ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2018.234.242

Research Article
Effect of Egg Disinfection by Silver Nanoparticles on Eggshell Microbial Load, Hatchability and Post-hatch Performance of Quail Chicks

[1]Faten A. Ibrahim, [2]Kout Elkloub M.El.Mousafa, [1]M.I. El Sabry, [3]Jihan M. Badr and [1]A.S.I. Hassan

[1]Department of Animal Production, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt
[2]Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt
[3]Department of Poultry Diseases, Bacteriology Unit, Animal Health Research Institute, Dokki, Giza, Egypt

Abstract

Background and Objective: Sanitation improvement of fertile eggs is an important point of research, as it is the goal of selection of the hygiene programs in commercial hatcheries. Silver nanoparticles (Ag-NPs) have new physical and chemical characteristics enabling it to have a strong antibacterial activity. This study was aimed to evaluate the effect of different concentrations of Ag-NPs as a disinfectant, on a microbial load on the quail eggshell, embryonic mortality, hatchability, chick quality and post-hatch performance. **Methodology:** Silver nanoparticles were synthesized and characterized by UV, concentration and transmission electron microscopy (TEM). A total of 1920 fertile quail eggs were randomly divided into 4 treatment groups, one control group disinfected by spraying with a commercial disinfectant (TH_4) and three treated groups sprayed with 30, 40 or 50 ppm of Ag-NPs. Thirty minutes after spraying, eggs in each group were sampled for determination of total bacterial and total coliform counts. **Results:** Results showed that bacterial loads on the eggshell were declined with the elevation of the concentration of Ag-NPs used. The 50 ppm concentration of Ag-NPs had the lowest bacterial load. Also, hatchability of the treated groups was increased by increasing Ag-NPs concentration. Hatchability of Ag-NPs 50 group was higher than the control group ($p \leq 0.05$). Embryonic mortality rates were decreased by increasing Ag-NPs concentration. Chick weight, length and quality of all Ag-NPs treated groups were numerically greater compared to the control ones. Performance of post-hatching quails constituted by body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) for all investigated groups was similar. Moreover, treatments had no negative effect on the structure of the liver. **Conclusion:** In conclusion, using Ag-NPs at different concentration (30, 40 and 50 ppm) in disinfecting quail embryonated eggs can effectively reduce bacterial load on eggshell. Also, using 40 ppm of Ag-NPs improved hatchability and chick quality without any adverse effect on the liver structure.

Key words: Nano-silver, Hatching eggs, sanitation, hygiene, antibacterial, quail

Citation: Faten A. Ibrahim, Kout Elkloub M.El.Mousafa, M. I. El Sabry, Jihan M. Badr and A. S. I. Hassan, 2018. Effect of egg disinfection by silver nanoparticles on eggshell microbial load, hatchability and post-hatch performance of quail chicks. Int. J. Poult. Sci., 17: 234-242.

Corresponding Author: Kout Elkloub M.El.Mousafa, Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Egg contamination may occur before and after oviposition. Typical contaminants on an eggshell surface are *Salmonella* Spp., coliforms and *E. coli*, yeast and molds[1-3]. Some of these micro-organisms can pass through the eggshell in contact with feces or bedding and finally lead to low hatchability and poor chick quality. Thus, hatchery sanitation is critical control point to have healthy and high-quality chicks by using efficient disinfectants.

Nanotechnology provides effective materials that can hinder the spread of diseases in the poultry industry. One of the potential Nano-materials that could be used as a disinfectant is nano-silver, which has a large number of health care applications. Silver-nano particles (Ag-NPs) has a wide spectrum of particle size, variable morphology, high stability and appropriate physicochemical properties have antimicrobial characteristics[4]. The arrangement of Nano-silver particles contributes to it's high bioavailability and, hence its strong bacteriostatic and bactericidal effects on a broad spectrum of microorganisms e.g. bacteria, fungi and virus[5,6]. For instance, Li *et al*.[7] found that exposing *E. coli* cells to different concentrations (10 and 50 μg mL^{-1}) of Ag-NPs resulted in many pores in bacterial cells and depression in the activity of some membranous enzymes, which eventually led to the death of *E. coli* bacteria. Moreover, different studies suggested the different applications of Ag-NPs and its beneficial effects in poultry production; Banach *et al*.[4] found that the use of nanosilver preparation to disinfect eggs and hatchers reduced microbial contamination by 86%. Bhanja *et al*.[8] indicated that the *in ovo* injection of Ag-NPs either alone or in a combination with some amino acids can be potential agents for the enhancement of innate and adaptive immunity without deleterious effect on embryonic growth in chicken. Also, Kout Elkloub *et al*.[9] found that broilers' diet supplemented with Ag-NPs (4 ppm kg^{-1}) enhanced the productive performance. In addition, Ag-NPs treatments (2-10 ppm) decreased the count of *E. coli* in cecum compared to the control. Interestingly, Viswanathan *et al*.[10] found that the storage of eggs in the Ag-NPs deposited paper egg trays improved the shelf-life of the eggs by more than 14 days compared to the conventional trays. They mentioned that silver nano particles containing egg trays showed a bactericidal effect against the commonly found bacteria on eggshells, *E. coli*, *S. aureus*, *Streptococcus* spp. and *Salmonella* spp.

