nutritional Studies on Nanoparticles (silver and graphene) in Broiler Diets 1-Broiler performance and digestive tract microbiota

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Abstract:

- Received: - Revised: - Accepted: - Published: Keywords: nanoparticles, broiler chicks, performance, and microorganism The present study aimed to investigate the effects of using nanoparticles (silver, SNaPs and graphene, GNaPs) as a supplementation in broiler diets on productive performance and microbial load in the small intestine (SI) and ceca of broiler chicks. A total of 360 one-day-old Indian River sex-mixed broiler chicks were used in nine treatments with eight replicates of five chicks per replicate. The experimental rations were prepared using 0.0, 2.5, 5.0, 7.5, and 10.0 ppm/ kg feed of two types of nanoparticles (silver and graphene). All diets were formulated to meet NRC minimal nutritional requirements. Data were analyzed using a randomized complete block design in factorial arrangement with types of nanoparticles of nanoparticles as main effects. The results were as follows:

- Productive performance including LBW, BWG, FI, PI, and EPEF differed significantly within treatments concerning the different levels of nanoparticles but wasn't affected by different types of nanoparticles during the overall trial period, also FCR wasn't differed significantly by different types or levels of nanoparticles at overall period.
- The count of Lactobacillus ssp. in both SI and Ceca was affected significantly by different types and levels of nanoparticles. In the same way, E. Coli in both SI and Ceca decreased significantly with increasing nanoparticle levels, while it wasn't affected significantly by different types of nanoparticles in SI it was affected significantly by different types of nanoparticles in Ceca.
- It can be concluded that there weren't significant differences between the two types of nanoparticles as feed additives on productive performance but microbiota in the digestive tract was affected significantly where GNaPs were better than SNaPs.

1. Introduction

The challenge that will face poultry science in the future includes increasing feed efficiency, safe growth promoter, modifying the contents of the egg-like cholesterol-free egg and full protein egg, tackling diseases, controlling the macro and micro-nutrient absorption, reducing the protein and energy loss in unproductive purposes thereby, improve the feed efficiency and reducing the price of the poultry production (meat and eggs). (**Taylor 2009**) Nanotechnology is playing an important and major role in a lot of research fields of poultry science. One of the minerals widely used as a nanomaterial is silver nanoparticles (SNaPs) because of their purification ability as it has antiseptic properties (**Chen et al**; **2007**). Silver compounds are considered a potential alternative to some food additives like oligosaccharides, organic acids, plant extracts, etc. The main objective of their use as additives in poultry feed is their effective role as anti-microbial, which is acting over potential pathogens, but not on the symbiotic microbial communities (**Fondevila et al., 2009**).

Silver nanoparticles have high effectiveness as an anti-microbial and are used as feed additives because it is compatible with the biological system in the body. It is known that silver nanoparticles have an impact on a wide elimination of harmful microbes such as *E. coli*, *Vibrio cholerae*, *Salmonellatyphi*, and *Pseudomonas* aeruginosa (Ahmadi *et al.* 2009).

Graphene nanoparticles (GNaPs), graphite nanoparticles, or charcoal nanoparticles are different structural combinations for carbon nanoparticles.

Graphene is a thick atom substance consisting of carbon bound to beta-sp2 in the structure of the honeycomb. Graphene reduces cell adhesion when it enters the cytoplasm and nucleus (**Wang** *et al.*, **2011**).

Saminathan *et al* (2018) studied the effect of fed broiler chick diets supplemented with graphene nanoparticles on the performance of broilers. The results showed that LBW and BWG improved significantly by using graphene nanoparticles in broiler diets.

The growth of Gram-positive, Gram-negative, and Escherichia coli bacteria has been affected significantly by the sharp edges of the reduced graphene nanoparticles (**Akhavan and Ghaderi, 2010**). **Park** (**2010**) also found that graphene is noncytotoxic to a mammalian cell.

This study aimed to investigate the effect of using different types and levels of nanoparticles (graphene and silver) in broiler feed on the Productive performance of broiler chicken, and Microorganism in the digestive tract.

