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Research Article

Nutrition Studies on Nanoparticles (silver and graphene) in Broiler Diets
2 -Carcass characteristics and bone measurements

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Keywords:

Nanoparticles, chicks, carcass traits, and bone measurements

Abstract:

This experiment was conducted to evaluate the effect of using nanoparticles (silver and graphene) supplementation at four different levels in broiler chicks fed corn-soy-based diets on carcass characteristics and some physical and chemical bone measurements. This study comprised three hundred and sixty unsexed one-day-old Indian river broiler chicks, which were divided randomly into (9) experimental treatments in 8 replicates with 5 chicks each. The tested nanoparticles (silver and graphene) were added on top to a basal diet (0.0 (basal diet), 2.5, 5.0, 7.5, and 10.0 ppm/kg), at the end of the experiment period (35 days of age) , the carcass and tibia bone characteristics of four chicks per treatments were determined. The results showed that:

- All carcass traits and cut percentages haven't affected either type or levels of nanoparticles except liver%.
- All physical and chemical bone measurements weren't affected significantly by different types or levels of nanoparticles except tibia width and ash% were affected significantly by different levels of nanoparticles.
- Conclusion: carcass characteristics and bone measurements haven't been affected significantly by different types or levels of nanoparticles except for liver, tibia width, and ash%.

1. Introduction

The field of nanotechnology is one of the fastest-growing areas of scientific research and development, with significant advances being made in a range of applications. Currently, the state of the art in nanotechnology covers a wide range of areas, including electronics, energy, materials science, biomedicine, and more.

Due to the rapid advancements that can be achieved by nanotechnology, it has been estimated that nanotechnology will impact the global economy by about three trillion dollars in the next years in all branches of science (**Roco et al, 2017)** could be attributed to the unique physicochemical properties of nanoparticles at the interface of chemistry, medicine, physics, and engineering.... etc.

Nanoparticles, which used in animal and poultry production for instance such as silicon dioxide, calcium, magnesium, silver, graphene nanoparticles for water purification or antimicrobial packaging or feed storage, and zinc as a feed colorant (**El Sabry et al. 2018).**

Nanosilver is one of the most commonly used nanomaterials because of its strong disinfection properties (**Chen et al, 2007).** 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 in membrane permeability, and de-activating cellular enzymes (**Lagman et al, 2012).**

Graphene oxide nanocomposites (MGO) have demonstrated efficiencies in the absorption of AFs, ochratoxin A, and zearalenone **(Pirouz *et al* 2017),** such as efficiencies of hydrated sodium calcium aluminosilicate (HSCAS), sodium bentonite and zeolites and modified materials Montmorillonites treated with organic cations14, chitosan polymers15, and yeast16. However, these adsorbents are effective against one or two specifics mycotoxins, and are relatively expensive, unlike MGO relatively cost is cheap **(Aly *et al* 2004, Var *et al* 2008, Papaioannou *et al* 2005, Jaynes and Zartman 2011, Deng 2013).** Graphene nanoparticles are the latest nanomaterial used in the field of animal production. Therefore, there is very little research on this substance in poultry feed or drinking water.

Saminathan *et al* (2018) showed that the weight of the gizzard, liver, and heart haven't been affected significantly by graphene nanoparticles in broiler feed.

Khah *et al* (2015) evaluated the effect of dietary different levels of nano zinc oxide (NP-Zno) in a broiler diet during the starter stage (from 1 to 21 days) on the carcass yield and quality. The results showed that the birds recorded the highest dressing weight (g), however, the weight of the breast, thigh, and wing weren't affected significantly by different levels.

Mohammadi *et al* (2015) investigated the effects of different levels of zinc oxide nanoparticles (ZnO-NPs) in broilers fed dry or wet diets during the starter period on the carcass yield. The study indicated that %carcass was affected significantly where the birds that fed Dry diet- 200mg Nano-Zno recorded the highest %carcass, however, %breast, %thigh, %wing, %gizzard, %liver, and %heart, weren't affected significantly by treatments.

Ahmdi*et al* (2018) observed that % the breasts and thighs with drumsticks increased with increasing nano-selenium levels in the diets. However, %wing, %liver, %gizzard, and %Heart wasn't affected by different levels of nano-selenium in diets.