Few types of research have been conducted to investigate the impacts of using Ag-NPs as a disinfectant for hatching eggs. Therefore, the objective of the present study was to evaluate the disinfectant effect of different concentrations of Ag-NPs (30, 40 and 50 ppm) compared to another commercial disinfectant (TH_4) and also, to study their effect on hatching traits, embryonic mortality and post-hatch performance in quail. In addition, to investigate their effect on the histological structure of the liver and the relative weights of carcass, liver, heart, spleen.

MATERIALS AND METHODS

This protocol was approved by Cairo University Ethics Committee for the Care and Use of Experimental Animals in Education and Scientific Research (Protocol number: CU-II-F-48-17). A total of 1920 fresh and clean fertile Japanese quail eggs were used in this study. Japanese quail eggs were obtained (strain was selected for high body weight) from private commercial farm hatchery. The Ag-NPs were prepared at Poultry Nutrition Department, Animal Production Research Institute, Agriculture Research Center, Egypt. The bacteriological examinations were submitted in Bacteriology Unit, Department of Poultry Diseases, Animal Health Research Institute, Dokki, Giza, Egypt. Embryonated quail eggs were equally distributed and sprayed with either TH_4 as a control (commercial disinfectant contains Didecyldimethyl Ammonium Chloride, Dioctyl dimethyl Dimethyl Ammonium Chloride and Octyl Decyl Dimethyl Ammonium Chloride as active ingredients) or three Ag-NPs solutions (30, 40 and 50 ppm). Thirty minutes after being sprayed, 40 eggs (10 per treatment) were assigned to bacteriological examination for determination of total bacterial and total coliform counts.

Preparation and characterization of silver nanoparticles

Preparation: Silver nanoparticles (Ag-NPs) were prepared by the reduction of silver nitrate ($AgNO_3$) with diluted aqueous solutions containing Cetyl trimethyl ammonium bromide (CTAB), which was used as a dispersing agent producing silver nanoparticles and hydrazine hydrate (HEN_2O). Changes in color were observed upon mixing the CTAB with Ag-NPs. These color changes arise because of the excitation of surface Plasmon vibrations in the Ag-NPs[11].

It is important to use stabilizer, during the preparation of metal nanoparticle, to avoid nanoparticles agglomeration[12,13]. The hydrazine acts as a reducing as well as an adsorbing agent in the preparation of roughly spherical and non-agglomerated Ag-NPs[14].

Characterization: Characterization of nanoparticles is important to understand and control nanoparticles synthesis and applications. Finally, Ag-NPs gross was assessed by UV-V

spectroscopy and the average of particle size and the size distribution were determined from transmission electron microscopy (TEM). Flam Atomic absorption spectrometry (Agilent Technologies 200 Series AA) was used to determine Ag-NPs concentration which was 1900 ppm L^{-1}.

Bacteriological examinations

Examination of egg incubator's air: The open-plate method was used to determine aerobic bacteria and coliform count in incubator cabin. Uncovered sterile Petri dishes containing either plate count agar (for the total bacterial count) or McConkey's agar (for total coliform count) were distributed at 3 different sites, one meter from the floor surface, for 10 min[15]. The plates were incubated at 37°C for 24-48 h then were enumerated.

Determination of the microbial load on the eggshell: The total egg wash method was used to determine the microbial load on the eggshell. Ten eggs were collected aseptically from each treatment in sterile plastic bags that contain sterile physiological saline. The egg was gently massaged for one minute then the egg was removed and the washing water was ten-fold serially diluted and 0.1 mL of each dilution sample was plated on two sterile plates for plate count and McConkey's agars[16], incubated at 37°C for 24-48 h then bacteria count was performed. Microbial loads were expressed as Colony Forming Units (CFU) per ml of sample. Isolation and identification of the suspected colonies were done according to Holt *et al.*[17].

Hatching performance: Eggs of all treatments received standard temperature (37.5°C) and 52% relative humidity (RH) for 18 days. During the last 3 days, eggs were incubated in the same hatcher at 36.5° C and 65% RH. All the chicks were counted and weighed within 45 min after hatch. Hatchability was calculated as a percentage of total eggs set. The unhatched eggs were broken out, the number of infertile, early death (0-7th days of incubation), middle death (8th-14th days of incubation) and late death (15th days to hatch) of embryos were recorded.

The quality of the chicks was assessed and they were categorized and counted as either A or B grade. The chicks that looked healthy and alert were graded A, whereas the chicks that showed unhealed navel, leg abnormalities or difficulties of standing were graded B.

Post-hatching performance: After hatching, a total of 480 chicks, representing the four treatment groups, were randomly distributed into equal four groups of 120 chicks/group in three replicates, 40 chicks each. All chicks received continuous lighting 23L: 1D for the three days, then 18L: 6D lighting program. Room temperature was set at 33°C for the first week and gradually reduced by 3°C/week. Feed and water were provided *ad libitum* throughout the experimental period (35 days of age). Quail chicks were fed a commercial diet (24% CP and 2900 kCal kg^{-1}).

