Effect of using colloidal silver in the disinfection of hatching eggs on some microbial, hatchability and performance traits in…

Article · January 2017

DOI: 10.1399/eps.2017.211

CITATIONS
0

READS
24

6 authors, including:

Justyna Batkowska
University of Life Sciences in Lublin
38 PUBLICATIONS **26** CITATIONS
SEE PROFILE

Antoni Brodacki
University of Life Sciences in Lublin
22 PUBLICATIONS **37** CITATIONS
SEE PROFILE

Łukasz Wlazło
University of Life Sciences in Lublin
30 PUBLICATIONS **21** CITATIONS
SEE PROFILE

Bożena Nowakowicz-Dębek
University of Life Sciences in Lublin
59 PUBLICATIONS **68** CITATIONS
SEE PROFILE

Europ.Poult.Sci., **81.** 2017, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2017.211

Effect of using colloidal silver in the disinfection of hatching eggs on some microbial, hatchability and performance traits in Japanese quail (*Coturnix cot. japonica*)

Einfluss der Verwendung von kolloidalem Silber zur Desinfektion von Bruteiern auf einige mikrobiologische, Brut- und Leistungsmerkmale bei der Japanischen Wachtel (*Coturnix cot. japonica*)

Justyna Batkowska[1*], **K.I.A. Al-Shammari**[2], **Magdalena M. Gryzinska**[1], **A. Brodacki**[1], **L. Wlazlo**[3] and **Bozena Nowakowicz-Debek**[3]

[1] Inst. of Biological Bases of Animal Production, University of Life Sciences in Lublin, 13 Akademicka St, 20–950 Lublin, Poland

[2] Dept. of Animal Production Techniques, Al-Musaib Technical College, Foundation of Technical Education, Babylon, Iraq

[3] Dept. of Animal Hygiene and Environment, University of Life Sciences in Lublin, Poland

*Correspondence: justyna.batkowska@up.lublin.pl

Manuscript received 1 July 2017, accepted 9 September 2017

Introduction

Good hygiene during the hatching is required to ensure embryonic vitality and proper development leading to maximum hatching rates and optimum chick quality and growth rate (Fueng-Lin et al., 1996; Cortés et al., 2004). Hatchery sanitation programs should include the use of disinfectants to inhibit the growth of microorganisms and maintain a desirable level of hatchability. Microorganisms found on the surface of hatching eggs can easily be distributed throughout the hatcher by air movements and thus contaminate or infect newly-hatched chickens. Search for alternative disinfection methods are necessary due to the fact that traditionally used formaldehyde is considered to be an irritant to the eyes and the nose, has a lingering noxious odour, carcinogenic effect, and venting of its vapours is difficult (Whistler and Sheldon, 1989; Debes and Basyony, 2011). Rashid et al. (2011) mentioned that different methods of shell treatment used during the pre-incubation and incubation stages are biologically effective on avian embryogenesis.

Colloidal silver (nanosilver) particles and its derivations have received attention by some researchers (Chen and Schluesener, 2008; Soltani et al., 2011; Arya et al., 2011; Underwood and Van Eps, 2012; Farzinpour and Karashi, 2012; Chmielowiec-Korzeniowska et al., 2013). Due to its antimicrobial properties, i.e. anti-bacterial, anti-fungal, anti-viral and anti-biotic, with no harmful side effects, it can prevent infections and diseases. It is used in a variety of healthcare products including therapeutics, biosensors, environmental remediation and dressing materials (Kong et al., 2000; Aitken et al., 2006; Lansdown, 2006). Kaye et al. (2004) showed that the antimicrobial resistance of microorganisms is an increasing global problem and emerging antimicrobial resistance has become a public health issue worldwide. That is why the nanoparticles are expected to be the alternative for traditionally used disinfectant, as well as potentially used on a wider scale in poultry hatcheries.

The aim of this study was to evaluate the effect of colloidal silver particles used for disinfecting Japanese quail (*Coturnix coturnix japonica*) hatching eggs on hatchability traits, egg shell conductance, hatched chickens, and microbial populations on the shell surface during incubation.

Material and methods

In total, 360 hatching eggs of Japanese quail were divided into 3 groups before incubation, with 120 eggs per treatment and 6 replicates for each. Before being placed into the incubator eggs of Group 1 were not disinfected (NC, negative control), eggs of Group 2 were disinfected by standard fumigation (using a mixture of formalin, permanganate and water) in a hermetic closed gas chamber (C, positive control) and eggs of Group 3 were disinfected with a commercial solution of colloidal silver (S, Nano-Koloid®). The solution contained 50 mg/kg colloidal silver and was applied by direct spraying (eggs were sprayed from all sides using a bottle with diffuser).

