



Effects of supplementing silver nanoparticles during early embryogenesis on the hatchability and post hatch performance of broilers

AKSHAT GOEL, SUBRAT KUMAR BHANJA*, NIBEDITA NAYAK, MANISH MEHRA, and KARUPPASAMY K

ICAR-Central Avian Research Institute, Izatnagar, Bareilly, Uttar Pradesh 243 122 India

Received: 27 June 2019 Accepted: 4 September 2019

ABSTRACT

The aim of the present study was to investigate the role of silver nanoparticles (AgNano) supplementation during early embryogenesis on the post-hatch performance and immunity in broilers. Increasing concentration of AgNano 0 (un-injected control), 12.5, 25 and 50 µg/egg, respectively were administered on 7th embryonic day at the broad end of broiler egg, following *in ovo* feeding techniques. A total of 60 eggs per treatment were used and five replicates of 8 birds each in all treatment were reared upto 42 days post-hatching. Egg weight, chick weight and ratio were similar in all the groups, however, the hatchability decreased with increasing AgNano concentration. Average daily weight gain was increased in all the AgNano supplemented chicks. Average daily feed intake and feed conversion ratio were not affected by AgNano supplementation. Pancreas weight has shown an increasing trend with increasing concentration of AgNano. The bursa and spleen weight were increased with increasing concentration of AgNano supplementation during embryogenesis. *In vivo* Immune-response to phytohaemagglutinin-P and sheep RBC was increased in AgNano supplemented chicks. Serum cholesterol level was decreased in AgNano supplemented chicks unlike glucose and total protein. Early embryonic supplementation of AgNano particles modulates the post-hatch growth performance and immunity in broilers.

Keywords: Broiler chicken, Embryo, Immunity, Performance, Silver nanoparticles

The embryonic and first week post-hatch represents a larger proportion (45%) of broilers lifespan. Unlike the mammalian embryo, the avian embryo develops in a carbohydrate free environment with finite amount of energy, i.e. closed nutrition (Foye *et al.* 2006). Moreover, there is a gap of 24–72 h till the chicks receive their first meal due to difference in hatch window and problems in logistic which hinders the immunity and further the growth process. Administering the developing embryo with appropriate supplements (by *in ovo* feeding) is a novel way to manipulate the composition and ‘jump-start’ development of the chick and has been tried previously for supplementing vitamins in eggs (Goel *et al.* 2013).

Nanotechnology deals with structures in the nano-size range (1–100 nm) with a large surface area and more atoms exposed on the surface of the nanoparticles. Being more reactive than larger particles, it has been demonstrated that toxicity can be minimized or eliminated using nano amounts of the active substance. These unique abilities bless them with electronic, optical and catalytic properties better than their macro counterparts (Paleo *et al.* 2011). Silver has been used since time immemorial for the treatment of burns, wounds and bacterial infections (Rai *et al.* 2009). Silver nanoparticles have effective biocidal activity against a broad spectrum of Gram negative as well as Gram positive bacteria

(Wright *et al.* 1999), modulates the expression of toll like receptors (Bhanja *et al.* 2015) and thus affect intestinal microbial populations and improve the health and immunological status of the birds (Pineda *et al.* 2012).

Considering the unique properties of AgNano particle, the present study was undertaken to study the effect of *in ovo* administration of AgNano on hatchability, post-hatch production, blood biochemical parameters and immune status of broiler chicken.

MATERIALS AND METHODS

The experiment was conducted at the facility of Central Avian Research Institute farms and was approved by the Institute Animal Ethics Committee of Central Avian Research Institute, Izatnagar, India. Proper ethical standards were followed during the experiment. The eggs were set in a vertical force draft incubator at temperature 37.5°C and relative humidity of 60%. On 19th day of incubation, the eggs were shifted to a hatcher and kept in pedigree hatching boxes.

Nanosolution: The colloidal AgNano solution (AgNano) *aXonnite* (100 mg per liter deionized water, 99.9999% purity) was obtained from Nano-Tech Poland Ltd (Warsaw, Poland). The average particle size, particle surface area, pH range and redox potential were 3.5 nm, 2.827 cm², 7.1–8.1 and 0.1 mV, respectively with high purity (>99%).