Body weight and feed intake were recorded. Feed conversion ratio was calculated on a pen basis at 7, 14, 21, 28, 35 days of age. Mortality rates were recorded daily on a pen basis. At day 35, five chicks were randomly taken from each treatment and were sacrificed by cervical dislocation. Carcass, heart, liver and spleen were weighed and relative weights were calculated.

Tissue samples were obtained from liver and were fixed in Bouins solution, dehydrated in ascending grades of ethyl alcohol, then cleared in Xylene and embedded in paraffin wax. Transverse sections were cut, mounted on glass slides and then stained with hematoxylin and eosin. Three serial histological sections of liver were investigated under light microscope XSZ-PW 146 (Proway Optics and Electronics, China) at magnification ×400.

Statistical analysis: Data were statistically analyzed using the linear model procedure according to SAS user guide[18]. Differences were considered significant at $p<0.05$. Differences among means were tested using Duncan's multiple range test[19]. Model applied was:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where:
Y_{ij} = Observations
μ = Overall mean
T_i = Effect of i^{th} treatments
E_{ij} = Experimental error

RESULTS AND DISCUSSION

Silver nanoparticles characterizations

Ultra-violet (UV) visible spectrophotometer analysis: The UV-Vis spectra of Ag-NPs sample is presented in Fig. 1. The peak at wavelength of 416 nm indicates that Ag-NPs have turned into silver nanoparticles[20]. The surface plasmon band of silver nanoparticles usually has a range of 400-450 nm in aqueous solutions, depending on the shape and size[21].

The UV-Vis spectrum of the brown suspension was recorded at about 416 nm (Fig. 1), showing a prominent peak. The brown color appears because of specific surface Plasmon

resonance arising due to the collective oscillation of free conduction electrons which is induced by electromagnetic field[20]. In the present study, the highest absorption was observed at 416 nm for the surface Plasmon resonance indicates the synthesis of Ag-NPs.

Transmission Electron Microscopy (TEM): Transmission Electron Microscopy (TEM) images showed that the size of Ag-NPs was ranged from 2.43-41.88 nm (Fig. 2).

Concentration: The concentration of Ag-NPs (1900 ppm L^{-1}) was measured by atomic absorption (Agilent Technologies 200 Series AA) at wavelength 328.1 nm. Thereafter, all different concentrations of Ag-NPs (30, 40 and 50 ppm) were prepared for egg sanitation.

Silver nanoparticles (Ag-NPs) are the most commonly used disinfectant materials because of their effective antimicrobial properties[22]. The Ag-NPs are characterized by smaller size and larger active area than silver in bulk size. Theses charicterisics make Ag-NPs more chemically and biologically reactive[23]. Lankveld *et al.*[24] and Arya *et al.*[25] suggested that Ag-NPs can deactivate bacterial cells through a different mechanism of functioning, such as inducing damage in the bacterial cell wall or interacting with thiol groups of respiration enzymes in bacteria that inhibit breathing and cause cell atrophy.

Incubator egg bacterial contamination: Several sources of microbial contamination of eggshell are found in the air of the poultry house, poultry diet or hatchery machine. *Salmonella* spp., coliforms and *E. coli*, yeast and molds[26-29] are among the predominant pathogenic contaminants. De Reu *et al.*[30] proved that higher microbial load on eggshell can increase the microbes penetration and contamination of egg contents that reduced hatchability. Therefore, the bacterial contamination of the incubator air and egg trolleys were determinated. In the current study, the total bacterial count in the air of incubater cabin was 3.64×10^2 CFU m^{-3} and a coliform count was zero, while the bacterial contamination on egg trolley surface was 13 CFU cm^{-2} and a coliform count was zero. Previous studies had shown a great fluctuation in the total bacterial count of air (CFU m^{-3}) and hatchery equipment (CFU cm^{-2}). Tymczyna *et al.*[31] found that Gram-negative bacteria made up 16% (651 CFU m^{-3}) of the total bacterial population in a hatchery. In addition, Kim and Kim[29] found different microbial load in the hatchery (rooms and equipment), when they studied the effect of different disinfectants on the bacterial contamination in different seasons.

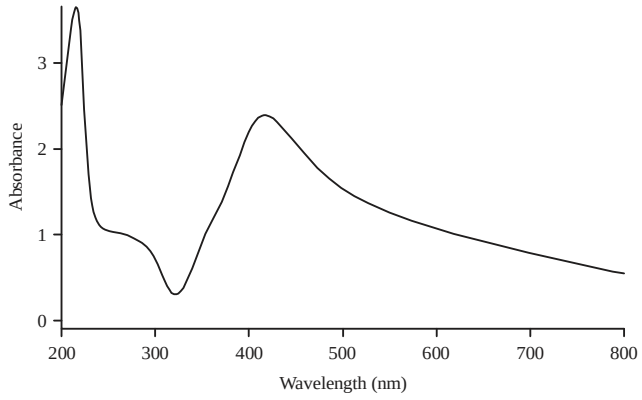

Fig. 1: UV-visible spectrophotometer analysis of silver nanoparticles synthesized