The eggs were incubated by using a BIOS hatching apparatus. Standard conditions of incubation were maintained, the temperature was 37.6–38.0°C with 50–65% relative humidity in the setting compartment, and 37.0–37.5°C with 75–80% relative humidity in the hatching compartment. The eggs were turned 8 times a day during the incubation period. After 14 days of incubation the eggs were candled to determine the number of infertile eggs and dead embryos, then the eggs were moved from the setter to the hatching compartment. Samples for microbial analysis were collected on the 14^{th} day of the experiment. Group 3 eggs were placed in sterile boxes containing 50 ml of sterile buffer salt solution (PBS) with 3 drops of TWEN 80. Containers with eggs were left on the stirrer for 1 h. Samples were serially diluted in PBS and plated on sterile medium in order to obtain the total number of aerobic mesophilic bacteria, the total number of bacteria, coliform bacteria, haemolytic bacteria, *Salmonella spp*. and *Staphylococcus spp*. (GENTRY and QUARLES, 1972; JONES et al., 2002; PN-EN ISO 4833:2004; PN-ISO 21528–1:2005). After incubation, colonies were counted and presented as cfu/ml of liquid from the egg. To identify the bacterial colonies microscopic examination was applied as well as Gram's staining and API biochemical tests (bioMérieuxPolska®). The media used in the course of the analysis are shown in Table 1.

Table 1. Incubation conditions used in the microbial analysis

Inkubationsbedingungen für die mirkobiellen Untersuchungen

Microorganisms	Applied medium
Aerobic mesophilic bacteria	Conditions agar
Total number of bacteria	Conditions agar
Haemolytic bacteria	Conditions agar + 5% sheep blood
Coliforme bacteria	Mac Conkey's
Staphylococcus spp.	Baird Parker agar (supplemented with 5% egg yolk-tellurite)
Salmonella spp.	agar *Salmonella-Shigella*

After 17.5 days of incubation normal, crippled and dead chickens were counted. Fertility, hatchability and periodical embryonic mortality were also calculated. Weight loss of eggs and eggshell conductance (CHRISTENSEN et al., 2001) during the incubation were also determined. After hatching, chickens were reared for 14 days and the mortality and body weight were recorded at the 7^{th} and 14^{th} day of rearing.

Statistical analyses

The data were analysed by the use of the statistical package SPSS 20.0PL (IBM, 2011). Normality of data was verified by using Kolmogorov-Smirnov test. A one-way ANOVA with Duncan's post-hoc test was carried out. The number of bacteria colony forming units were verified using non-parametrical χ^2 test.

Results

Application of disinfection solutions affected total number of bacteria significantly ($P \leq 0.05$) (Figure 1). In the S group, the smallest value of 1.15 Log_{10} CFU/ml of egg liquid was recorded compared to 1.65 Log_{10} CFU/ml egg liquid in Group NC. Group C (1.39 CFU/ml) did not differ significantly from these groups. At the same time, the number of bacteria decreased in group S very significantly ($P \leq 0.01$), at most for the important bacteria *Staphylococcus spp.* (% of total isolates) (Table 2). Nevertheless, in group S a significant increment ($P \leq 0.05$) in *Bordetella spp.* and a highly significant increment ($P \leq 0.01$) in *Non identified* bacteria was observed. No *E. Coli* and *Salmonella spp.* bacteria were found among the treatment groups. No other significant differences were observed between treatments for other strains of bacteria.