Experimental design and in ovo feeding: The eggs were

*Corresponding author e-mail: subratcari@gmail.com

collected (n=240) from a single flock (50 weeks old) of white plumaged synthetic broiler dam line (SDL) that has been developed from synthetic base population at Experimental broiler farm, Central Avian Research Institute, Izatnagar, by selective breeding and crossing of six elite crosses based on their economic traits for growing in tropical region. The base population were stabilized after two generation of cross-breeding and was maintained on a balanced ration as per the US National Research Council recommendations (NRC, 1994). The eggs were then weighed, marked and distributed into four groups of 60 eggs each. The treatments were *in ovo* administration of AgNano with 0, 12.5, 25 and 50 µg/egg, respectively. The total volume of 500 µl AgNano was administered at the broad end in each egg on 7th day of incubation using 26-gauge 13 mm needles targeting extra embryonic cavity.

Animals and management: On the day of hatch, chicks were weighed, wing banded, sexed and transferred to 4-tier electrically heated battery brooders placed in an open-sided house. Each treatment group had five replicates of eight birds each and the chicks were reared up to 42 day of age and provided with standard broiler rations as per NRC (1994). Feed and water was available *ad lib*. Standard management practices were followed while rearing the birds.

Production parameters: Hatchability was calculated on fertile egg set basis. On day of hatch, chick weight was taken to 0.1 g accuracy, and mean chick weight and egg weight to chick weight ratio were calculated. For the growth assay, the individual bird weights and feed disappearance by a group of 8 birds in each pen were recorded biweekly for the determination of ADG (average daily gain), ADFI (average daily feed intake) and FCR (feed conversion ratio). Daily record of mortality, if any, for each treatment was maintained.

In vivo cell-mediated immune response: *In vivo* cutaneous basophilic hypersensitivity response to the lectin phytohaemagglutinin-P from *Phaseolus vulgaris* (PHA-P) was assessed in ten 21-day-old chicks following the protocol previously published in our laboratory (Bhanja and Mandal 2005).

Humoral immune response to sheep red blood cells (SRBC): To evaluate the humoral immune response to sheep red blood cells (SRBC), the fresh sheep blood was collected, washed and adjusted to provide a final SRBC suspension of 1% (v/v). At 21st day of age 10 chicks from each treatment group were injected intravenously with 1 ml of SRBC suspension; 5 days later, blood sample (1 ml) was obtained and serum was separated from each chick to evaluate the antibody response to SRBC following the protocol previously published in our laboratory (Bhanja and Mandal 2005, Goel *et al.* 2013).

Blood bio-chemicals: At 21st d post-hatch, 2.0 ml blood was collected from six birds (equal sexes) of each treatment group and allowed to clot at room temperature. Serum was separated and subjected to blood biochemical analysis. Serum glucose, total protein and total cholesterol were

estimated using standard kits.

Immune organ and digestive organ study: At 42nd day post-hatch, six birds (equal sexes) were killed and weight of the bursa, thymus and spleen were recorded and expressed as mg/bwt. The weight of the digestive organs, i.e. empty gizzard plus proventriculus, intestine without chyme, liver and pancreas were recorded and expressed as mg/bwt.

Statistical analyses: Data were subjected to statistical analysis for ANOVA using standard procedure (Snedecor and Cochran 1980). Average daily gain, average daily feed intake and FCR were calculated for each pen. Individual birds were the experimental unit for all immunological, blood biochemicals and organ study. Duncan Multiple Range Test was used for verifying significant difference among treatment means. Each pen served as the experimental unit. The initial BW was used as a covariate for ADFI and ADG, and initial value was used as a covariate for blood profile. Variability in the data was expressed as the pooled standard error (SE) and a $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

In ovo injection of AgNano had no adverse effect on hatching parameters like per cent hatchability (76.5–85.0), hatch weight (37.85–38.48 g), and egg weight to chick weight ratio (65.66–66.78%). It has been reported that *in ovo* feeding on 14th and 18th day of incubation had a negligible effect on hatchability (Uni and Ferket 2004, Goel *et al.* 2013). The previous reports also suggested that *in ovo* injection of amino acids have no adverse effect when injected into yolk sac or extra embryonic cavity, but with positive influence on the growth of developing embryo and little impact on the hatchability (Ohta *et al.* 2002, Bhanja and Mandal 2005). In another study, *in ovo* feeding of AgNano also reported no impact on the embryo weight until late incubation period (Bhanja *et al.* 2015).

