Egyptian J. Nutrition and Feeds (2023), 26(2) Special Issue:9-17

EFFECTS OF DIFFERENT SOURCES AND LEVELS OF NANOPARTICLES IN BROILER DRINKING WATER ON SOME PHYSICAL AND CHEMICAL BONE MEASUREMENTS

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(Received 13/09/2023, accepted 01/11/2023)

SUMMARY

The aim of research was to investigate the effects of (silver or graphene) nanoparticles in concentrations of (0.0, 2.5, 5.0, 7.5 and 10.0) ppm/L drinking water in broiler chickens on some physical and chemical tibia bone measurements and some blood plasma parameters. For this purpose, 270 one day broiler chicks (Cobb 500) were randomly assigned to 9 experimental treatments (3replicates/ treatment, with 10/ chicks/replicate). In the end of experimental period (35 days) of age, tibia bone and blood plasma characteristics were determined. The results showed that: Tibia bone physical and chemical measurements weren't affected significantly by different types or levels of nanoparticles except organic matter%. There weren't significant differences between experimental treatments in all plasma total protein, Albumin, Globulin and A/G ratio related to nether types or levels of nanoparticles but, cholesterol, triglycerides and AST were differed significantly by types or levels of nanoparticles, however, ALT affected by different types of nanoparticles only. In addition, inclusion of different types or levels of nanoparticles in drinking water for broiler chickens didn't have any significant effects on tibia bone physical or chemical measurements and blood plasma parameters except tibia bone organic matter%, plasma cholesterol, triglycerides and AST.

Keywords: *broiler chicken, physical and chemical bone measurements, blood plasma and nanoparticles.*

INTRODUCTION

Nanotechnology is one of the most promising fields that can generate new applications in a lot of science branches. This technology deals with particle sizes that ranging between 1:100 nm (Loghman *et al.,* 2012 and Ahmadi *et al.,* 2013). When these materials turn to nanoparticles, the chemical and physical properties of these molecules change compared to the same materials and natural size. Nano minerals have unique properties that attracted a lot of attention for their distinct characteristics that are absent in traditional substances (Al-Nori, 2012). Nanotechnology is playing an important and major role in a lot of research fields of poultry science.

One of the most minerals widely used as a nanomaterial is silver nanoparticles because of its ability to purification 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 its effective role as anti-microbial, which is acting over potential pathogens, but not on the symbiotic microbial communities (Fondevila *et al.,* 2009).

Graphene nanoparticles, 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 chicks diets supplemented with graphene nanoparticles on 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 have 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

No data is available in the scientific literature directly to study the effects of different types and levels of nanoparticles (silver and graphene) in broiler drinking water. Therefore, the objective of our current research was to evaluate the effects of (silver or graphene) nanoparticles in concentration of (0.0, 2.5, 5.0, 7.5 and 10.0 ppm/L) drinking water of broiler chickens on some physical and chemical tibia bone measurements and some blood plasma parameters.

MATERIALS AND METHODS

The experiment work was carried out at poultry experimental unit, Agriculture experiment and research station at Shalakan, Faculty of Agriculture, Ain Shams University.

Experiment designed management:

A total number of 270 unsexed one day-old (Cobb 500) broiler chicks were distributed randomly into 9 treatments group, 30 chicks were assigned to each treatment group, 3 replicates of 10 chicks. The tested nanoparticles treatments were (0.0 nanoparticles) as a control, treatments, 2.5, 5.0, 7.5 and 10.0 ppm/L drinking water of two types of nanoparticles (silver or graphene).

Experimental diets:

Three periodical diets in mash form were formulated in the experiment includes a starter (1-14 days), a grower (15-28 days) and finisher (29-35 days) of age (Table 1).

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 day old), Grower (15-28 days- old) and finisher (29-35 day 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 (CaCO3) to 3 kg.*

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

Bone measurements and analysis:

At the end of the experiment (35 days) of age, 4 chickens from each treatment were randomly selected and sacrificed for tibia traits.