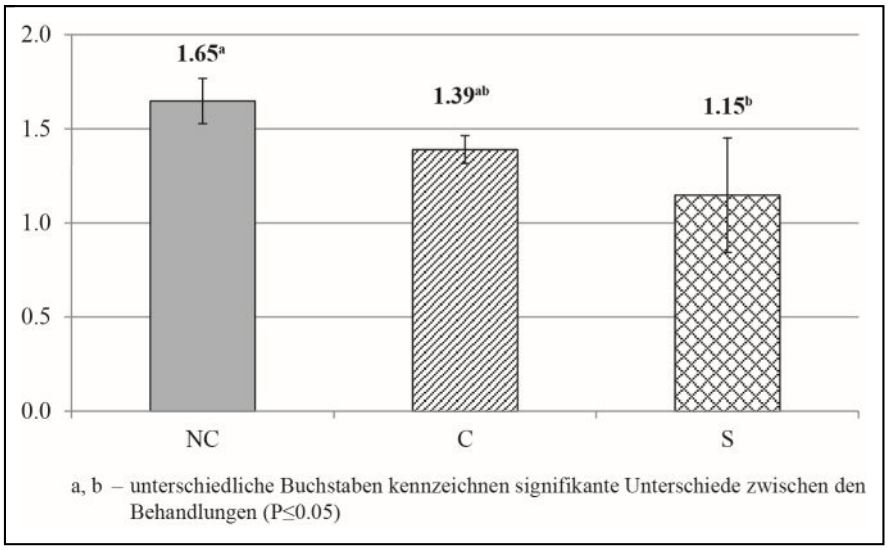

Figure 1. Total number of bacteria (Log_{10} CFU ml^{-1} liquid from egg). NC: negative control, C: control and S: colloidal silver. a, b – differences between mean values for treatments are significant ($P \leq 0,05$)

Gesamtanzahl der Bakterien (Log_{10} CFU/ml Eiinhalt). NC: negative control, C: control (disinfected with formaldehyde) and S: colloidal silver.

Table 2. The most common bacteria isolated from different groups of eggs (% of total isolates)

Häufigste, bei den Eiern der Behandlungen gefundene Bakterien-Spezies (% der Gesamtisolate)

Bacteria strain	Treatment			X^2
	NC	C	S	(*P*-value)
Cellulomonas spp.	8.3			*0.073*
Corynebacterium spp.	2.8	2.0		*0.805*
Kocuria kristinae	5.6		8.3	*0.244*
Micrococcus spp.	8.3		8.3	*0.148*
Staphylococcus aureus	33.3	10.0	16.7	*0.073*
Staphylococcus spp.	11.1	86.0		*0.000*
Streptococcus spp.	5.6	2.0	16.7	*0.280*
E. coli				
Salmonella spp.				
Non indentified	25.0		50.0	*0.001*

NC: negative control, C: control (disinfected with formaldehyde) and S: colloidal silver.

The results of eggshell conductance during incubation days 15 and 17.5 of embryonic development are given in Table 3. No significant differences among treatments were observed in respect to infertile and dead embryos at the 15th day of incubation and for unhatched chickens. Group S, however, showed a positive significant decrement ($P \leq 0.05$) in

number of fertile eggs at 15 days of incubation and for healthy chickens (%). This might be explained by minimisation of water loss through occluded egg pores after silver spraying. This result suggests the possibility to obtain bigger and, at the same time, better chickens due to considerably smaller water loss during incubation.

Table 3. Egg shell conductance constant (mg H_2O/day/mm Hg)

Schalendurchlässigkeits-Konstante

Trait		Treatment			SEM
		NC	C	S	
Day 15	Fertile eggs	1.79ab	2.47b	1.31a	0.163
	Infertile eggs	2.16	2.34	1.59	0.152
	Dead embryos	3.65	4.08	1.40	0.566
Day 17.5	Unhatched chickens	6.61	7.62	6.60	0.443
	Healthy chickens	3.98b	2.52b	2.02a	0.165

a, b – differences between mean values for treatments are significant at $P \leq 0.05$
NC: negative control, C: control (disinfected with formaldehyde) and S: colloidal silver.

The results (shown in Table 4) found no significant differences between controls (NC and C) and experimental treatments (S) in all hatchability parameters such as proportion of fertile eggs, hatchability and mortality of fertile and set eggs. The embryonic periodical mortality also did not differ significantly (Figure 2).

Table 4. Hatching results

Schlupfergebnisse

Trait	Treatment			SEM
	NC	C	S	
Eggs fertility (%)	83.53	85.87	87.76	3.127
Hatchability of fertile eggs (%)	81.71	78.22	79.34	2.078
Hatchability of set eggs (%)	69.40	70.99	73.51	2.910
Mortality of fertile eggs (%)	18.29	21.78	20.66	2.078
Mortality of set eggs (%)	14.13	14.88	14.25	1.543

NC: negative control, C: control (disinfected with formaldehyde) and S: colloidal silver.

