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THE IMPACT OF SILVER AND GRAPHENE NANOPARTICLES IN BROILER DRINKING WATER ON PERFORMANCE AND INTESTINAL MICROFLORA.

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SUMMARY

The current study aimed to determine the impact of using silver nanoparticles (SNaPs) or graphene nanoparticles (GNaPs) as a water supplementation in broiler chicken on productive performance and microbial load in small intestine and ceca of broiler chicken at thirty five days of age. The study comprised of 270 unsexed broiler chicks (Cobb 500) one-day old, which were divided randomly into nine experimental treatments in 3 replicates for each treatment. Four levels of each types (SNaPs or GNaPs) of nanoparticles (2.5, 5, 7.5 and 10 ppm/ L) were used as well as control group. At thirty five days of age, four birds from each treatment were slaughtered to obtain intestinal content samples. Results showed that live body weight (LBW) and body weight gain (BWG) were differed significantly by different levels and different types of nanoparticles. However, LBW, BWG feed consumption (FC) were significantly higher in birds received drinking water supplemented with GNaPs than those received SNaPs, but they were not affected by different levels of nanoparticles. Also, feed conversion ratio (FCR) was not affected by neither types nor levels of nanoparticles. On the other hand, a bacterial count in small intestine was affected significantly by both types and levels of nanoparticles. Also bacterial counts in ceca was affected significantly by both types and levels of nanoparticles *Lactobacillus spp.* and *E. Coli* counts were decreased in small intestine and ceca with increasing nanoparticles levels in drinking water. However, the birds received drinking water supplemented with SNaPs, recorded significantly higher *Lactobacillus spp.* and *E.Coli* counts than those received GNaPs in both small intestine and ceca. It can be concluded that GNaPs have highest effects than SNaPs on productive performance and microorganism in small intestine and ceca.

***Keywords**: nanoparticle, broilers, performance, microorganism*

INTRODUCTION

Recently, nanotechnology had rapidly developed having the most impact on human, animal, environmental and industrial life (Ahmadi and Kurdestany 2010). Researchers used nanoparticles as additives in broiler feeding to achieve positive effects on poultry production, these products such as: nanosilver, nanographene, nanoselenium and zinc oxide nanoparticles (Ahmadi and Rahimi 2011; Sawosa et al., 2007). Silver has been used since ancient times for jewelers, utensils, monetary currency, dental alloy, photography, and explosives (Oberdörster, 2010) because it can control microbial proliferation even against antibiotic-resistant bacteria (Wadhera and Fung 2005). Ahmedi and Rahimi (2011) studied the effect of different levels of SNaPs as supplementation in drinking water of broilers on LBW at 42 days of age. Treatments were 0, 4, 8 and 12 ppm/ L. The results showed significantly decreased LBW and FI also, FCR was affected significantly with increased SNaPs levels. Kvitek *et al.* (2011) compared in-vitro between low concentration of SNaPs with different sizes and concentrations of ionic silver as antibacterial agents. As the effect of SNaPs decreases with larger particle diameter. The greatest effect of SNaPs as antibacterial agents was with diameter of 25 nm. Vadalasetty *et al.* (2018) studied the effect of silver nanoparticles on broiler performance at 30 days of age. Chicks were divided into two groups, first group was control group (chicks were offered drinking water without SNaPs) and treated group (chicks were offered drinking water with 50 ppm SNaPs). The results showed that daily water intake, daily FI, daily BWG and FCR were not affected by treatment at 30 day of age. However, total number of aerobic bacteria in treated group was considerably higher than in the control. Also, it was found that there was a decrease in the number of *E. coli* bacteria in treated group compared with control group.