2. Materials and Methods

The present study was carried out at the Poultry Nu-

trition Farm, Poultry Production Department, Faculty of Agriculture, Ain Shams University, Shalakan, Kaliobia, Egypt. This experiment was conducted to evaluate the effect of using nanoparticles (silver and graphene nanoparticles) as a supplementation in broiler diets on productive performance and microorganisms in the digestive tract. 360 unsexed one-day-old IR (Indian River) broiler chicks were used in this study; obtained from a commercial hatchery divided into 9 treatments, each treatment comprised 40 chicks divided into eight replicates of five chicks. The tested nanoparticles treatments were (0 nanoparticles) as a control treatment, 2.5, 5.0, 7.5, and 10.0 ppm/ Kg feed of two types of nanoparticles) silver and graphene nanoparticles), of starter, grower, and finisher diets, respectively. Chicks were fed on the experimental corn-soybean (starter, grower, and finisher) diets in which the composition and calculated analysis are in Table (1).

Live body weight (LBW) and feed intake (FI) were recorded weekly till 35 days of age. Body weight gain (BWG), feed conversion ratio (FCR), performance index (PI), and European production efficiency factor (EPEF) were calculated at (1-35) days of age. At the end experiment, four chickens from each treatment were randomly selected and fasted for about 10 hours then slaughtered to determine the bacterial count in the small intestine and ceca. Statistical analysis was done as treatments were assigned as the main factor; the statistical model was performed as follows:

> $Yij = \mu + Ti + Lj + (T^*L) ij + Eijk$ Where

- Yij= is the effect of the observation
- μ = overall mean.
- Ti = the effect of ith levels of nanoparticles.
- Lj= the effect of the jth type of nanoparticles.
- (T*L) ij = interaction between types and levels of nanoparticles.
- Eijk = random error.

	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 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			

Table (1): Composition and calculated analysis of starter grower and minister
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* Starter (1-14 days old), Grower (15-28 days- old), and finisher (29-35 days old).

** Each 3 kg contains: Vit A 12 000 000 IU, Vit D3 2 000 000 IU, Vit E 1g, Vit K3 2 g, Vit B1 1 g, Vit B2 5 g, Vit B6 1.5 g, Vit B12 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, Iodine 1 g, Selenium 0.1 g, Cobalt 0.1 g and carrier (CaCO3) to 3 kg.

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

3. RESULTS AND DISCUSSION

Live body weight (LBW) and body weight gain (BWG)

Table 2 shows the main effect of either type of nanoparticles (silver or graphene) or levels (0.0, 2.5, 5.0, 7.5, and 10.0 ppm/ kg diets) on (LBW) and (BWG) of broilers, chicks fed different types of nanoparticles during studied periods (1 - 35 days)reflected insignificant values in both (LBW) and (BWG). On the other hand, chicks fed control diets reflected the highest significance (LBW) and (BWG) compared with those fed different levels of nanoparticles. Besides, the chicks fed the diet supplemented with 5 ppm/kg diets reflected the lowest (LBW and BWG) compared with other treatments. These results agree with Ahmadi (2013), Shabani et al (2010), and Mohammadi et al. (2011) who found that body weight gain was affected at 42 days of age with ionic solver and H2O2 but the interaction was not significantly affected. On the other hand, the obtained data disagree with those reported by Ahmadi (2009), Kout Elkloub et al (2015), Saleh and A. El-Magd (2018) who

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showed that significant improvement in LBW and BWG of broiler chicks with silver nanoparticles supplementation. Also, **Odunsi** *et al* (2007), **Abu Bakr (2008), and Majewska** *et al* (2009) found that the inclusion of charcoal at different levels in broiler diets reflected significantly higher LBW and BWG compared with control groups.

Feed intake (FI) and Feed conversion ratio (FCR)

Data showed that no significant differences were observed for (FI and FCR) within different types of nanoparticles (silver or graphene). In the same order, the figures of (FI and FCR) indicated significant differences between chicks fed diets containing 5 ppm/kg diet compared with those fed other levels of nanoparticles or control diets. On the other hand, the best FCR was detected for the chicks fed control diets (1.52) and the worst FCR was found in chicks fed diets containing 7.5 ppm/kg, (1.58 and 2.5 ppm/kg, 1.57) during the whole experiment period, without any significant differences. These results agreed with those obtained by **Ahmadi (2009), Shabani** *et al* (2010), Mohammad et al. (2011), and Andi et al. (2011) who found that FI was affected at 42 days of age with type of feed, supplementation, and interaction. The FCR was affected at 42 days of age by the type of feed, and interaction but supplementation hasn't affected the FCR. This result disagrees with Ahmadi et al (2010), Pineda et al (2012) Felehgari et al. (2013), and Kout Elkloub et al. (2015) who found that FCR was affected significantly by different levels of silver nanoparticles supplementation. The best FCR has been recorded by the 4ppm/kg treatment. In addition to Odunsi et al (2007), Abu Bakr (2008), Majewska et al (2011), khadem (2012), and Saminathan et al (2018) showed that using active charcoal or graphene nanoparticles in broiler diets led to significant improvement in FI and FCR.