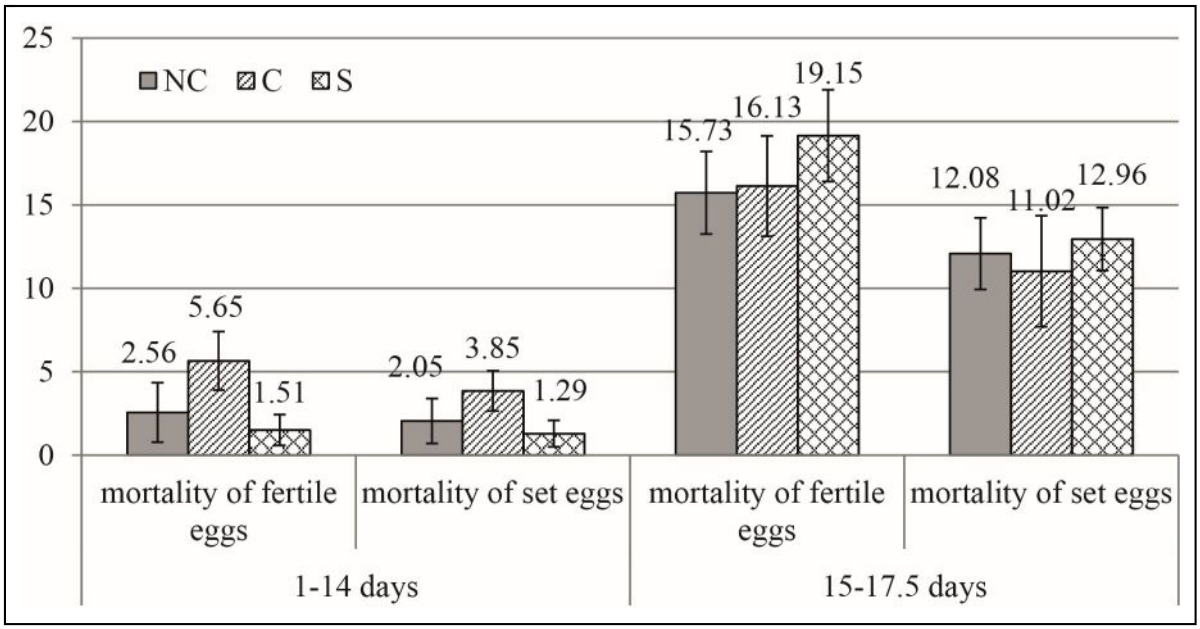

Figure 2. Mean values (±SE) of embryonic periodical mortality (%). NC: negative control, C: control and S: colloidal silver.

Mittelwerte (±SE) für die Embryonensterblichkeit in den Brutperioden (%). NC: negative control, C: control (disinfected with formaldehyde) and S: colloidal silver.

The body weight of newly hatched chickens ranged from 5.89 to 6.05 g depending on group and did not differ significantly between treatments. However, chickens were significantly heavier ($P \leq 0.05$) (27.5 and 45.0 g) in group S compared to groups NC (24.6 and 44.7 g) and C (25.3 and 45.9 g) at the 7th and 14th day of rearing, respectively (Figure 3). Table 5 shows survivability rates during the 1st week, with significant differences ($P \leq 0.05$) between treatments. NC had the biggest value. However, survivability was also insignificantly better in Group S (93.4 and 82.6%) compared to groups NC (86.9 and 80.2%) and C (88.2 and 67.4%) in the 2nd week and during the 14 days of rearing, respectively.