Sohair *et al* (2017) found that %dressing, %gizzard, %heart, and %liver wasn't affected significantly by hydroxyapatite (NHA) nanoparticles in broiler diets as a source of calcium and phosphorous.

El-Rayes *et al* (2019) found that the percentages of heart, and liver haven't been affected significantly by different levels of zinc nanoparticles (ZnO-NPs) in diets. On the other hand, percentages of breast, and abdominal fat differed significantly by different levels of zinc nanoparticles (ZnO-NPs) where the highest percentage of breast recorded by birds injected with 0 mg /egg ZnO-NPs and fed a diet supplemented with 0 mg/kg ZnO-NPs. The highest percentage of abdominal fat was recorded by birds injected with 80mg /egg ZnO-NPs and fed a diet supplemented with 30 mg/kg ZnO-NPs.

2. Materials and Methods

The current study aimed to investigate the effects of using different sources and levels of nanoparticles (silver and graphene) in broiler diets on carcass and bone characteristics.

Experimental design and basial diets

This study was conducted at the poultry production department, faculty of Agriculture, Ain Shams University, Kaliobia, Egypt.

A total number of 360 one-day-old Indian River broiler chicks were used in this experiment. The chicks were randomly distributed into 9 experimental groups in 2×4 factorial treatment with the control group. Each treatment had 8 replicates with 5 chicks each. The factors were two trace minerals (nanoparticles silver and graphene) at four levels (2.5, 5.0, 7.5, and 10.0 ppm/kg feed) of two types of nanoparticles, control treatment (0.0 nanoparticles) of starter, grower, and finisher diets, respectively. The corn-soybean meal-based diet was fed throughout the experiment till 35 days of age. The composition and nutrient content of the diets according to NRC (1994) were presented in Table (1).

Slaughter procedure, carcass, bone preparation, and measurements.

At the end of the experiments, four chicks from each treatment (with no visible abnormalities) were randomly selected and fasted for about 10 hours then slaughtered. After complete bleeding was over, birds were scalded at 50° C water. Feathers were removed by de-feathering machine and birds were reweighed and eviscerated. Viscera were removed manually without disrupting abdominal fat. They were feather picked and total inedible parts (head, legs, and inedible viscera) were taken aside and then, the remaining carcass (dressed weight), liver, gizzard, heart, and abdominal fat were weighed per g.

The data on carcass yield, abdominal fat, and relative weights of liver, gizzard, and heart were calculated as a percentage of live body weight.

Total cool carcass weight was recorded then each carcass was split into its cuts, breast, drumstick, thighs, and wings where each cut weight was recorded.

Bone measurements and analyses.

Chemical bone measurements

The Tibia was dried in a drying oven at 60° C overnight, weighed, and some chemical compositions of tibia were determined **(AOAC, 2012).**

Bone dimensions.

Tibia was firstly thawed at room temperature for about one hour and then measured for their length (from proximal to distal end) and width using a Hardened Stainless Steel digital micrometer according to the method described by **Samejima (1990).**

Seedor index (SI) (g/mm).

The Seedor index is a value that expresses the bone mineral density (BMD). It is obtained when a tibia dry weight (in grams) is divided by its length (in mm), as proposed by **Seedor *et al*. (1991).**

Tibia breaking strength (TBS).

Tibia breaking strength (TBS) was determined on tibiae on a wet basis following the method of **Crenshaw *et al.* (1981)** by applying the simple three-point bending concept. This determination was made at the Research Center of Properties and Testing of Materials and Quality Control, Engineering Consulting Center, Faculty of Engineering, Ain Shams University, with an Instron Universal Testing Machine, which was set at a maximum load of 50 Kg and a crosshead speed of mm/ min.

Statistical analysis

Results of carcass characteristics, carcass cut parts, and physical and chemical bone measurements were statistically analyzed by using the statistical model performed as follows:

$$Y_{ij} = \mu + T_i + L_j + (T*L)_{ij} + E_{ijk}$$

Where:

- Y_{ij}= is the effect of the observation
- μ = overall mean.
- T_i = the effect of ith levels of nanoparticles.
- L_j = the effect of the jth type of nanoparticles.
- $(T*L)_{ij}$ = interaction between types and levels of nanoparticles.
- E_{ijk} = random error.