In ovo injection had enhanced the ADG in AgNano supplemented eggs throughout the experiment (Table 1). Our results agree with the earlier study where significant improvement in the weight gain was reported after supplementing in feed Nano-silver (silver nanoparticles) (Andi *et al.* 2011). In continuation, higher concentration of AgNano in feed had increased the live body weight in chicken and daily growth of pigs after weaning, respectively (Ahmadi 2009, Fondevila *et al.* 2009). In contrast, *in ovo* feeding of upto 50 mg/kg AgNano did not have any effect on the growth of the embryos (Sawosz *et al.* 2009). The exact mechanism of AgNano to act as growth modulator in chickens is yet to be established. Since, the role of Ag salt has been noticed for its antibacterial effects since long time (Silver and Phung 1996). They may act through the electrostatic attraction between negative charged cell membrane of microorganism in intestine and resulted in healthy hindgut and better absorption of nutrients (Dibrov *et al.* 2002). In continuation AgNano may act as a carrier of available oxygen (O₂) and with high surface reactivity

Table 1. Effect of *in ovo* injection of AgNano on average daily gain, feed intake and FCR of broilers

Parameter	0	12.5	25	50	SEM	P value ¹
<i>Phase 1 (d 0–d 21)</i>						
ADG ²	16.10 ^a	16.80 ^b	16.20 ^a	17.70 ^b	0.230	0.032
ADFI ³ (g/d)	28.45	29.09	28.07	29.69	0.382	0.512
FCR ⁴	1.77	1.73	1.74	1.68	0.014	0.107
<i>Phase 2 (d 21–d 42)</i>						
ADG ²	41.50 ^a	43.90 ^b	44.00 ^b	44.00 ^b	0.380	0.014
ADFI ³ (g/d)	96.18	102.79	106.03	101.84	1.872	0.341
FCR ⁴	2.32	2.34	2.41	2.31	0.030	0.743
<i>Overall (d 0–d 42)</i>						
ADG ²	28.80 ^a	30.30 ^b	30.10 ^b	30.80 ^b	0.270	0.021
ADFI ³ (g/d)	62.32	65.94	67.05	65.76	1.041	0.459
FCR ⁴	2.16	2.17	2.23	2.13	0.023	0.590

¹Means within a row did not differ significantly at $P < 0.05$;

²Average daily gain; ³ADFI, Average daily feed intake; ⁴Feed conversion ratio; SEM, Standard error of mean.

may increase O₂ consumption, enhance fat uptake (FU), and thus stimulates growth (Pineda *et al.* 2012). In the present study, the ADFI and FCR were not affected by *in ovo* AgNano during incubation. This is in correlation with the earlier study where the feed intake and feed conversion ratio for the broilers were similar and was not affected by supplementing AgNano at 5th day of incubation at up to 45 mg/kg in egg (Saki and Salary 2015).

At 42nd d of age, relative weights of the bursa and spleen was higher ($P < 0.05$) in *in ovo* fed nano-particle groups against control (Table 2). Previous studies also reported higher bursa and spleen weight when fed with AgNano in mash/pellet form (Andi *et al.* 2011). However, contrary to this investigation, Ahmadi and Kurdestani (2010) reported smaller lymphoid organs after feeding. This might be due to the use of AgNano produced by different methods and their stability in solutions. For example, during chemical synthesis of nanoparticles a certain quantity of active silver ions may be adsorbed on their surface, whereas AgNano produced by physical methods has no (or very few) active silver ions on its surface (Ognik *et al.* 2016). In another study, 10 ppm of AgNano reduces the number and size of the lymph follicles in bursa of Fabricius without effecting weight of thymus (Grodzik and Sawosz 2006). Lymphoid organs such as bursa of Fabricius, thymus and spleen are