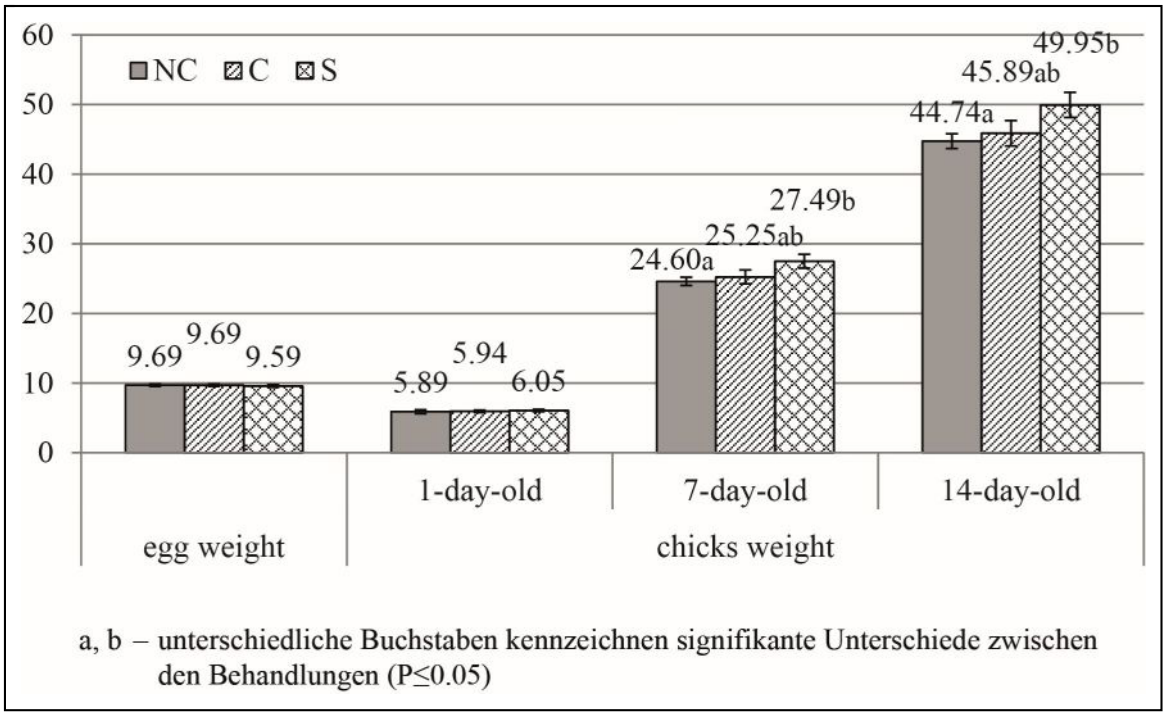

Figure 3. Mean values (±SE) of egg weight and body weight of quail chickens after 1, 7 and in 14 days of rearing depending on treatment. NC: negative control, C: control and S: colloidal silver. a, b – differences between mean values for treatments are significant at *P*≤0.05

Mittelwerte (±SE) für das Eigewicht und das Körpergewicht der Wachtelküken am 1., 7. und 14. Lebenstag in Abhängigkeit von der Behandlung. NC: negative control, C: control (disinfected with formaldehyde) and S: colloidal silver.

Table 5. Survivability of chickens (% of hatched chickens)

Überlebensrate der Küken (% der geschlüpften Küken)

Survivability after hatching	Treatment			χ^2 (*P*-value)
	NC	C	S	
1.-7. days	92.3	76.4	88.4	0.026
8.-14. days	86.9	88.2	93.4	0.447
Total (1.-14.days)	80.2	67.4	82.6	0.641

NC: negative control, C: control (disinfected with formaldehyde) and S: colloidal silver.

Discussion

The observed effects on the microflora could result from the mechanism behind the antimicrobial activity of nanosilver particles. Silver can reduce the total bacteria population by an interaction with sulphur containing proteins in the bacterial cell wall and by an interaction with phosphorus and sulphur containing compounds like DNA. Cell lysis, death and damage occurs as a result of disrupting membrane permeability, deactivating cellular enzymes, impairing and preventing energy cycle and DNA replication (Feng et al., 2000; Jeon et al., 2003; Sondi and Salopek-Sondi, 2004; Morones et al., 2005; Song et al., 2006). In addition, various forms of silver ions have been developed to increase antimicrobial activity and disrupt membranous function of bacterial cells. For example, nitrogen silver was applied for disinfection and silver citric acid was utilised for removal of *Pseudomonas aeruginosa* (Thurman and Gerba, 1989). Generally, silver nanoparticles exhibit stronger biocidal effects against a wide spectrum of harmful microorganisms, i.e. bacteria, fungi and vira by affecting gene expression. Deactivation of enzymes responsible for DNA replication may stop ATP synthesis in microorganisms (Chmielowiec-Korzeniowska et al., 2013). Arya et al. (2011) stated that silver nanoparticles exert their antibacterial effects by anchoring to and penetrating the bacterial cell wall, and by modulating cellular signalling via dephosphorylating putative key peptide substrates on trypsin residues. This is the main mechanism by which silver nanoparticles exhibit antibacterial properties. Moreover, it was noted that nanosilver particle chemistry, including size distribution, morphology, surface area, charge, surface modifications and chemical composition (purity) has an important role in the possible mechanisms of antimicrobial action (Soltani et al., 2011).

The eggshell conductance constant is defined as the conductance per unit of egg mass and incubation period which is referred to as egg weight loss. Therefore, the eggshell conductance constant is an important parameter in incubation of eggs. A too fast moisture loss is disadvantageous for the active embryonic development (Geng and Wang, 1990; Christensen et al., 2001). The cuticle of the eggshell might be affected by the application of sanitisers leading to altered eggshell permeability and embryonic development (Bagley et al., 1988; Yildirim et al., 2003). The variation in the ability of embryos to adjust their water content and eggshell conductance is essential to estimate the link between egg weight loss and embryonic survivability (Shahein and Sedeek, 2014). The lowest values were found in eggs treated with colloidal silver and this may result from the impact of the disinfectant on the cuticle layer and shell porosity. The water loss during incubation depends on many factors such as species of bird, genotype, size of egg or eggshell properties (Ar et al., 1974; Christensen et al., 2001; Shafey, 2002). Too much as well as too little water loss may negatively influence hatchability results. The effect of colloidal silver application may be due to the blocking of pores. However, worse hatching results were not noticed in this group and at the same time body weight of one-day-old chick were the highest (non-significantly). It may prove that although pores have possibly been blocked, the shell conductivity was still in an optimal range.