Tibia dimensions:

Tibia bones were removed and cleaned of all soft tissues and weight, tibia length and width were determined using digital micrometer according to the methods described by Samejima (1990). Tibia was dried in drying oven at 60 ^{o}C overnight, weighed (AOAC, 2012).

Blood sampling:

At the end of 35 days of age, individual blood samples were collected in dry clean centrifuge tubes from four chicks within each treatment. Blood samples were taken from the slaughtered birds and plasma was separated by centrifugation at 3000 rpm for 15 minutes and assigned for subsequent determination. plasma samples were harvested after centrifugation of the clotted blood, stored at –20o C in the deep freezer until the time of chemical determinations.

Blood constituents:

Biochemical analyses of some blood plasma of experiment was conducted in Poultry Nutrition Laboratory, Faculty of Agriculture, Ain Shams University. Quantitative determination of blood total protein Gornall *et al.* (1949)., albumin Doumas (1971)., Globulin = (Total protein - Albumin), cholesterol Richmond (1973), triglycerides Fassati and Prencipe (1982)., AST, and ALT Reitman and Frankel (1957).. All biochemical parameters of blood were calorimetrically determined using commercial diagnosing kits (Produced by Bio-Diagnostics Company, Egypt).

Treatments were assigned as the main factor, the statistical model performed as follow:

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

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

RESULTS ND DISCUSION

Tibia bone measurements and chemical composition:

Tibia bone measurements:

The results in table (2) showed the relationship between different types (SNaPs and GNaPs) or levels of nanoparticles and tibia bone physical measurements.

As shown in table (2) no significant differences have observed in all tibia bone physical measurements. Numerically, the chickens received drinking water supplemented with SNaPs had bone physical measurements values higher than the chickens received drinking water supplemented with GNaPs being (tibia length, 87.76 vs. 86.12mm, tibia width, 7.06 vs. 6.91mm, tibia wet weight, 16.80 vs. 14.95g, tibia dry weight, 7.95 vs. 7.30g, seedor index, 0.89 vs. 0.83 and tibia breaking strength 344.37 vs. 341.04 Newton) respectively. Besides, the differences between the two types were insignificant

On the other hand, the different levels of nanoparticles had no significant effects on studied parameters. Numerically the chickens drink water with 5.0ppm/l of nanoparticles had the highest tibia length and wet tibia weight values compared with the control group, Moreover, the control group recorded the highest tibia breaking strength and lowest tibia length, seedor index, wet and dry tibia weight compared with the treated group. However, the differences failed to be significant between treatments.

Bone chemical composition:

As shown in Table (3) the effect of using different types or levels of nanoparticles as a supplementation in broilers drinking water on some bone chemical composition.

Data in the table (3) indicate that no significant differences have observed between the two types of nanoparticles (SNaPs and GNaPs) in percentages of ash, calcium and phosphorus. However, the percentage of organic matter was higher significantly in birds drink water supplemented with GNaPs than birds drink water supplemented with SNaPs (58.29 vs. 53.49% respectively).

On the other hand, the different levels of nanoparticles hadn't a significant effect on all studied parameters (ash, organic matter, calcium and phosphorus).

Blood plasma parameters:

Table (4) showed the effect of using different types or levels of nanoparticles in broiler diets on blood plasma parameters.

Plasma total protein and its fractions:

Results in Table (4) showed the effect of different types and levels of nanoparticles on total protein, albumin, and globulin. It is worth to note that broiler chickens received drinking water supplemented with GNaPs has been linearly insignificant increased plasma total protein (5.18 vs. 4.93 g/dl) and its fractionation (albumin, 1.61 vs. 1.57 g/dl and globulin, 3.57 vs. 3.36g/dl), while, the A/G ratio was insignificantly decreased (0.47 vs. 0.48) compared with those received drinking water supplemented with SNaPs. On the other hand, plasma total protein and its fractions and A/G ratio haven't differed significantly with the different levels of nanoparticles as a supplementation in drinking water. These results differed with Shabani *et al.* (2010), Ahmadi (2012) and Kout Elkloub*et al.* (2015) who showed significant differences in total protein and its fractions and A/G ratio with silver nanoparticles supplement.