Table 2. Effect of *in ovo* injection of AgNano on 42nd day lymphoid organ weight (g/100 g live weight) and immune response of broilers

Parameter	0	12.5	25	50	SEM	P value
Thymus	0.155	0.140	0.134	0.134	0.007	0.725
Bursa	0.240 ^a	0.298 ^b	0.292 ^b	0.331 ^b	0.011	0.005
Spleen	0.158 ^a	0.186 ^{bc}	0.174 ^{ab}	0.204 ^c	0.006	0.010
Foot web index	0.580 ^a	0.62 ^{ab}	0.780 ^b	0.760 ^b	0.031	0.032
SRBC titer	8.120 ^a	9.25 ^{ab}	9.120 ^{ab}	10.000 ^b	0.240	0.038

¹Values with the different superscript in the same row are statistically different ($P < 0.05$).

the initiation sites of B and T lymphocytes during embryogenesis and thus play an important role in imparting immunity to chicks (Erf 1997). Thus, improvement in the weight of bursa and spleen can be treated as the augmentation of immunity in *in ovo* AgNano supplemented birds.

Cell mediated immunity at 21 d of age with respect to mean skin thickness to phytohaemagglutinin differ significantly ($P < 0.05$) in *in ovo* nano particles supplemented groups. The highest immune response was obtained in AgNano treatment at 25 and 50 µg/egg compared with the control (Table 2). This is in correlation with the data of the lymphoid organ weight. Previous studies had also reported enhanced cellular immune response when immunized with either 2 mg/kg or 15 mg/kg AgNano in mice and in chicken, respectively (Bhanja *et al.* 2015, AL-Rhman *et al.* 2016). Our study shows the dose related increase in cell-mediated immune response.

There was a significant increase ($P < 0.05$) in the humoral immune response to SRBC in 50 µg/egg AgNano treatment. Previous studies also reported enhanced humoral immunity in response to *in ovo* AgNano supplemented chicks by modulating the major components of the pattern recognition receptor system and pathogen associated molecular patterns (Bhanja *et al.* 2015). It could be due to the fact that, AgNano enhances the susceptibility of macrophages to inflammatory stimulation generated in response to SRBC after releasing pro-inflammatory cytokines (Castillo *et al.* 2008). In addition, the antibacterial effectiveness of silver and its compounds is known to be directly proportional to the amount of biologically active Ag⁺ ions released. Silver in form of colloidal solution or as 5 to 100 nm nanoparticles is more stable, absorbed at much lower extent and thus minimum toxic, and exerts higher antimicrobial effect (Wright *et al.* 1999).

Relative weights of the digestive organs, i.e. empty gizzard plus proventriculus, intestine without chyme, liver did not differ significantly ($P < 0.05$) among the control and *in ovo* fed groups (Table 3). In contrast, previous study conducted on silver especially in the form of nano-particles

Table 3. Effect of *in ovo* injection of AgNano on 42nd day visceral organ weight (g/100 g live weight) and serum biochemical of broiler chickens

Parameter	0	12.5	25	50	SEM	P value
Liver	2.323	2.584	2.502	2.461	0.064	0.579
Gizzard	3.174	3.393	2.993	3.043	0.068	0.153
Intestine	4.934	4.914	5.096	5.097	0.130	0.946
Pancreas	0.279 ^a	0.299 ^a	0.357 ^b	0.330 ^{ab}	0.011	0.034
Heart	0.615	0.618	0.541	0.618	0.019	0.406
Glucose (mg/dl)	119.390	131.460	132.150	140.190	4.630	0.500
Total protein (%)	2.240	1.980	2.570	2.140	0.110	0.290
Total cholesterol (mg/dl)	99.780 ^b	86.970 ^a	100.30 ^b	101.260 ^b	2.130	0.030

¹Values with the different superscript in the same row are statistically different ($P < 0.05$).

had reported negative effects on relative liver weight (Shabani *et al.* 2010). Since oxidative stress and lipid peroxidation which occurs as a result of *in ovo* injection of AgNano are important processes that can cause damage to liver cells. The results of this study indicate that AgNano had significantly affected the relative weight of pancreas ($P < 0.05$). The reason behind this could be that AgNano positively modulated the expression of FGF gene which is involved in the pancreatic development in chicken (Hotowy *et al.* 2012). This could be due to the effect of AgNano into the interior of cell and thus accumulated in pancreas.