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 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 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, Iodine1 g, Selenium 0.1 g, Cobalt 0.1 g and carrier ($CaCO_3$) to 3 kg.

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

3. RESULTS AND DISCUSSION

Carcass characteristics

As shown in Table 2 there weren't significant differences observed in percentages of carcass, giblets, and total edible parts between different dietary treatments. The corresponding values for carcass percentage ranged between (67.12 and 69.56 %), also TEP % ranged between (71.58 and 74.24 %). The differences failed to be significant compared to different dietary treatments.

Numerically, chicken-fed GNaPs had the highest percentage for carcass and total edible parts compared with chicken-fed SNaPs (68.88 vs. 67.44 and 73.32 vs. 72.07 % respectively).

These results are in agreement with **Majewska *et al* (2011), Jiya *et al* (2014) Ognik *et al* (2016), and Swaminathan *et al* (2018)** they found that the percentage of dressing% in broiler chicks hadn't been affected significantly by different levels of SNaPs or active charcoal or GNaPs.

On the other hand, these results disagree with **Kout Elkloub *et al.* (2015)** who found significant differences in carcass percentage with silver nanoparticles supplement. Moreover, **Odunsi *et al* (2007)**, showed that the inclusion of different levels of charcoal in the broiler diets reflected a negative effect on percentages of carcass and abdominal fat.

However, the figures of liver % indicated significant differences between chicks fed different levels of nanoparticles with those fed control diets. The corresponding figures were (2.80, 2.60, 2.77 and 2.73 vs. 2.19 %) respectively. This result agrees with **Mohammadi *et al.* (2011) Ahmedi *et al.* (2013), and Felehgari *et al.* (2013)** who found that treatments had a significant effect on some carcass characteristics where the liver percentage increased significantly with different levels. These results might be related to the function of the liver as a blood filter for minerals that silver and graphene can depose.

On the other hand, these findings are in contrast with the results obtained by **Vadalasetty *et al* (2018), Ognik *et al* (2016), Kout Elkloub *et al.* (2015), and Saminathan *et al* (2018)** found that the percentage of the liver wasn't affected significantly with a different type of nanoparticles. These results might be attributed to the fact that both silver and graphene nanoparticles have similar properties.

Carcass parts percentages

As shown in Table 3 there weren't significant differences were observed in the percentage of carcass parts due to types or levels of nanoparticles. The average values of breast % for different types ranged between 27.00 and 28.13% respectively and breast percent for different levels ranged between 26.37 and 29.52% respectively, however, the differences failed to be significant.

Also, no significant differences were observed in thigh% and the values of different treatments ranged between 17.4 and 19.51 % respectively. Drumstick and wing% showed the same trend and the corresponding figures were (8.80 and 10.08 %) and (5.93 and 7.45 %) respectively without significant differences between treatments. These results agree with **Majewska *et al* (2011), and Ognik *et al* (2016)** who found that the percentages of breast muscles, and thigh muscles hadn't been affected significantly by treatments.

Tibia bone measurements and chemical composition

Tibia bone measurements

The results in Table (4) show the effect of dietary nanoparticles (types and levels) in broiler diets on some physical and chemical bone measurements.

Tibia width was affected significantly by different levels of nanoparticles, but it wasn't affected by the type of nanoparticles. Broiler chickens fed diets supplemented with (2.5 ppm/ kg diet) nanoparticles reflected the lower significant figures compared with those fed control diets (6.27 Vs. 7.79 mm respectively).

While the other physical measurements (tibia length (mm), seedor index, wet tibia weight (g), dry tibia weight (g), and tibia breaking strength (Newton)) were almost the same when chickens fed different types or levels of the nanoparticle.

Bone chemical composition

Data in Table 4 indicate that Ash % increased by feeding different levels of nanoparticles compared with the control group. The corresponding figures were (41.23, 42.12, 45.07, and 41.96 vs. 35.06 %), respectively, with significant differences between treatments.