Plasma Lipids (Cholesterol and Triglycerides):

Data in Table (4) had shown the effect of different types and levels of nanoparticles on plasma lipid metabolites (Total cholesterol and triglycerides) of broiler chickens. Plasma cholesterol and triglyceride showed that the chickens received drinking water supplemented with GNaPs were recorded plasma lipid metabolites values higher than chickens received drinking water supplemented with SNaPs and the corresponding figures (cholesterol, 100.08 vs. 82.57 mg/dl and triglyceride, 88.34 vs. 83.09 mg/dl) with significant differences between the two types of nanoparticles.

On the other side, a significant difference has detected for total cholesterol and triglycerides due to different levels of nanoparticles. Significantly, the lower value of cholesterol recorded by birds received drinking water supplemented with 5.0 ppm/l (79.67 mg/dl) while birds in the control group were the highest (102.91mg/dl).

Also, the lower significant value of triglycerides recorded by birds in the control group (75.00 mg/dl), while, birds received drinking water supplemented with2.5 ppm/l (97.10). These results were similar to Ahmadi (2012) and Kout Elkloub*et al.* (2015) who showed that total lipid decreasing significantly with silver nanoparticles supplement. Cholesterol also differs significantly with silver nanoparticles. These results differed with what found by Ahmedi *et al.* (2013) who recorded that cholesterol hasn't affect by treatments.

Liver enzymes in plasma (AST and ALT):

The values of plasma liver enzymes activities AST and ALT at 35 days of age have shown Table (4). The figures for AST and ALT indicated significant differences between the two types of nanoparticles (SNaPs and GNaPs). The birds drink water supplemented with SNaPs were recorded AST and ALT values higher (AST, 26.06 vs. 22.17µ/dl and ALT, 19.82 vs. 18.17 µ/dl) compared with birds drink water supplemented with GNaPs. Besides, the differences between the two types were significant in AST levels only.

On the other hand, significant differences were observed in both of AST and ALT values with the different nanoparticles levels where the birds drink water with 2.5 ppm/l recorded lowest ALT value (14.98µ/dl) and birds drink water with 10.0 ppm/l recorded lowest AST value(21.01µ/dl) compare with the control group.

These results in similar to what found by Ahmadi (2012) recorded that ALT was a significant decrease in plasma with increasing Nano-silver (SNaPs) levels in the diet. Also, AST achieved the same results in plasma. However, Ahmadi (2009) who showed that there wasn't a significant effect on blood enzymes (AST or ALT) at different levels.

CONCLUSION

In addition, inclusion of different types or levels of nanoparticles in drinking water for broiler chickens didn't have any significant effects on tibia bone physical or chemical measurements and blood plasma parameters except tibia bone organic matter%, plasma; cholesterol, triglycerides and AST.

Table (2): Supplementation of nanoparticles (SNaPs & GNaPs) to broiler drinking water on some bone measurements.

Item	Type	Control	Levels				overall	MSE	Sig.		
			T1	T2	T3	T4			T	L	T*L
Tibia length (mm)	SNaPs	84.72	86.61	86.55	86.32	86.40	87.76	3.70	NS	NS	NS
	GNaPs	84.72	88.91	89.44	87.37	88.34	86.12				
	overall	84.72	87.76	88.00	86.85	87.37					
Tibia Width (mm)	SNaPs	7.27	6.90	6.79	7.17	7.14	7.06	0.92	NS	NS	NS
	GNaPs	7.27	7.25	6.38	6.59	7.09	6.91				
	overall	7.27	7.08	6.59	6.88	7.11					
MSEedor index	SNaPs	0.09	0.08	0.09	0.09	0.08	0.09	0.01	NS	NS	NS
	GNaPs	0.09	0.08	0.08	0.09	0.08	0.08				
	overall	0.09	0.08	0.08	0.09	0.08					
Wet Tibia Weight(g)	SNaPs	14.50	15.50	17.50	20.50	16.00	16.80	3.40	NS	NS	NS
	GNaPs	14.50	14.50	9.50	18.50	15.00	14.40				
	overall	14.50	15.00	13.50	19.50	15.50					
Dry Tibia Weight(g)	SNaPs	7.18	8.18	8.00	9.02	7.40	7.95	1.40	NS	NS	NS
	GNaPs	7.18	7.43	4.94	8.82	8.12	7.30				
	overall	7.18	7.80	6.47	8.92	7.76					
Tibia breaking strength (Newton)	SNaPs	373.14	318.35	385.07	343.53	301.75	344.37	100.08	NS	NS	NS
	GNaPs	373.14	281.88	295.87	395.87	358.40	341.04				
	overall	373.14	300.12	340.48	369.71	330.08					