Graphene nanoparticles is a one-atom-thick material consisting of sp2-bonded carbon with a honeycomb structure (Ying Wang *et al*., 2011) and can be act as antimicrobial activity against both of

Gram-negative and Gram-positive bacteria. Additionally, graphene oxide (GO) nanoparticles presented potent microbial properties. These new allotropic type of carbon have been discovered in the last twenty years, and, since then, they have been used in many fields of science (Solmaz, 2015). GO nanoparticles has strong antimicrobial activity on E. coli (Kang *et al.,* 2007). Carbone nanoparticle is one of the most applied nanoparticles in water purification as carbone surface area is large in addition to its higher absorption ability (Ortiz-Ibarra *et al.,* 2007). Carbone can remove some species of bacteria such as *E. Coli* from water systems (Quinlivan and Knappe, 2005). Also, carbone particles can be associated with microorganisms by Lifshitz van der Waals forces (Jucker *et al.,* 1996). The growth of Gram-positive, Gram-negative and *E. coli* bacteria are affected significantly by the sharp edges of the reduced graphene nanoparticles. Majewska *et al.* (2011) studied the effect of using carbon in broiler diets on performance. The treatments were control group fed basal diet and treated group fed diet supplemented with 0.3% carbon and found that carbon can improve LBW significantly at 42 days old compared with control and, improve FCR insignificantly but FI decreased around 2%. Amprako *et al*. (2018) investigated the effect of using different levels of wood charcoal in broiler diets at 42 days of age. The experimental diets were 0, 1.5, 3, and 6% wood charcoal and the results showed that LBW, BWG, FI and FCR were not significantly affected by treatments.

The present study is aimed to determine the impact of using different types of nanoparticle (SNaPs) or (GNaPs) in broiler drinking water on performance and microbial load in gut at 35 days of age.

MATERIALS AND METHODS

The study was conducted by using 270 unsexed broiler chicks (Cobb 500) one-day old which were randomly distributed into nine treatments (3 replicate in 10 birds each) to examine using two types of nanoparticles (silver and graphene) at 4 levels (2.5, 5, 7.5, and 10 ppm/ L drinking water) and control group without any supplementation. The experiment lasted till 35 days of age in three periodical diets (starter, grower, and finisher). Diets were formulated to meet broiler requirements based on manual guide of Cobb 500 strain. The composition and nutrient content of diets are presented in Table (1).

Table (1): Composition and calculated chemical analysis of starter, grower, and finisher diets.

Ingredients	Experimental diets		
	Starter*	Grower*	Finisher*
Yellow corn grains	55.76	59.70	63.70
Soybean meal 48%	37.84	33.10	28.22
Soybean 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 (NaCl)	0.30	0.30	0.30
Premix**	0.30	0.30	0.30
Total	100	100	100
Calculated chemical analysis***			
Crude protein (%)	23.01	21.04	18.99
ME (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 day 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 chemical according to NRC (1994).*

Live body weight (LBW) of each replicate was recorded weekly till 35 days of age in the early morning before feeding. Feed consumption (FC) of each replicate was also recorded weekly till 35 days of age. Both body weight gain (BWG) and feed conversion ratio (FCR) were calculated at 14, 28 and 35 days of age. Performance index (PI) and European production efficiency factor (EPEF) are calculated at 35 days of age. At the end of experiment, four chicks of each treatment were taken randomly and slaughtered to determine the bacterial count in small intestine and ceca. Statistical analysis of data obtained from the present study was conducted using the general linear model (GLM) procedure of SAS® (SAS, 2006), by applying test using two ways ANOVA. Means were compared using Duncan´s range test (Duncan, 1955) where the level of significance was set at minimum ($P \leq 0.05$).

Treatments were assigned to statistical analysis with type of additives (T) and level of additives (L) and the statistical model was performed as fallow:

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

Where: Y_{ij}= is the effect of the observation, M= overall mean, T_i= the effect of ith type of nanoparticles, L_j= the effect of jth level of nanoparticles, $(T*L)_{ij}$ = interaction between type and level of nanoparticles and E_{ijk}= random error.

RESULTS AND DISCUSSIONS

Productive performance

Data in Table (2) showed the effect of using different types (SNaPs or GNaPs) and levels of nanoparticles as supplementation in drinking water on productive performance of broilers.