No significant effect was seen on the concentrations of glucose and total protein at 21 d of age, indicating that the treatment did not influence related metabolic pathways (Tables 3). In comparison to the control, the concentration of serum cholesterol was significantly decreased ($P < 0.05$) by 12.5 µg AgNano supplementation. This correlates the earlier study where 8 ppm/kg and 12 ppm/kg AgNano supplementation reduced HDL cholesterol (Ahmadi and Kurdestany 2010). This is because Ag-NPs induced oxidative stress and adversely affect the structure and peroxidation of lipid in the cell membranes and function leading to rupture of lipid membrane. In another study, the level of malondialdehyde (MDA), the end product of lipid peroxidation in plasma was enhanced due to supplementation of 20, 40 and 60 ppm/kg of nanosilver in broilers feed (Ahmadi 2012).

Finally, our results suggest that the *in ovo* injection of AgNano during early incubation period enhances the post-hatch growth and immune-competence of the birds. Immunity parameters had shown a dose dependent increase though hatchability was slightly reduced with increasing concentration of AgNano. However, more research is required to confirm its status as growth promoter.

ACKNOWLEDGEMENTS

The research work presented in this article is funded by Science and Engineering Research Board, Department of Science and Technology, Government of India.

REFERENCES

- Ahmadi F and Kurdestany A H. 2010. The impact of silver nano particles on growth performance, lymphoid organs and oxidative stress indicators in broiler chicks. *Global Veterinaria* **5**: 366–70.
- Ahmadi F. 2012. Impact of different levels of silver nanoparticles (Ag-NPs) on performance, oxidative enzymes and blood parameters in broiler chicks. *Pakistan Veterinary Journal* **32**: 325–28.
- Ahmadi J. 2009. Application of different levels of silver nanoparticles in food on the performance and some blood parameters of broiler chickens. *World Applied Science Journal* **7**(1): 24–7.
- AL-Rhman R M, Ibraheem S R and A L-Ogaidi I. 2016. The effect of silver nanoparticles on cellular and humoral immunity of mice *in vivo* and *in vitro*. *Iraqi Journal of Biotechnology* **15**(2): 21–9.
- Andi M A, Mohsen H and Farhad A. 2011. Effects of feed type with/without nanosil on cumulative performance, relative organ weight and some blood parameters of broilers. *Global Veterinaria* **7**: 605–09.
- Bhanja S K, Hotowy A, Mehra M, Sawosz E, Pineda L, Vadalasetty K P, Kurantowicz N and Chwalibog A. 2015. *In ovo* administration of silver nanoparticles and/or amino acids influence metabolism and immune gene expression in chicken embryos. *International Journal of Molecular Science* **16**: 9484–503.
- Bhanja S K and Mandal A B. 2005. Effect of *in ovo* injection of critical amino acids on pre- and post-hatch growth, immunocompetence and development of digestive organs in broiler chickens. *Asian Australasian Journal of Animal Science* **18**: 524–31.
- Castillo P M, Herrera J L, Fernandez-Montesinos R, Caro C, Zaderenko A P, Mejias J A and Pozo D. 2008. Tiopronin monolayer-protected silver nanoparticles modulate IL-6 secretion mediated by Toll-like receptor ligands. *Nanomedicine* **3**(5): 627–35.
- Dibrov P, Dzioba J, Gosink K K and Hase C C. 2002. Chemiosmotic mechanism of antimicrobial activity of Ag(+) in *Vibrio cholera*. *Antimicrobial Agents Chemotherapy* **46**: 2668–70.
- Erf G F. 1997. *Immune system function and development in broilers*. Center of Excellence for Poultry Science., ed. Univ. Arkansas, Fayetteville.
- Fondevila M, Herrera R, Casallasa M C, Abecia L and Duchab J J. 2009. Silver nanoparticles as a potential antimicrobial additive for weaned pigs. *Animal Feed Science and Technology* **150**: 259–69.
- Foye O T, Uni Z, McMurtry J P and Ferket R P. 2006. The effects of amniotic nutrients administration, *in ovo* feeding of arginine and/or β-hydroxy β-methylbutyrate (HMB) on insulin-like growth factors, energy metabolism and growth in turkey poults. *International Journal of Poultry Science* **5**: 309–17.
- Goel A, Bhanja S K, Pande V, Mehra M and Mandal A. 2013. Effects of *in ovo* administration of vitamins on post hatch-growth, immunocompetence and blood biochemical profiles of broiler chickens. *Indian Journal of Animal Science* **83**(9): 916–21.
- Grodzik M and Sawosz E. 2006. The influence of silver nano particles on chicken embryo development and bursa of Fabricius morphology. *Journal of Animal and Feed Science* **15**: 111–14.
- Hotowy A, Sawosz E, Pineda L, Sawosz F, Grodzik M and Chwalibog A. 2012. Silver nanoparticles administered to chicken affect VEGFA and FGF2 gene expression in breast muscle and heart. *Nanoscale Research Letters* **7**(1): 418.
- National Research Council 1994. *Nutrients Requirements of Poultry*, 8th revised ed., Washington, DC, USA, National Academy Press.
- Ognik K, Cholewińska E, Czech A, Kozłowski K, Wlazło L, Nowakowicz-Debek B, Szlajak R and Tuta K. 2016. Effect of silver nanoparticles on the immune, redox, and lipid status of chicken blood. *Czech Journal of Animal Science* **61**: 450–61.
- Ohta Y, Yoshida T, Tsushima N and Kidd M T. 2002. The needle bore diameter for *in ovo* amino acid injection has no effect on hatching performance in broiler breeder eggs. *The Journal of Poultry Science* **39**: 194–97.
- Paleo A J, Martinez-Boubeta C, Balcells L, Costa C M, Sencadas V and Lanceros-Mendez S. 2011. Thermal, dielectrical and