*a,b: Means in the same row or column with the same letters are not significantly different. MSE: Mean standard error NS: Non-significant **: ($P\leq 0.01$) SNaPs= silver nanoparticles, GNaPs = graphene nanoparticles, T*L= the interaction between types and levels of nanoparticles.*

Table (3): Supplementation of nanoparticles (SNaPs & GNaPs) to broiler drinking water on some bone components.

Item	Type	Control	Levels				overall	MSE	Sig.		
			T1	T2	T3	T4			T	L	T*L
% Moisture	SNaPs	52.01	53.66	58.66	55.34	52.04	54.34	4.67	NS	*	NS
	GNaPs	52.01	52.85	58.55	50.85	48.52	52.56				
	Overall	52.01[b]	53.26[b]	58.61[a]	53.09[b]	50.28[b]					
% Ash	SNaPs	41.85	43.90	48.18	48.18	47.76	45.97	4.79	NS	NS	NS
	GNaPs	41.85	44.93	43.87	40.32	43.77	42.95				
	Overall	41.85	44.42	46.03	44.25	45.76					
% Organic mater	SNaPs	56.25	54.00	51.90	51.60	53.70	53.49[b]	4.00	*	NS	NS
	GNaPs	56.25	62.00	55.55	60.75	56.90	58.29[a]				
	Overall	56.25	58.00	53.72	56.17	55.30					
% Calcium	SNaPs	15.89	15.46	15.89	14.91	16.11	15.65	1.38	NS	NS	NS
	GNaPs	15.89	15.89	15.24	14.80	16.00	15.57				
	Overall	15.89	15.67	15.57	14.85	16.06					
% Phosphorus	SNaPs	9.42	8.79	8.66	8.87	9.00	8.95	0.81	NS	NS	NS
	GNaPs	9.42	9.07	9.86	9.32	9.56	9.45				
	Overall	9.42	8.93	9.26	9.10	9.28					

*a,b: Means in the same row or column with the same letters are not significantly different. MSE: Mean standard error NS: Non-significant **: ($P \leq 0.01$) SNaPs= silver nanoparticles, GNaPs = graphene nanoparticles, T*L= the interaction between types and levels of nanoparticles.*

Table (4): Supplementation of nanoparticles (SNaPs & GNaPs) to broiler drinking water on some blood plasma components.