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

The results in Table (2) showed significant increases in both LBW and BWG in treated groups compared 10 ppm with control group. These results might be related to that nanoparticles destroy harmful bacteria in small intestine and ceca. On the other hand, the groups received drinking water supplemented with GNaPs recorded higher LBW and BWG than groups received SNaPs by 2.07% and 2.12%, respectively. These results might be related to that the harmful bacteria in small intestine and ceca were lower in the groups received drinking water supplemented with GNaPs than groups received SNaPs. Ahmedi and Rahim (2011) studied the effect of different levels of SNaPs as supplementation in drinking water of broiler on LBW at 42 days old and found significantly decreased LBW with increase the SNaPs levels. However, Pinedaa *et al.* (2012) evaluated the effect of using SNaPs in broiler on LBW during the period from 7-35 days of age. The experimental design was using SNaPs in levels 0, 10 and 20 mg/ kg in broiler drinking water. The results recorded that the different levels of SNaPs had no effect on LBW and BWG of broiler chicks. Mookiah Saminathan (2018) studied the effect of Nano magnetic graphene oxide with chitosan (MGO-CTS) adsorbents as anti-aflatoxin in feed contaminated with aflatoxin (AF) on LBW and BWG of broilers. Treatments were, AF nor MGO-CTS added, T1), basal diet + 0.25% MGO-CTS (T2), basal diet+ 0.50% MGO-CTS (T3), AF diet + 0.25% MGO-CTS (T4), AF diet + 0.50% MGO-CTS (T5), and AF diet (T6). Results showed that (MGO-CTS) improved LBW and BWG in diets contaminated with AF.

Feed consumption (FC) and feed conversion ratio (FCR):

As shown in Table 2, both FC and FCR were not affected significantly with different levels of nanoparticles (SNaPs or GNaPs) in drinking water. However, the groups received drinking water supplemented with GNaPs recorded significant higher FC than the groups received SNaPs but, FCR was not affected by different types of nanoparticles. Vadalasetty et al. (2018) studied the effect of SNaPs on broiler performance at 30 days old. The chick divided into two groups, first group was control group (chicks drinking water without SNaPs) and second treated group (chicks drinking water with 50 ppm SNaPs). The results showed that FI and FCR were not affected by treatment at 30 days old. Mookiah Saminathan (2018) studied the effect of Nano magnetic graphene oxide with chitosan (MGO-CTS) adsorbents as anti-aflatoxin in feed contaminated with aflatoxin (AF) on FC and FCR. Results showed that (MGO-CTS) both of FI and FCR were affected significantly in diets contaminated with AF.

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

The obtained data for PI and EPEF are summarized in Table 2 showed that insignificant differences were observed during the overall period (1-35) days of age between different types of nanoparticles (SNaPs and GNaPs). However, chicks received GNaPs in drinking water recorded insignificant increased values (PI, 122.11 vs. 119.80) and (EPEF, 340.64 vs. 334.05) compare with those received SNaPs in drinking water.

Microbial count in small intestine and ceca

The results in Table 3 showed that there was significant decreased for microbial count in small intestine and ceca in all treatments compared with the control group. These results might be related to the role of nanoparticles as antimicrobial for both types of Gram-positive and Gram-negative bacteria including antibiotic-resistant strains (Keller et al., 2018). On the other hand, birds consumed water supplemented with GNaPs recorded lower *E. Coli* count in both of small intestine and ceca than those get water supplemented with SNaPs. However, the birds received water supplemented with SNaPs recorded higher *Lactobacillus spp.* count in ceca than birds received water supplemented with GNaPs but the opposite occurred in small intestine. These results might be related to the effect of both types of nanoparticles as antimicrobial against gram-positive and gram-negative bacteria (Kang et al 2007, Sawosz et al., 2007; Egger et al., 2009; Prabhu and Poulose, 2012)). Ognik et al. (2016) studied the effect of SNaPs on broiler microflora in small intestine at 42 day old via a tube into the crop. The control group receives distilled water. The group II received an aqueous solution of SNaPs at a dose of 5 mg/ kg body weight/ day. The group III received an aqueous solution of lipid-coated SNaPs a dose of 5 mg/ kg body weight/ day. The results showed that the total number of aerobic bacteria in second and third groups of broilers was considerably higher than in the control. Also, it was found that there was decrease in the number of *E. coli* group bacteria in second and third groups compared with control group. Pinedaa et al. (2012) evaluated the effect of using SNaPs in broiler on microorganisms in digestive tract of broiler chicks during the period from 7-35 days old. The experimental design involved SNaPs in levels of 0, 10 and 20 mg/ kg in broiler drinking water. The results recorded that the different levels of AgNano had no effect on microorganisms in ileum or cecum of broiler chicks. Dong et al. (2012) who studied the properties of single-walled carbon nanotubes (SWCNTs) as antibacterial in different surfactant against E. coli and found that SWCNTs exhibited antibacterial activity against E. coli, and it was improved with the increased nanotube concentrations. Also, Arias and Yang (2009) investigated the effect of SWCNTs as antimicrobial with different surface groups towards rod-shaped or round-shaped Gram-negative and Gram-positive bacteria and found that SWCNTs with surface groups of -OH and -COOH indicated improved antimicrobial activity to both Gram-positive and Gram-negative bacteria. Their results showed that, formation of cell-CNTs aggregates, caused to damage the cell wall of bacteria and then release of their DNA content.