- mechanical response of α and β -poly (vinylidene fluoride)/Co-MgO nanocomposites. *Nanoscale Research Letters* **6**: 257–60.
- Pineda L M, Chwalibog A, Sawosz E, Lauridsen C, Engberg R G, Elnif J, Hotowy A M, Sawosz F, Gao Y, Ali A and Moghaddam H S. 2012. Effect of silver nanoparticles on growth performance, metabolism and microbial profile of broiler chickens. *Archive of Animal Nutrition* **66**(5): 416–29.
- Rai M, Yadav A and Gade A. 2009. Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances* **27**: 76–83.
- Saki A A and Salary J. 2015. The impact of *in ovo* Injection of silver nanoparticles, thyme and savory extracts in broiler breeder eggs on growth performance, lymphoid-organ weights, and blood and immune parameters of broiler chicks. *Poultry Science Journal* **3**: 165–72.
- Sawosz E, Grodzik M, Zielinska M, Niemiec T, Olszanska B and Chwalibog A. 2009. Nanoparticles of silver do not affect growth, development and DNA oxidative damage in chicken embryos. *European Poultry Science* **73**: 208–13.
- Shabani A, Dastar B, Khomeiri M, Shabanpur B and Hassani S. 2010. Effects of zeolite_hydrocolloidal silvernanoparticles on the performance and serum biochemical parameters in broiler during experimental aflatoxicosis. *Proceedings of the Australian Society of Animal Production* **28**: 98.
- Silver S and Phung L T. 1996. Bacterial heavy metal resistance: new surprises. *Annual Review of Microbiology* **50**: 753–89.
- Snedecor G W and Cochran W G. 1980. *Statistical Methods*. 6th edn. Iowa State Univ Press, Ames, Iowa.
- Uni Z and Ferket P R. 2004. Methods for early nutrition and their potential. *World Poultry Science Journal* **60**: 101–11.
- Wright J B, Lam K, Hansen D and Burrell R E. 1999. Efficacy of topical silver against fungal burn wound pathogens. *American Journal of Infection Control* **27**: 344–50.