Hatchability results may suggest that treating hatching eggs with silver as disinfectant did not have any negative impact compared with traditional sanitation treatment (NC) and group C. However, silver can be used as an alternative to formaldehyde, which is considered hazardous to humans (Whistler and Sheldon, 1989, Debes and Basyony, 2011). Thus, effective alternative disinfectants are needed to replace formaldehyde. Silver is an effective, safe, and nontoxic natural hatching egg disinfectant improving or at least maintaining the hatchability of eggs at an acceptable level.

Poultry eggs are vulnerable to microbial infections affecting health and performance of hatched chickens. Good sanitation procedures for hatching eggs mitigate high weights of hatched chickens and increase survival of the hatched chickens (Turblin, 2011). Rashid et al. (2011) found that sanitation of Fayoumi and crossbred (Rhode Island Red male × Fayoumi female) chicken eggs by commercial sanitisers (Sanisquad, Beloran, TH4, QC Standar, Prills and Supersept) via swabbing, dipping, fumigation, spraying or cleaning with a piece of cloth soaked by these sanitisers did not have any effect on body weight at hatching. The results of this study also disagree with the findings of Fasenko et al. (2009), who reported no significant differences with respect to sanitisation of chicken eggs by electrolysed oxidising water on the following body weight. Likewise, Aygun et al. (2012) showed that spraying of quail eggs with propolis, ethyl alcohol and benzalkonium solutions did not affect body weight.

Conclusions

The microbial analyses partially confirmed the antimicrobial properties of colloidal silver. Using this preparation limited the number of bacteria colonies of almost all species after 15 days of incubation. It did not create preferential conditions for the development of any particular bacteria strain, as opposed to formalin. In group C, *Staphylococcus spp*. dominated. Hatching results were not affected by treatments. Silver nanoparticles changed egg shell conductivity of fertile eggs and of hatched chickens, and thus to a lesser loss of moisture. Chickens hatched from eggs disinfected by silver nanoparticles were characterised by lower mortality and considerably higher body weight during the first 14 days of rearing than birds from other treatments. Colloidal silver can be used as an alternative to traditional disinfection methods applied in hatcheries.

Summary

The aim of this study was to evaluate the effect of colloidal silver as disinfectant of Japanese quail hatching eggs on hatchability traits, eggshell conductance, hatched chicken quality and microbial populations on the shell surface. In total, 360 Japanese quail hatching eggs were distributed into 3 equal groups before incubation. Before being placed into the incubator the eggs from Group 1 were not disinfected (NC) and eggs of Group 2 were disinfected with formalin and permanganate (C). In Group 3, colloidal silver (S, Nano-Koloid®) was applied in 50 mg/kg concentration by spraying. The eggs were hatched in a BIOS hatching apparatus under standard conditions. After 14 days of incubation the eggs were candled to determine the number of infertile eggs and dead embryos. Samples for microbial analyses were collected. After 17.5 days of incubation fertility, hatchability, periodical embryonic mortality and eggshell conductance were determined. Thereafter, chickens were reared for 14 days and mortality and body weight (bw) were registered at 7^{th} and 14^{th} day of rearing.

The results of the microbial analyses partially confirmed the antimicrobial properties of colloidal silver. Using this preparation reduced the number of bacterial colonies of almost all species after 15 days of incubation. It did not create preferential conditions for development of any particular bacteria strain, as opposed to formalin. In group C *Staphylococcus spp*. dominated. Hatching results of eggs disinfected with colloidal silver were similar to groups C and NC. The use of colloidal silver resulted in significant changes in eggshell conductivity of fertile eggs and thus in a lower loss of moisture. In group S lower mortality and considerably higher body weights were reached within 14 days of rearing.

Key words

Japanese quail, colloidal silver, hatching eggs, disinfection, hatchability, microflora, performance

Zusammenfassung

Einfluss der Verwendung von kolloidalem Silber zur Desinfektion von Bruteiern auf einige mikrobiologische, Brut- und Leistungsmerkmale bei der Japanischen Wachtel (*Coturnix cot. japonica*)