CONCLUSION

Graphene nanoparticles have the highest significant effect compare with silver nanoparticle on broiler chicken performance (LBW, BWG and FC) and E.Coli count in small intestine and ceca, advised doing more research in this area to improve the utilization of nanoparticles.

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تاثير اضافة جزيئات النانو (فضة وجرافين) لماء شرب بداري التسمين على الاداء الانتاجى والكائنات الحية الدقيقة فى الامعاء.

أحمد محمد تمام و سيد عبد الرحمن ابراهيم و علاء الدين عبد السلام و فتحي عبد العظيم و احمد ابراهيم الفحام و نعمة الله جمال الدين و وسام سالم

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اجريت تجربة للتعرف على تاثير استخدام نوعين من جزيئات النانو (فضة اوجرافين) فى ماء شرب بداري التسمين على الاداء الانتاجي وميكروبات الامعاء الدقيقة والاعور فى تجربة استمرت 35 يوم. واستخدم فى التجربة 270 كتكوت غير مجنس من عمر يوم من سلالة (Cobb 500) وتم تقسيمها الى 9 معاملات (3 مكررات) لكل معاملة حيث استخدم 4 مستويات من كل نوع (2.5 , 5.0 , 7.5 و 10.0) جزء فى المليون/لتر ماء شرب مع مجموعة كنترول بدون اضافة.

النتائج : تأثر الوزن الحى والوزن المكتسب معنويا بالمستويات والانواع المختلفة من جزيئات النانو وتحسن الوزن الحى والوزن المكتسب واستهلاك العلف معنويا للطيور المعاملة بالجرافين بالمقارنة بالطيور المعاملة بالفضة ولكن لم تتاثر بالمستويات المختلفة. لم يتاثر معامل التحويل الغذائى بنوع او مستوى جزيئات النانو. وكذلك لم يتاثر المحتوى البكتيري فى الامعاء والاعور معنويا بالنوع والمستوى وجزيئات النانو. وقد انخفضت اعداد *Lactobacillus spp.* و *E.Coli* فى الاعور والامعاء بصورة معنوية بارتفاع مستوى جزيئات النانو. تزداد اعداد *Lactobacillus spp* و *E.Coli* فى الاعور والامعاء بصورة معنوية باستخدام جزيئات النانو فضة بالمقارنة بجزيئات النانو جرافين.

الخلاصة: جزيئات النانو جرافين أكثر تاثيرا على الاداء الانتاجى والبكتيريا فى الامعاء والاعور بالمقارنة بجزيئات الفضة.

Table (2): Effect of nanoparticles (SNaPs and GNaPs) as a supplementation in drinking water on productive performance of broiler chicks