Performance index (PI) and European production efficiency factor (EPEF)

Data showed that PI or EPEF haven't been affected significantly by nanoparticle types. On the other hand, there were significant decreases in PI or EP-EF with increasing nanoparticle levels until 5 ppm/kg diet then increase again. The birds fed a diet with a 5 ppm/kg diet recorded the lowest PI or EPEF values (106.45 and 300.44 respectively) and the control group recorded the highest PI value (117.94) and EPEF value (336.98). This result may be related to the effect of nanoparticles on BWG where the same trend was achieved with both BWG and PI or EPEF. This result agrees with KoutElkloub et al. (2015) who found significant differences between silver nanoparticle levels in EPEF where the highest EPEF value was recorded by birds fed diet with 4 ppm SNaPs (374) and the lowest EPEF value recorded by birds fed control group.

Microbiology measurements of digestive tract content

Data in Table 3 showed the effects of different types or levels of nanoparticles on microorganisms in the small intestine and ceca of broiler chicks.

Results had shown that increased significantly in Lactobacillus spp. count in the small intestine with increasing nanoparticle levels in a diet where birds fed the diet with 5.0 ppm/ kg diet recorded the highest Lactobacillus spp. count and control group was the lowest (2.79 X10⁴ and 0.68X10⁴ respectively). On the other hand, E. Coli decreased significantly in the small intestine with increasing nanoparticle levels in a diet where birds fed the control diet recorded the highest E. Coli count and birds fed the diet with 7.5 ppm/ kg diet nanoparticles was the lowest (1.50 X104 and 0.20X104 respectively). These results agree with Pineda et al (2012) and Kout Elkloub et al. (2015) who found that there were significant effects on total count bacteria, lactobacillus ssp., and E. Coli bacteria. The study showed a decrease in harmful bacteria such as E. Coli compared with the control group.

In the same order, there was a significant difference between the different types of nanoparticles in *lactobacillus ssp.* in the small intestine where the birds that were fed diets supplemented with graphene nanoparticles recorded *lactobacillus ssp.* count higher than those fed diets supplemented with silver nanoparticles (2.24 X10⁴ and 1.49 X10⁴, respectively). *E. Coli* wasn't affected significantly by different types of nanoparticles in the small intestine whereas the two types have the same effect on *E. Coli* count in the small intestine (0.54X10⁴ and 0.56X10⁴ respectively).

In ceca *Lactobacillus spp.* count increase significantly with increasing nanoparticle levels where the bird feed diet supplemented with 7.5 ppm/kg diet was recorded highest *Lactobacillus spp.* count and control group was the lowest (5.04 X10⁴ and 0.44 X10⁴respectively). *E. coli* has been affected significantly by treatments where the control group recorded the highest *E. Coli* count and birds fed diet supplemented with a 7.5 ppm/kg diet was recorded lowest *E. Coli* count (1.62 X10⁴ and 0.08 X10⁴, respectively).

On the other hand, there were significant differ-

ences between the different types of nanoparticles in Lactobacillus spp. and E. coli where the groups fed diets supplemented with graphene nanoparticles recorded a higher count than the groups fed diets

supplemented with silver nanoparticles (4.86X10⁴ vs. 2.71X10⁴) and (0.46X10⁴ vs. 0.42X10⁴), respectively.