Im Rahmen der Studie sollten die Auswirkungen des Einsatzes von kolloidalem Silber zur Desinfektion von Bruteiern der Japanischen Wachtel auf das Schlupfergebnis, die Schalendurchlässigkeit, die Qualität der geschlüpften Küken und die mikrobielle Besiedelung der Eischale untersucht werden. Hierzu wurden 360 befruchtete Wachteleier vor der Einlage in den Brutapparat in 3 Behandlungsgruppen aufgeteilt: die erste Gruppe wurde nicht desinfiziert (NC), die zweite Gruppe wurde mit Formalin und Permanganat (C) und die dritte mit kolloidalem Silber (S; Nano-Koloid®; Besprühen mit 50 ppm Lösung) desinfiziert. Die Eier wurden unter Standardbedingungen in einem BIOS-Brutapparat ausgebrütet. Bei der Umlage der Eier in den Schlupfbrüter wurden diese durchleuchtet, um die Anzahl der unbefruchteten Eier und der abgestorbenen Embryonen zu ermitteln. Proben für die mikrobiellen Analysen wurden gezogen. Nach 17,5 Tagen Bebrütung wurden die Befruchtungsrate, der Schlupferfolg, die Embryonensterblichkeit in den einzelnen Brutperioden und die Schalendurchlässigkeit bestimmt. Danach wurden die Küken über 14 Tage aufgezogen und die Mortalität zum 7. bzw. 14. Lebenstag erfasst.

Die Ergebnisse der mikrobiellen Untersuchungen haben die antimikrobiellen Eigenschaften von kolloidalem Silber bestätigt. Die Silberlösung reduzierte bis zum 15. Bruttag nahezu alle ermittelten Bakterienspezies. Im Gegensatz zu Formalin hat Silber die Entwicklung von keiner einzigen Bakterienspezies gefördert. In der Behandlungsgruppe C dominierten *Staphylococcus Spp*. Die Schlupfergebnisse der mit Silber behandelten Eier unterschieden sich nicht von

den Gruppen C und NC. Die Verwendung von Silber veränderte die Schalendurchlässigkeit signifikant und verminderte so den Wasserverlust. In der Behandlungsgruppe S wurde in den ersten 14 Tagen nach dem Schlupf eine geringere Mortalität und deutlich höhere Lebendgewichte beobachtet.

Stichworte

Japanische Wachtel, kolloidales Silber, Bruteier, Desinfektion, Brutergebnisse, Mikroflora, Leistung

References

AITKEN, R.J., M.Q. CHAUDHRY, A.B.A. BOXALL, M. HULL, 2006: Manufacture and use of nanomaterials: current status in the UK and global trends. Occup. Med. - Oxford **56**, 300-306.

AR, A., C.V. PAGANELLI, R.B. REEVES, D.G. GREENE, H. RAHN, 1974: The avian egg: water vapor conductance, shell thickness, and functional pore area. The Condor **76(2)**, 153-158.

ARYA, V., R. KOMAL, M. KAUR, A. GOYAL, 2011: Silver nanoparticles as a potent antimicrobial agent: A Review. Pharmacologyonline **3**, 118-124.

AYGUN, A., D. SERT, 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.

BAGLEY, L.G., V.L. CHRISTENSEN, R.A. BAGLEY, 1988: Effect of altering eggshell permeability on the hatchability of turkey eggs at high altitude. Poult. Sci. **69**, 451-456.

CHEN, X., H.J. SCHLUESENER, 2008: Nanosilver: a nanoproduct in medical application. Toxicol. Lett. **176(1)**, 1-12.

CHMIELOWIEC-KORZENIOWSKA, A., Ł. KRZOSEK, L. TYMCZYNA, M. PYRZ, A. DRABIK, 2013: Bactericidal, fungicidal and virucidal properties of nanosilver. Mode of action and potential application. A review. Annales UMCS Sec. EE **31 (2)**, 1-11.

CHRISTENSEN, V.L., J.L. GRIMES, M.J. WINELAND, L.G. BAGLEY, 2001: Effects of turkey breeder hen age, strain, and length of the incubation period on survival of embryos and hatchlings. J. Appl. Poultry Res. **10**, 5-15.

CORTÉS, C.R., G.T. ISAÍAS, C.L. CUELLO, J.M.V. FLORES, R.C. ANDERSON, C.E. CAMPOS, 2004: Bacterial isolation rate from fertile eggs, hatching eggs, and neonatal broilers with yolk sac infection. Revista Latinoamericana de Microbiologia **46(1)**, 12-16.

DEBES, A., M. BASYONY, 2011: The use of oregano (*Origanum vulgare* L) and ginger (*Zingiber officinale*) oils as alternative hatching egg disinfectant versus formaldhyde fumigation in leghorn and matrouh eggs. Egypt. Poult. Sci. **31**, 755-765.

FARZINPOUR, A., N. KARASHI, 2012: The effects of nano-silver on egg quality traits in laying Japanese Quail. Appl. Nanosci., 311-315.