In addition,, Chick-fed diets supplemented with SNaPs showed ash% lower than those fed GNaPs diets (39.52 and 42.66 respectively). However, differences failed to be significant.

Phosphorous % showed the same trend since chickens fed control diets have the lowest compared with those fed different levels of nanoparticle diets without significant differences. On the contrary, supplementation of (10 ppm/ kg diet) nanoparticles in broiler diets reflected an insignificant reduction in calcium % compared with their fed different dietary treatments.

Conclusion: The obtained results showed that the supplementation of nanoparticles (silver and graphene) by different levels in broiler diets didn't affect carcass characteristics and bone measurements except for liver%, tibia width, and ash% were affected significantly by different levels of nanoparticles.

Table (2): Effect of nanoparticles (silver and graphene) in broiler diets on carcass characteristics %

Items	Carcass (%)	Liver (%)	Gizzard (%)	Heart (%)	Total giblets (%)	Total edible parts (%)	Abdominal fat (%)
Type							
SNaPs	67.44	2.71	1.4	0.49	4.62	72.07	0.98
GNaPs	68.88	2.53	1.39	0.51	4.44	73.32	1.03
SEM	2.71	0.32	0.22	0.07	0.43	2.53	0.29
p-value	Ns	Ns	Ns	Ns	Ns	Ns	Ns
Levels							
0 PPM	67.3	2.19[b]	1.56	0.51	4.28	71.58	0.97
2.5 PPM	68.36	2.80[a]	1.27	0.53	4.61	72.97	0.96
5 PPM	68.47	2.60[a]	1.43	0.47	4.51	72.98	1.04
7.5 PPM	67.12	2.77[a]	1.31	0.49	4.58	71.71	0.91
10 PPM	69.56	2.73[a]	1.42	0.51	4.67	74.24	1.14
SEM	2.71	0.32	0.22	0.07	0.43	2.53	0.29
p-value	Ns	**	Ns	Ns	Ns	Ns	Ns
Interaction							
SNaPs&0 PPM	67.3	2.19	1.56	0.51	4.28	71.58	0.97
SNaPs&2.5 PPM	68.9	2.76	1.3	0.48	4.56	73.47	0.86
SNaPs&5 PPM	66.99	2.57	1.39	0.45	4.43	71.42	0.9
SNaPs&7.5 PPM	64.73	3.07	1.35	0.5	4.93	69.67	0.76
SNaPs&10 PPM	69.3	2.96	1.41	0.51	4.89	74.2	1.43
GNaPs&0 PPM	67.3	2.19	1.56	0.51	4.28	71.58	0.97
GNaPs&2.5 PPM	67.81	2.84	1.24	0.57	4.66	72.48	1.06
GNaPs&5 PPM	69.94	2.63	1.46	0.49	4.59	74.54	1.19
GNaPs&7.5 PPM	69.51	2.47	1.26	0.48	4.22	73.74	1.06
GNaPs&10 PPM	69.83	2.51	1.42	0.52	4.46	74.29	0.85
SEM	2.71	0.32	0.22	0.07	0.43	2.53	0.29
p-value	Ns	Ns	Ns	Ns	Ns	Ns	Ns

a,b: Means in the same column with the same letters are not significantly different. MSE: Mean standard error NS: Non-significant *:($P \leq 0.05$) **: ($P \leq 0.01$) SNaPs = silver nanoparticles, GNaPs = graphene nanoparticles.

Table (3): Effect of nanoparticles (silver and graphene) in broiler diets on carcass cuts parts