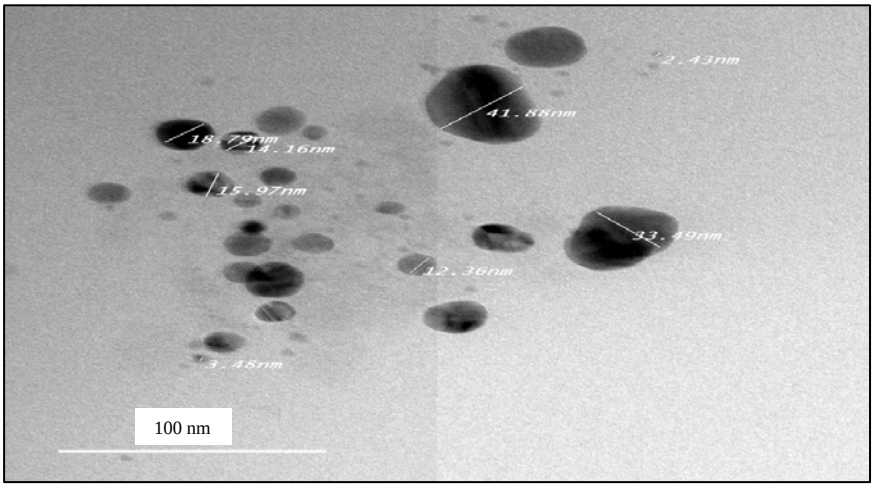

Fig. 2: TEM image of silver nanoparticles

Table 1: Total bacterial count (TCC) and total coliform on the eggshell surface of control (TH4) and Ag-NPs (30, 40, 50 ppm) disinfected groups

Groups	TCC (log CFU/egg)	Coliform count (log CFU/egg)	Isolates
Control (TH_4)	3.25	0.48	G+*Bacillus* spp.
			G+cocci (*Staphylococcus* spp.)
			G-Coliform bacteria
Ag-NPs 30	3.09	0.42	G+*Bacillus* spp.
			G+cocci (*Staphylococcus* spp.)
			G-Coliform bacteria
Ag-NPs 40	2.78	0.30	
Ag-NPs 50	1.95	Absent	G+*Bacillus* spp.
			G+cocci (*Staphylococcus* spp.)

G+: Gram positive and G-: Gram negative

Table 2: Effect of disinfecting quail hatching eggs with TH_4 (control) and different concentrations of Ag-NPs (30, 40 and 50 ppm) on hatchability

Groups	Hatchability of set eggs (%)
Control (TH4)	66.38[b]
Ag-NPs 30	71.06[ab]
Ag-NPs 40	72.12[ab]
Ag-NPs 50	78.93[a]
p-value	0.05
±MSE	2.57

[a,b]Means with different superscripts differ significantly ($p<0.05$)

Table 3: Effect of disinfecting quail hatching eggs with TH_4 (control) and different concentrations (30, 40 and 50 ppm) of Ag-NPs on embryonic mortality

	Embryonic mortality (%)			
Groups	Early	Mid	Late	Piped chicks
Control (TH_4)	4.19	6.26	7.10	0.26
Ag-NPs 30	3.31	3.53	7.26	0.50
Ag-NPs 40	3.84	3.08	5.64	0.77
Ag-NPs 50	2.25	2.27	3.48	0.48
p-value	0.39	0.13	0.27	0.47
±MSE	0.79	1.09	1.39	0.22

In addition, clean fertile eggs with minimum microbial contamination are required for successful hatching process. Board and Tranter[32] reported a great variation in the levels of contamination of hatching eggs that were started from 10^2-10^7 CFU m^{-3}. It is well known that eggs contaminated by pathogenic bacteria have an important role in the spreading of diseases. These harmful bacteria can result in embryonic mortality, lower hatchability and increased early chick mortality.

Smith *et al.*[26] mentioned that the bacterial contamination of eggshell was affected by several factors such as the concentration of bacteria in the air of the poultry facility. In some studies, the total count of bacteria in the air of poultry houses was found to be positively correlated with the initial bacterial eggshell contamination in the birdhouse[27,28].

In the current study, the eggs were mostly contaminated by Gram-Positive bacteria. Similar results were reported by De Reu *et al.*[33] and De Reu *et al.*[34], who found that the natural eggshell contamination was dominated by G+ *Staphylococcus* spp. Board and Tranter[32] elucidated that Gram-positive bacteria may be initiated in dust, soil or feces and they wide spread may be due to their tolerance to the dry conditions. On the other hand, contaminant bacterial load (total bacterial and total coliform counts) of the eggshell in groups disinfected with Ag-NPs (40 or 50 ppm) was lower than that of eggs disinfected with TH_4 (control). Also, the bacterial load on eggshells of the group treated with Ag-NPs at 30 ppm was close to that of the control group. Moreover, the decline in total bacterial count with the absence of coliform was found in the group treated with Ag-NPs at 50 ppm (Table 1). These results proved the effective antimicrobial properties of Ag-NPs[22]. Lankveld *et al.*[24] suggested that Ag-NPs deactivate bacteria through catalytic oxygenation, reactions with the bacterial cell wall, protein denaturation and binding with bacterial DNA.