Item	Type	Control	Levels				overall	MSE	Sig.		
			T1	T2	T3	T4			T	L	T*L
AST µ/dl	SNaPs	27.46	28.64	24.78	27.98	21.47	26.06[a]	3.21	**	**	*
	GNaPs	27.46	19.19	20.88	22.75	20.55	22.17[b]				
	overall	27.46[a]	23.91[bc]	22.83[bc]	25.37[ab]	21.01[c]					
ALT µ/dl	SNaPs	20.98	15.53	19.87	24.30	18.42	19.82	3.08	NS	**	NS
	GNaPs	20.98	14.42	12.21	23.96	19.28	18.17				
	overall	20.98[ab]	14.98[d]	16.04[bc]	24.13[a]	18.85[bc]					
Cholesterol (mg/dl)	SNaPs	102.91	82.12	80.14	82.74	64.96	82.57[b]	8.21	**	**	**
	GNaPs	102.91	78.48	79.20	121.10	118.71	100.08[a]				
	overall	102.91[a]	80.30[c]	79.67[c]	101.92[a]	91.84[b]					
Triglyceride (mg/dl)	SNaPs	75.00	84.68	90.00	88.59	77.18	83.09[b]	6.00	**	**	**
	GNaPs	75.00	109.53	100.31	81.25	75.62	88.34[a]				
	overall	75.00[c]	97.10[a]	95.15[a]	84.92[b]	76.40[c]					
Total Protein (g/dl)	SNaPs	5.36	5.02	4.88	4.78	4.64	4.93	0.53	NS	NS	NS
	GNaPs	5.36	5.75	4.67	4.86	5.26	5.18				
	overall	5.36	5.39	4.77	4.82	4.95					
Albumin (g/dl)	SNaPs	1.59	1.65	1.55	1.63	1.42	1.57	0.13	NS	NS	NS
	GNaPs	1.59	1.60	1.48	1.73	1.64	1.61				
	overall	1.59	1.63	1.51	1.68	1.53					
Globulin (g/dl)	SNaPs	3.77	3.36	3.32	3.15	3.22	3.36	0.56	NS	NS	NS
	GNaPs	3.77	4.15	3.19	3.12	3.62	3.57				
	overall	3.77	3.75	3.25	3.13	3.42					
A/G Ratio	SNaPs	0.42	0.50	0.48	0.54	0.44	0.48	0.11	NS	NS	NS
	GNaPs	0.42	0.38	0.48	0.58	0.46	0.47				
	overall	0.42	0.44	0.48	0.56	0.45					

*a,b: Means in the same row or column with the same letters are not significantly different. MSE: Mean standard error NS: Non-significant **: ($P \leq 0.01$) SNaPs= silver nanoparticles, GNaPs = graphene nanoparticles, T*L= the interaction between types and levels of nanoparticles*

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دراسة تاثير استخدام انواع ومستويات مختلفة من جسيمات النانو فى مياة شرب دجاج اللحم على بعض القياسات الفيزيائية والكيميائية للعظم

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الهدف من البحث هو دراسه تاثير اضافه جسيمات النانو(فضه او جرافين) في ماء شرب دجاج اللحم بمستويات (0.0 و2.5 و 5.0 و 7.5 و 10.0) جزء في المليون/لتر علي بعض قياسات العظم الفيزيائية و الكيميائية وبعض قياسات بلازما الدم.

استخدام في التجربه 270 كتكون تسمين في سلاله كب 500 عمر يوم قسمت الي 9 مجموعات بكل منها (3 مكرر/ 10 كتاكيت لكل مكررة)

أوضحت النتائج أن

1. صفات عظام الساق الفيزيائية و الكيميائية لم تتأثر بالانواع أو المستويات المختلفه من جسيمات النانو باستثناء ٪للماده العضويه حيث تأثرت معنويا بالانواع المختلفه.
2. -قياسات بلازما الدم (٪للبروتين الكلي و الالبيومين و الجلوبيولين و نسبه (G/A) لم تتأثر بالانواع أو المستويات المختلفه من جسيمات النانو لكن٪ للكولسترول والدهون الثلاثيه وASTاختلف معنويا بالانواع او المستويات المختلفه من جسيمات النانوية.بينما النسبة المئوية لل ALT تاثر فقط بالمستويات المختلفة من جسيمات النانو.

الخلاصه: اضافه المصادر المختلفه من جسيمات النانو(فضه او جرافين)بمستويات مختلفه لماء شرب دجاج اللحم لم يكن له تأثير علي صفات العظم الفيزيائية و الكيميائية و كذلك لبلازما الدم باستثناء الماده العضويه بعظمه الساق و كذلك مستوي الكولسترول و الدهون الثلاثيه وAST لبلازما الدم.