Items	Initial LBW	LBW 35 d	BWG 1- 35 d	FI 1-35 d	FCR 1-35 d	PI	EPEF
Туре							
SNaPs	43.76	1726.77	1683.40	2601.72	1.54	112.32	316.18
GNaPs	43.71	1720.04	1676.55	2612.57	1.55	111.31	314.86
SEM	0.39	36.68	36.67	114.6	0.05	6.7	18.84
p-value	Ns	Ns	Ns	Ns	Ns	Ns	Ns
Levels							
0 PPM	43.30	1787.63ª	1744.73 ^a	2665.31ª	1.52	117.94ª	336.98 ^a
2.5 PPM	43.86	1710.57 ^b	1667.34 ^b	2617.85 ^a	1.57	109.08 ^{bc}	311.66 ^b
5 PPM	43.77	1656.57°	1612.97°	2491.72 ^b	1.54	106.45°	300.44 ^c
7.5 PPM	43.95	1723.18 ^b	1679.28 ^b	2669.45 ^a	1.58	109.78 ^{bc}	305.83 ^{bc}
10 PPM	43.78	1739.09 ^b	1695.54 ^b	2591.42 ^{ab}	1.53	115.83 ^{ab}	322.69 ^{ab}
SEM	0.39	36.68	36.67	114.6	0.05	6.7	18.84
p-value	Ns	**	**	*	Ns	**	**
Interaction							
SNaPs&0 PPM	43.30	1787.62ª	1744.73 ^a	2665.30 ^c	1.52	117.94	336.98
SNaPs&2.5 PPM	44.00	1702.52 ^d	1659.52°	2512.83 ^e	1.51	111.85	319.59
SNaPs&5 PPM	43.62	1626.93^{f}	1583.33^{f}	2481.67^{f}	1.56	104.01	289.76
SNaPs&7.5 PPM	44.10	1781.42ª	1737.47ª	2774.67ª	1.59	112.38	313.06
SNaPs&10 PPM	43.77	1735.37 ^b	1691.92 ^b	2574.12 ^d	1.52	115.42	321.54
GNaPs&0 PPM	43.30	1787.62ª	1744.73 ^a	2665.30°	1.52	117.94	336.98
GNaPs&2.5 PPM	43.72	1718.61°	1675.16 ^c	2722.86 ^b	1.62	106.31	303.74
GNaPs&5 PPM	43.92	1686.21 ^e	1642.61 ^d	2501.76 ^e	1.52	108.89	311.13
GNaPs&7.5 PPM	43.80	1664.94 ^e	1621.09 ^e	2564.21 ^d	1.58	107.19	298.61
GNaPs&10 PPM	43.80	1742.80 ^b	1699.15 ^b	2608.72°	1.53	116.25	323.83
SEM	0.39	36.68	36.67	114.60	0.05	6.7	18.84
p-value	Ns	**	**	*	Ns	Ns	Ns

Table (2): Effect of nanoparticles (types and levels) in broiler diets on productive performance

a,b: Means in the same column with the same letters are not significantly different. MSE: Mean standard error NS: Non-significant *: (P≤0.05), SNaPs = silver nanoparticles, GNaPs = graphene nanoparticles, PI= Performance index, EPEF= European production efficiency factor

The mechanism of the inhibitory effects of nanoparticles was higher in the case of Gram-negative bacteria. This might be related to the thickness of the peptidoglycan layer in Gram-positive bacteria cell walls, which may prevent to some extent the increase in Gram-negative bacteria KoutElkloubet al (2015). The increase in Lactobacillus ssp. may be related to the competitive relationship between harmful bacteria and beneficial bacteria in the digestive tract and the increase in the total count of bacteria may be related to the increase in beneficial bacteria in the digestive tract. **Singh** *et al.* (2008) assumed a higher sensitivity of Gram-negative bacteria to treatment with nanoparticles. This result agrees **Sawosz** *et al.* (2007) examined the effects of silver nanoparticles (SNaPs) on the microbial profile of the caecum of Japanese quail. Quails were fed with granulated diets given ad libitum and had free access to drinking water. SNaPs were added to drinking water at concentrations of 0, 5, 15, and 25 mg/kg. The results showed that 25 mg/kg of SNaPs supplemented in quail drinking water significantly increased the number of gram-positive bacteria (*Lactobacillusspp*, *Leuconostoclactis*, *Actinomycesnaeslundii*) compared to control birds.

Table (3) Effect of nanoparticles (silver and graphene) in broiler diets on digestive microorganisms in the digestive tract