Hatching performance: Hatchability rates of the eggs disinfected by different concentrations of Ag-NPs solution were higher compared to the control group. The high concentration of Ag-NPs resulted in higher hatchability rate. However, hatchability of Ag-NPs 50 group was significantly higher compared to the control group (Table 2). The results of hatchability matched with the results of bacterial loads after disinfection. The group treated with Ag-NPs (50 ppm) had the lowest bacterial count on the eggshell surface and the highest hatchability. This indicates that the efficiency of using Ag-NPs as disinfecting agent increases with the increase of Ag-NPs concentration. Minimizing the bacterial load on the eggshell can significantly reduce the bacteria penetration that may influence the embryos. In this respect, Ibrahim *et al.*[35] reported that inhibition of the pathogen activity on the eggshell surface resulted in lowering embryonic mortality by 10%, consequently, resulted in increased hatchability.

Table 3 shows that the embryonic mortalities (early, mid and late) are numerically decreased by the increase in concentrations of Ag-NPs and no significant difference was found between the percentage of piped chicks of the control

and Ag-NPs (30, 40 or 50 ppm) groups. Sawosz *et al.*[36] found that supplementing hatching eggs with Ag-NPs by *in ovo* injection had no harmful effect on embryos viability and may accelerate growth and maturation of muscle cells of chicken embryos. In addition, Beck *et al.*[37] found that *in ovo* injection of experimental solutions [Ag-NPs, hydroxyproline solution (Hyp) and a complex of silver nanoparticles with hydroxyproline (AgHyp) into albumen] did not harm embryos. Therefore, it could be suggested that disinfecting eggs with Ag-NPs may not affect hatchability.

Chick quality: Chick weight and length are good indicators for chick performance throughout the growing period[38,39]. In the current study, body weight and length of one-day-old chicks in all treatments were similar. The categorization of chicks to either category A or category B in each treatment group showed non-significant differences between the groups in the percentage of each category (Table 4). The percentage of category A chicks was however numerically greater in all Ag-NPs groups than the control group and increased with the increase in Ag-NPs concentration. The main reason for culling the chicks was the un-healing navel. The increase in category A percentage in the groups treated with Ag-NPs could be then due to the reduction in the bacterial load on the eggshell surface and consequently the increase in hatchability into healthy chicks.

Post-hatch performance: Body weights of the chicks in different Ag-NPs groups were similar to those in the control group at 7, 14, 21, 28 and 35 days of age (Table 5). This indicates that the Ag-NPs as an egg disinfectant has no harmful effect on the post-hatch performance of the quail chicks. No differences were also found in body weight gain, feed intake and feed conversion ratio between the chicks in different Ag-NPs treated groups and the control group (Table 6).

The carcass weight percentages in the treated groups and the control group were similar (Table 7). These results indicate that Ag-NPs had no harmful residues that may negatively affect the muscle and therefore, the muscle development and post-hatch performance in all treated groups were normal.

Heart, liver and spleen weight percentages could be used as stress indicators for environmental stressors[40]. The increase in liver weight and decrease in spleen and heart weights were recorded when birds were exposed to stress[41-43]. In the present study, the weight percentages of heart, liver and spleen for TH_4 and Ag-NPs treated groups were similar. This showed that sanitizing quail eggs using 30, 40 or 50 ppm of Ag-NPs solution had no deleterious effect on the organs (Table 7).

Liver histological structure: Histological examination of liver section revealed that chicks from the Ag-NPs 30 (Fig. 3b) and 40 (Fig. 3c) groups and the control group (Fig. 3a) show normal liver structure. The liver of the chicks from the Ag-NPs 50 group showed notable fat accumulation (Fig. 3d). Chmielowiec-Korzeniowska *et al.*[6] revealed that using 50 ppm of Ag-NPs as an eggshell disinfectant resulted in no Ag bioaccumulation in the liver of newly hatched chicks in comparison to the control. Also, Elkloub *et al.*[9] reported normal plasma transaminase activity (ALT and AST) in response to adding Ag-NPs at 2-10 ppm kg^{-1} feed of quail, which revealed normal liver functions. The results of the current study show that the use of Ag-NPs in lower concentrations (30 and 40 ppm) is safe, while a high concentration of Ag-NPs might work as a co-factor for the observed changes.

Table 4: Effect of sanitizing quail eggs with TH4 (control) and different concentrations (30, 40 and 50 ppm) of Ag-NPs on chick weight (g), chick length (cm) and chick quality

			Chick quality (%)	
Groups	Chick weight (g)	Chick length (cm)	Grade (A)	Grade (B)
Control (TH4)	11.83	12.09	76.96	5.22
Ag-NPs 30	11.97	12.25	81.48	3.90
Ag-NPs 40	12.12	12.17	83.63	3.05
Ag-NPs 50	12.15	12.12	87.28	4.24
p-value	0.08	0.95	0.19	0.82
±MSE	0.08	0.19	3.01	1.62