Items	Types	Control	Level				Overall	MSE	Sig.		
			2.5 PPM	5 PPM	7.5 PPM	10 PPM			T	L	T*L
LBW (35 day)	SNaPs	1829.15	1875.97	1834.45	1771.55	1834.07	1829.04^{b}	46.89	*	*	NS
	GNaPs	1829.15	1871.62	1876.94	1811.36	1949.78	1867.77^{a}				
	Overall	1829.15^{bc}	1873.80^{ab}	1855.70^{ab}	1791.46^{c}	1891.93^{a}					
BWG (1-35 day)	SNaPs	1784.99	1832.03	1832.03	1728.32	1789.64	1785.02^{b}	46.52	*	*	NS
	GNaPs	1784.99	1826.58	1832.61	1767.80	1905.95	1823.59^{a}				
	Overall	1784.99^{bc}	1829.31^{ab}	1811.37^{ab}	1748.06^{c}	1847.80^{a}					
FC (1-35 day)	SNaPs	2751.38	2767.78	2770.03	2674.78	2672.06	2727.21^{b}	82.99	*	NS	NS
	GNaPs	2751.38	2768.75	2829.12	2702.50	2901.32	2790.62^{a}				
	Overall	2751.38	2768.27	2799.58	2688.65	2786.70					
FCR (1-35 day)	SNaPs	1.54	1.51	1.54	1.54	1.49	1.52	0.04	NS	NS	NS
	GNaPs	1.54	1.51	1.54	1.52	1.52	1.53				
	Overall	1.54	1.51	1.54	1.53	1.50					
Performance index	SNaPs	118.66	124.35	118.55	114.56	122.86	119.80	5.25	NS	*	NS
	GNaPs	118.66	123.53	121.59	118.57	128.20	122.11				
	Overall	118.66^{bc}	123.94^{ab}	120.07^{bc}	116.57^{c}	125.53^{a}					
European production efficiency factor	SNaPs	330.86	346.98	330.55	319.33	342.54	334.05	14.74	NS	*	NS
	GNaPs	330.86	344.46	339.21	330.63	358.06	340.64				
	Overall	330.86^{bc}	345.72^{ab}	334.88^{bc}	324.98^{c}	350.30^{a}					

a, b, c: Means in the same row or column with the same letters are not significantly different.
MSE: Mean standard error NS: Non-significant: ($P \leq 0.05$)*
*SNaPs= silver nanoparticles, GNaPs = graphene nanoparticles, T*L= the interaction between types and levels of nanoparticles.*

Table (3): Effect of nanoparticles (SNaPs and GNaPs) as a supplementation in drinking water on microbial count in the gut of broilers chicks.

	Item	Type	Control	Level				Overall	MSE	Sig.		
				2.5 PPM	5 PPM	7.5 PPM	10 PPM			T	L	T*L
Small intestine		SNaPs	0.76 X10^4	0.36 X10^4	0.31 X10^4	0.08 X10^4	0.60 X10^4	0. 42^b X10^4				
	Lactobacillus spp.	GNaPs	0.76 X10^4	0.46 X10^4	0.75 X10^4	0.65 X10^4	0.11 X10^4	0.54^a X10^4	0.20X10^4	**	**	**
		Overall	0.76^a X10^4	0.41^c X10^4	0.53^b X10^4	0.36^d X10^4	0.35^d X10^4					
		SNaPs	0.18X10^4	0.22 X10^4	0.20 X10^4	0.00 X10^4	0.00 X10^4	0.12^{a}X10^4				
	E. Coli	GNaPs	0.18X10^4	0.02 X10^{4b}	0.06 X10^{4b}	0.12 X10^{4c}	0.14 X10^{4c}	0.10^{b}X10^4	0.00X10^4	**	**	*
		Overall	0.18^a X10^4	0.12^c X10^4	0.13^b X10^4	0.06^e X10^4	0.07^d X10^4					
Ceca		SNaPs	0.58 X10^4	0.78 X10^4	0.01 X10^4	0.17 X10^4	0.01 X10^4	0.31^a X10^4				
	Lactobacillus spp.	GNaPs	0.58 X10^4	0.11 X10^4	0.04 X10^4	0.64 X10^4	0.02 X10^4	0.28^{b}X10^4	0.01X10^4	**	**	**
		Overall	0.58^a X10^4	0.45^b X10^4	0.40^c X10^4	0.02^d X10^4	0.02^d X10^4					
		SNaPs	0.17 X10^4	0.35 X10^4	0.18 X10^4	0.15 X10^4	0.06 X10^4	0.18^{a}X10^4				
	E. Coli	GNaPs	0.17 X10^4	0.02 X10^4	0.00 X10^4	0.00 X10^4	0.00 X10^4	0.03^{b}X10^4	0.01X10^4	**	**	**
		Overall	0.17^a X10^4	0.18^a X10^4	0.09^b X10^4	0.07^b X10^4	0.03^d X10^4					

$^{a, b, c:}$Means in the same row or column with the same letters are not significantly different.
*MSE: Mean standard error NS: Non-significant **: (P≤ 0.01) *: (P≤ 0.05).*
*SNaPs= silver nanoparticles, GNaPs= graphene nanoparticles, T*L= the interaction between types and levels of nanoparticles.*