Items	% Breast	% Thigh	% Drumstick	% Wing
Type				
SNaPs	28.13	18.27	9.60	6.83
GNaPs	27.00	18.36	9.30	6.87
SEM	5.38	2.12	1.29	0.87
p-value	Ns	Ns	Ns	Ns
Levels				
0 PPM	29.52	18.59	10.08	7.22
2.5 PPM	26.39	19.21	9.55	7.45
5 PPM	27.73	17.22	8.8	6.71
7.5 PPM	26.37	19.51	9.43	6.92
10 PPM	27.83	17.04	9.39	5.93
SEM	5.38	2.12	1.29	0.87
p-value	Ns	Ns	Ns	Ns
Interaction				
SNaPs&0 PPM	29.52	18.59	10.08	7.22
SNaPs&2.5 PPM	28.54	19.25	9.85	7.12
SNaPs&5 PPM	28.06	17.86	9.5	6.68
SNaPs&7.5 PPM	24.85	20.04	9.02	6.68
SNaPs&10 PPM	29.69	15.62	9.53	6.43
GNaPs&0 PPM	29.52	18.59	10.08	7.22
GNaPs&2.5 PPM	24.24	19.17	9.26	7.79
GNaPs&5 PPM	27.41	16.58	8.11	6.74
GNaPs&7.5 PPM	27.88	18.98	9.83	7.17
GNaPs&10 PPM	25.96	18.46	9.24	5.42
SEM	5.38	2.12	1.29	0.87
p-value	Ns	Ns	Ns	Ns

MSE: Mean standard error NS: Non-significant, SNaPs = silver nanoparticles, GNaPs = graphene nanoparticles.

Table (4): Effect of nanoparticles (silver and graphene) in broiler diets on some physical and chemical bone measurements

Items	Tibia length (mm)	Tibia width (mm)	Seedor index	Wet tibia weight (g)	Dry tibia weight (g)	Tibia breaking strength (Newton)	Ash%	Organic matter%	Calcium%	Phosphorus %
Type										
SNaPs	85.52	6.90	0.86	15.20	7.33	287.68	39.52	59.86	15.59	8.87
GNaPs	84.74	7.20	0.85	13.30	6.54	310.70	42.66	58.77	15.50	8.57
SEM	3.51	0.77	0.13	3.57	1.04	69.18	5.35	4.15	0.97	0.62
p-value	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
Levels										
0 PPM	87.15	7.79[a]	0.86	14.00	7.11	354.08	35.06[b]	65.14	15.46	8.27
2.5 PPM	84.89	6.27[b]	0.81	14.25	6.14	264.34	41.23[a]	58.90	15.46	9.00
5 PPM	83.31	6.96[ab]	0.90	13.75	7.16	261.30	42.12[a]	57.22	15.84	8.60
7.5 PPM	86.08	6.99[ab]	0.84	15.00	7.21	310.83	45.07[a]	56.96	15.57	8.98
10 PPM	84.21	7.22[a]	0.87	14.25	7.07	305.40	41.96[a]	58.35	15.40	8.75
SEM	3.51	0.77	0.13	3.57	1.04	69.18	5.35	4.15	0.97	0.62
p-value	Ns	**	Ns	Ns	Ns	Ns	*	Ns	Ns	Ns
Interaction										
SNaPs&0 PPM	87.15	7.79	0.86	14.00	7.11	354.08	35.06	65.14	15.46	8.27
SNaPs&2.5 PPM	85.44	6.28	0.84	15.00	6.66	277.25	36.22	63.55	15.89	9.32
SNaPs&5 PPM	84.54	7.11	0.83	14.00	6.83	237.00	42.15	55.50	15.89	8.20
SNaPs&7.5 PPM	85.26	6.37	0.92	17.00	8.3	239.41	43.45	56.22	15.13	9.40
SNaPs&10 PPM	85.20	6.95	0.87	16.00	7.77	330.65	40.69	58.87	15.57	9.15
GNaPs&0 PPM	87.15	7.79	0.86	14.00	7.11	354.08	35.06	65.14	15.46	8.27
GNaPs&2.5 PPM	84.35	6.26	0.77	13.50	5.61	251.42	46.23	54.24	15.02	8.69
GNaPs&5 PPM	82.08	6.81	0.97	13.50	7.49	285.60	42.09	58.93	15.78	9.00
GNaPs&7.5 PPM	86.9	7.62	0.75	13.00	6.13	382.25	46.69	57.70	16	8.55
GNaPs&10 PPM	83.23	7.50	0.87	12.50	6.36	280.15	43.22	57.82	15.24	8.35
SEM	3.51	0.77	0.13	3.57	1.04	69.18	5.35	4.15	0.97	0.62
p-value	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns

standard error NS: Non-significant *: ($P\leq 0.05$), SNaPs= silver nanoparticles, GNaPs = graphene nanoparticles.

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