Table 5: Effect of disinfecting quail eggs with TH_4 (control) and different concentrations of Ag-NPs (30, 40 and 50 ppm) on body weight (BW, g) of quail chicks at 7, 14, 21, 28, 35 days of age

	BW (days)					
Groups	1	7	14	21	28	35
Control (TH4)	11.89	45.35	124.91	180.00	256.46	312.69
Ag-NPs 30	11.99	46.76	122.00	188.17	261.35	312.72
Ag-NPs 40	12.11	45.59	124.77	179.53	257.57	312.91
Ag-NPs 50	12.02	45.48	127.66	187.71	260.25	312.50
p-value	0.69	0.53	0.67	0.25	0.84	0.99
±MSE	0.13	0.73	3.18	3.69	4.34	3.94

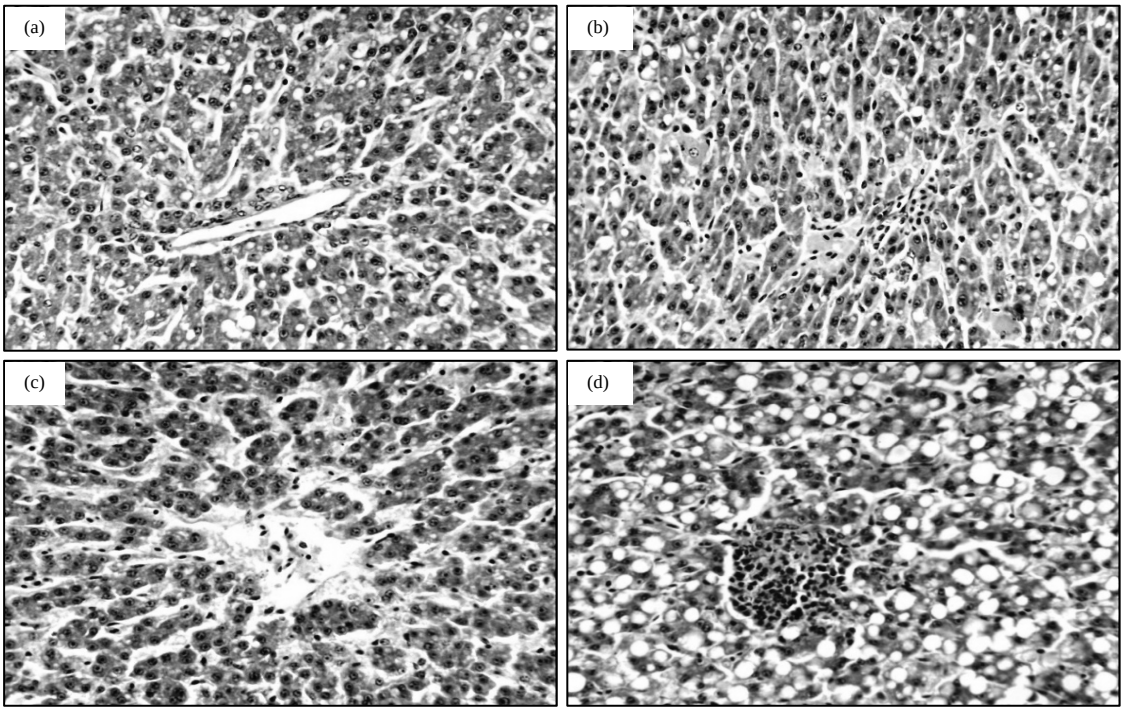

Fig. 3(a-d): Effect of disinfecting quail eggs with TH4 as (a) Control, (b) 30, (c) 40 and (d) 50 ppm Ag-NPs on the histological structure of the liver

Table 6: Effect of disinfecting quail eggs with TH4 (control) and different concentrations of Ag-NPs (30, 40 and 50 ppm) on total body weight gain (BWG, g), total feed intake (FI, g) and total feed conversion ratio (FCR) of quail chicks

Groups	BWG (1-35 days)	FI (1-35 days)	FCR (1-35 days)
Control (TH4)	300.80	787.30	2.62
Ag-NPs 30	300.73	797.14	2.65
Ag-NPs 40	300.80	793.77	2.64
Ag-NPs 50	300.48	782.37	2.61
p-value	0.99	0.39	0.78
±MSE	3.94	6.20	0.03

Table 7: Effect of disinfecting quail eggs with TH4 (control) and different concentrations of Ag-NPs (30, 40 and 50 ppm) on carcass, liver, gizzard, heart and spleen relative weight percentages

	Relative weight (%)			
Groups	Carcass	Heart	Liver	Spleen
Control (TH4)	69.47	0.82	2.35	0.07
Ag-NPs 30	69.57	0.93	2.17	0.09
Ag-NPs 40	69.93	0.87	1.91	0.06
Ag-NPs 50	69.99	0.82	2.32	0.07
p-value	0.88	0.81	0.27	0.27
±MSE	0.55	0.09	0.16	0.01

Finally, well prepared Ag-NPs is a perquisite for improving the percentage of hatchability of eggs. However, further studies are necessary to evaluate other sources and doses of nano-particles as disinfectants in hatcheries.