T 4	Small integ	stine	Ceca		
Items	Lactobacillus ssp.	E.Coli	Lactobacillus ssp.	E.Coli	
Туре					
SNaPs	1. 49 ^b X10 ⁴	$0.54 X 10^4$	2.71 ^b X10 ⁴	$0.42^{b}X10^{4}$	
GNaPs	$2.24^{a} \mathrm{X} 10^{4}$	$0.56 X 10^4$	$4.86^{a} \mathrm{X}10^{4}$	$0.46^{a}X10^{4}$	
SEM	$0.10 X 10^4$	0.13X10 ⁴	$0.08 X 10^4$	$0.04 X 10^4$	
p-value	**	Ns	**	*	
Levels					
0 PPM	$0.68^{d} \mathrm{~X10^{4}}$	$1.50^{a} \mathrm{X} 10^{4}$	$0.44^{d} \ X10^{4}$	1.62 X10 ^{4a}	
2.5 PPM	1.39 ^c X10 ⁴	$0.41^{b} X10^{4}$	4.34° X10 ⁴	$0.26^{b} \mathrm{X10^{4}}$	
5 PPM	$2.72^{a} \mathrm{X10^{4}}$	0.39 ^b X10 ⁴	4.38° X104	$0.17^{\circ} \mathrm{X}10^{4}$	
7.5 PPM	$1.84^{\rm b} {\rm X10^4}$	$0.20^{\circ} \mathrm{X} 10^{4}$	$5.04^{a} X 10^{4}$	$0.08^{d} \mathrm{~X10^{4}}$	
10 PPM	$2.69^{a} \mathrm{X} 10^{4}$	$0.25^{bc} X 10^4$	$4.74^{b} \mathrm{X}10^{4}$	$0.07^{d} \mathrm{~X10^{4}}$	
SEM	$0.10 X 10^4$	0.13X10 ⁴	$0.08 X 10^4$	$0.04 X 10^4$	
p-value	**	**	**	**	
Interaction					
SNaPs&0 PPM	$0.68^{d} \mathrm{~X10^{4}}$	$1.50^{a} \mathrm{X} 10^{4}$	$0.44^{h} X 10^{4}$	$1.62^{a} \mathrm{X} 10^{4}$	
SNaPs&2.5 PPM	1.29 ^c X10 ⁴	0.37° X10 ⁴	$2.87^{f}X10^{4}$	$0.32^{b} \mathrm{X10^{4}}$	
SNaPs&5 PPM	$1.60^{\rm c} { m X} 10^4$	$0.26^{d} X 10^{4}$	$1.05^{\rm g} {\rm ~X10^{4}}$	$0.17^{\circ} X10^{4}$	
SNaPs&7.5 PPM	$1.74^{\circ} \mathrm{X}10^{4}$	$0.24^{d} \ X10^{4}$	$4.99^{d} X 10^{4}$	$0.00^{d} \mathrm{~X10^{4}}$	
SNaPs&10 PPM	$2.13^{b} X10^{4}$	$0.35^{\circ} \mathrm{X10^{4}}$	4.21 ^e X10 ⁴	$0.00^{d} \mathrm{~X10^{4}}$	
GNaPs&0 PPM	$0.68^{d} \mathrm{~X10^{4}}$	$1.50^{a} \mathrm{X} 10^{4}$	$0.44^{h} X 10^{4}$	$1.62^{a} X10^{4}$	
GNaPs&2.5 PPM	$1.50^{\circ} \mathrm{X} 10^{4}$	$0.45^{b} X10^{4b}$	$5.81^{b} X 10^{4}$	$0.20^{\circ} \mathrm{X10^{4}}$	
GNaPs&5 PPM	$3.84^{a} \mathrm{X}10^{4}$	$0.55^{b} X10^{4b}$	$7.71^{a} \mathrm{X} 10^{4}$	$0.17^{\circ} X10^{4}$	
GNaPs&7.5 PPM	1.94 ^c X10 ⁴	$0.15^{e} X 10^{4c}$	$5.09^{d} X 10^{4}$	$0.15^{\circ} \mathrm{X10^{4}}$	
GNaPs&10 PPM	$3.26^{a} X 10^{4}$	0.15 ^e X10 ^{4c}	5.27° X10 ⁴	0.17° X10 ⁴	
SEM	$0.10 X 10^4$	$0.13X10^{4}$	$0.08X10^{4}$	$0.04 X 10^4$	
p-value	**	*	**	**	

a,b: Means in the same column with the same letters are not significantly different. MSE: Mean standard error NS: Non-significant $*: (P \le 0.05)$ SNaPs = silver nanoparticles, GNaPs = graphene nanoparticles.

Fondevila *et al.* (2009) studied SNaP supplementation levels of 0, 25, 50, and 100 ppm in vitro and recorded that the proportion of coliforms small intestine content decreased and lactobacillus bacteria and *E. Coli.* Also, **Ognik** *et al* (2016) found that there was a decrease in the number of *E. Coli* group bacteria in treated groups compared with the control group.

The study showed decreasing in harmful bacteria such as *E. Coli* compared with the control group, however, **Pineda** *et al* (2012) found that different levels of SNaPs haven't affected microorganisms in the cecum of broiler chicks. These results may be related to the antimicrobial effect of SNaPs and their role as a regulation factor of microorganisms in the digestive tract where the pathogenic bacteria count is little (Fondevila, 2009).

Conclusion:

There weren't significant differences between the two types of nanoparticles as feed additives on productive performance but microorganisms in the digestive tract were affected significantly where GNaPs were better than SNaPs.

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