CONCLUSION

Silver nanoparticles (Ag-NPs) improved the post-hatch performance of quails as it decreased the eggshell microbial contamination and enhanced the hatchability percentage. The Ag-NPs solution with 40 ppm could be used as an effective disinfectant in egg hatcheries.

REFERENCES

1. Singh, R., K.M. Cheng and F.G. Silversides, 2009. Production performance and egg quality of four strains of laying hens kept in conventional cages and floor pens. Poult. Sci., 88: 256-264.
2. Jones, D.R., K.E. Anderson and M.T. Musgrove, 2011. Comparison of environmental and egg microbiology associated with conventional and free-range laying hen management. Poult. Sci., 90: 2063-2068.
3. Aygun, A., D. Sert and G. Copur, 2012. Effects of propolis on eggshell microbial activity, hatchability and chick performance in Japanese quail (*Coturnix coturnix* Japonica) eggs. Poult. Sci., 91: 1018-1025.
4. Banach, M., L. Tymczyna, A. Chmielowiec-Korzeniowska and J. Pulit-Prociak, 2016. Nanosilver biocidal properties and their application in disinfection of hatchers in poultry processing plants. Bioinorg. Chem. Applic., Vol. 2016, 10.1155/2016/5214783.

5. Panyala, N.R., E.M. Pena-Mendez and J. Havel, 2008. Silver or silver nanoparticles: A hazardous threat to the environment and human health? J. Apploed Biomed., 6: 117-129.
6. Chmielowiec-Korzeniowska, A., L. Tymczyna, M. Dobrowolska, M. Banach and B. Nowakowicz-Debek *et al*., 2015. Silver (Ag) in tissues and eggshells, biochemical parameters and oxidative stress in chickens. Open Chem., 13: 1269-1274.
7. Li, W.R., X.B. Xie, Q.S. Shi, H.Y. Zeng, Y.S. Ou-Yang and Y.B. Chen, 2010. Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*. Applied Microbiol. Biotechnol., 85: 1115-1122.
8. Bhanja, S.K., A. Hotowy, M. Mehra, E. Sawosz and L. Pineda *et al*., 2015. *In ovo* administration of silver nanoparticles and/or amino acids influence metabolism and immune gene expression in chicken embryos. Int. J. Mol. Sci., 16: 9484-9503.
9. Elkloub, K., M.E. Moustafa, A.A. Ghazalah and A.A.A. Rehan, 2015. Effect of dietary nanosilver on broiler performance. Int. J. Poult. Sci., 14: 177-182.
10. Viswanathan, K., M.L.M. Priyadharshini, K. Nirmala, M. Raman and G.D. Raj, 2016. Bactericidal paper trays doped with silver nanoparticles for egg storing applications. Bull. Mater. Sci., 39: 819-826.
11. Thakkar, K.N., S.S. Mhatre and R.Y. Parikh, 2010. Biological synthesis of metallic nanoparticles. Nanomed. Nanotechnol. Biol. Med., 6: 257-262.
12. Oliveira, M.M., D. Ugarte, D. Zanchet and A.J. Zarbin, 2005. Influence of synthetic parameters on the size, structure and stability of dodecanethiol-stabilized silver nanoparticles. J. Colloid Interface Sci., 292: 429-435.
13. Bai, J., Y. Li, J. Du, S. Wang, J. Zheng, Q. Yang and X. Chen, 2007. One-pot synthesis of polyacrylamide-gold nanocomposite. Mater. Chem. Phys., 106: 412-415.
14. Khan, Z., S.A. Al-Thabaiti, A.Y. Obaid and A.O. Al-Youbi, 2011. Preparation and characterization of silver nanoparticles by chemical reduction method. Colloids Surfaces B: Biointerfaces, 82: 513-517.
15. Berrang, E., N.A. Cox and J.S. Bailey, 1995. Measuring air-borne microbial contamination of broiler hatching cabinets. J. Applied Poult. Res., 4: 83-87.
16. Willinghan, E.M., J.E. Sander, S.G. Thayer and J.L. Wilson, 1996. Investigation of bacterial resistance to hatchery disinfectant. Avian Dis., 40: 510-515.
17. Holt, J.G., N.R. Kreig, P.H.A. Sneath, J.T. Staley and S.T. Williams, 1994. Bergey's Manual of Determinative Bacteriology. 9th Edn., Lippincott Williams and Wilkins, Baltimore, USA., ISBN-13: 9780683006032, Pages: 787.
18. SAS., 2001. SAS User's Guide: Statistics. Version 9th Edn., SAS Instituse Inc., Cary NC. USA.
19. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.
20. Govindaraju, K., V. Kiruthiga, V.G. Kumar and G. Singaravelu, 2009. Extracellular synthesis of silver nanoparticles by a marine alga, *Sargassum wightii* Grevilli and their antibacterial effects. J. Nanosci. Nanotechnol., 9: 5497-5501.
21. Shankar, S.S., A. Ahmad, R. Pasricha and M. Sastry, 2003. Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes. J. Mater. Chem., 13: 1822-1826.
22. Cho, K.H., J.E. Park, T. Osaka and S.G. Park, 2005. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochimica Acta, 51: 956-960.
23. Choi, O., C.P. Yu, E.G. Fernandez and Z. Hu, 2010. Interactions of nanosilver with *Escherichia coli* cells in planktonic and biofilm cultures. Water Res., 44: 6096-6103.
24. Lankveld, D.P.K., A.G. Oomen, P. Krystek, A. Neigh and A.T. de Jong *et al*., 2010. The kinetics of the tissue distribution of silver nanoparticles of different sizes. Biomaterials, 31: 8350-8361.
25. Arya, V., R. Komal, M. Kaur and A. Goyal, 2011. Silver nanoparticles as a potent antimicrobial agent: A review. Pharmacology, 3: 118-124.
26. Smith, S., S.P. Rose, R.G. Wells and V. Pirgozliev, 2000. The effect of changing the excreta moisture of caged laying hens on the excreta and microbial contamination of their egg shells. British Poult. Sci., 41: 168-173.
27. Protais, J., S. Queguiner, E. Boscher, J.C. Piquet, B. Nagard and G. Salvat, 2003. Effect of housing systems on the bacterial flora of egg shells. Br. Poult. Sci., 44: 788-790.
28. De Reu, K., K. Grijspeerdt, M. Heyndrickx, J. Zoons and K. de Baere *et al*., 2005. Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems for laying hens. Br. Poult. Sci., 46: 149-155.
29. Kim, J.H. and K.S. Kim, 2010. Hatchery hygiene evaluation by microbiological examination of hatchery samples. Poult. Sci., 89: 1389-1398.
30. De Reu, K., K. Grijspeerdt, W. Messens, M. Heyndrickx, M. Uyttendaele, J. Debevere and L. Herman, 2006. Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including *Salmonella enteritidis*. Int. J. Food Microbiol., 112: 253-260.
31. Tymczyna, L., A. Chmielowiec-Korzeniowska and A. Drabik, 2007. The effectiveness of various biofiltration substrates in removing bacteria, endotoxins and dust from ventilation system exhaust from a chicken hatchery. Poult. Sci., 86: 2095-2100.
32. Board, R.G. and H.S. Tranter, 1995. The Microbiology of Eggs. In: Egg Science and Technology, Stadelman, W.J. and O.J. Cotterill (Eds.)., Food Products Press, New York, pp: 81-103.

33. De Reu, K., M. Heyndrickx, K. Grijspreedt, B. Rodenburg and F. Tuyttens *et al*., 2007. Estimation of the vertical and horizontal bacterial infection of hen's table eggs. Proceedings of the 18th European Symposium on the Quality of Poultry Meat and 12th European Symposium on the Quality of Eggs and Egg Products Conference, September 5-7, 2007, Prague, pp: 55-56.
34. De Reu, K., W. Messens, M. Heyndrickx, T.B. Rodenburg, M. Uyttendaele and L. Herman, 2008. Bacterial contamination of table eggs and the influence of housing systems. World Poult. Sci. J., 64: 5-19.
35. Ibrahim, J.I., D.H. Mansour and H.A. Abdelrahman, 2014. Prevalence and inhibition of microbial load on chicken eggs with special references to egg quality and hatchability. Am. J. Anim. Vet. Sci., 9: 294-302.
36. Sawosz, F., L. Pineda, A. Hotowy, S. Jaworski, M. Prasek, E. Sawosz and A. Chwalibog, 2013. Nano-nutrition of chicken embryos. The effect of silver nanoparticles and ATP on expression of chosen genes involved in myogenesis. Arch. Anim. Nutr., 67: 347-355.
37. Beck, I., A. Hotowy, E. Sawosz, M. Grodzik and M. Wierzbicki *et al*., 2015. Effect of silver nanoparticles and hydroxyproline, administered *in ovo*, on the development of blood vessels and cartilage collagen structure in chicken embryos. Arch. Anim. Nutr., 69: 57-68.
38. Willemsen, H., N. Everaert, A. Witters, L. De Smit and M. Debonne *et al*., 2008. Critical assessment of chick quality measurements as an indicator of posthatch performance. Poult. Sci., 87: 2358-2366.
39. El Sabry, M.I., S. Yalcin and G. Turgay-Izzetoglu, 2013. Interaction between breeder age and hatching time affects intestine development and broiler performance. Livestock Sci., 157: 612-617.
40. Bayram, A. and S. Ozkan, 2010. Effects of a 16-hour light, 8-hour dark lighting schedule on behavioral traits and performance in male broiler chickens. J. Applied Poult. Res., 19: 263-273.
41. Puvadolpirod, S. and J.P. Thaxton, 2000. Model of physiological stress in chickens 1. Response parameters. Poult. Sci., 79: 363-369.
42. Malheiros, R.D., V.M. Moraes, A. Collin, E. Decuypere and J. Buyse, 2003. Free diet selection by broilers as influenced by dietary macronutrient ratio and corticosterone supplementation: 1. Diet selection, organ weights and plasma metabolites. Poult. Sci., 82: 123-131.
43. Lin, H., S.J. Sui, H.C. Jiao, J. Buyse and E. Decuypere, 2006. Impaired development of broiler chickens by stress mimicked by corticosterone exposure. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 143: 400-405.