FASENKO. G.M., E.E.O. CHRISTOPHER, L.M. MCMULLEN, 2009: Spraying hatching eggs with electrolyzed oxidizing water reduces eggshell microbial load without compromising broiler production parameters. Poult. Sci. **88**, 1121-1127.

FENG, Q.L., J. WU, G.Q. CHEN, F.Z. CUI, T.N. KIM, J.O. KIM, 2000: A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J. Biomed. Mater. Res. **52**, 662-668.

FUENG-LIN, W.O., J.B. CAREY, S.C. RICKEY, S. DHA, 1996: Peroxidase catalyzed chemical dip, egg shell surface contamination and hatchability. J. Appl. Poultry Res. **5**, 6-13.

GENG, Z.Y., X.L. WANG, 1990: Relationship of hatchability and the percentage of egg weight loss and shell pore concentration during incubation. Chin. J. Anim. Sci. **26**, 12–14 [in Chinese, with English abstract].

GENTRY, R.F., C.L. QUARLES, 1972: The measurement of bacterial contamination on eggshells. Poult. Sci. **51**, 930-933.

IBM Corp. Released, 2011: IBM SPSS Statistics for Windows", Version 20.0. Armonk, NY: IBM Corp.

JEON, H.J., S.C. YI, S.G. OH, 2003: Preparation and antibacterial effects of Ag–SiO_2 thin films by sol–gelmethod. Biomaterials **24**, 4921-4928.

JONES, D.R., K.E. ANDERSON, P.A. CURTIS, F.T. JONES, 2002: Microbial contamination in inoculated shell eggs: I. Effects of layer strain and hen age. Poult. Sci. **81**, 715-720.

KAYE, K.S., J.J. ENGEMANN, H.S. FRAIMOW, E. ABRUTYN, 2004: Pathogens resistant to antimicrobial agents: Epidemiology, molecular mechanisms, and clinical management. Infect. Dis. Clin. N. Am. **18**, 467-511.

KONG, J., N.R. FRANKLIN, C. ZHOU, M.G. CHAPLINE, S. PENG, K. CHO, H. DAI, 2000: Nanotube molecular wires as molecular sensors. Science **287(5453)**, 622-625.

LANSDOWN, A.B., 2006: Silver in health care: antimicrobial effects and safety in use. Curr. Probl. Dermatol. **33**, 17-34.

MORONES, J.R., J.L. ELECHIGUERRA, A. CAMACHO, K. HOLT, J.B. KOURI, J.T. RAMÍREZ, M.J. YACAMAN, 2005: The bactericidal effect of silver nanoparticles. Nanotechnology **16**, 2346-2353.

PERCIVAL, S.L., P.G. BOWLER, D. RUSSELL, 2005: Bacterial resistance to silver in wound care. J. Hosp. Infect. **60**, 1-7.

POLISH NORM PN-EN ISO 4833, 2004: Mikrobiologia żywności i pasz - Horyzontalna metoda oznaczania liczby drobnoustrojów - Metoda płytkowa w temperaturze 30 stopni C [in Polish, available in English].

POLISH NORM PN-ISO, 21528–1, 2005: Mikrobiologia żywności i pasz - Horyzontalna metoda wykrywania i oznaczania liczby *Enterobacteriaceae*. Część 1: Wykrywanie i oznaczanie liczby metodą NPL z przednamnażaniem" [in Polish, available in English].

RASHID, A., S.H. KHAN, M. AURANGZEB, M.B. BHATTI, 2011: Effects of different disinfectants on chick body weight and hatchability traits in Fayoumi and F1 crossbred (Rhode Island Red male × Fayoumi female) chickens. Asian J. Phar. Biol. Res. **1(4)**, 518-524.

SHAFEY, T.M., 2002: Effects of egg size and eggshell conductance on hatchability traits of meat and layer breeders flocks. Asian-Australas. J. Anim. Sci. **15(1)**, 1-6.

SHAHEIN, E.H.A., E.K. SEDEEK, 2014: Role of spraying hatching eggs with natural disinfectants on hatching characteristics and eggshell bacterial counts. Egypt. Poult. Sci. J. **34(1)**, 213-230.

SOLTANI, M., M. ESFANDIARY, M.M. SAJADI, S. KHAZRAEENIA, A.R. BAHONAR, H. AHARI, 2011: Effect of nanosilver particles on hatchability of rainbow trout (*Oncorhynchusmykiss*) egg and survival of the produced larvae. Iran. J. Fish. Sci. **10(1)**, 167-178.

SONDI, I., B. SALOPEK-SONDI, 2004: Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for gram-negative bacteria. J. Colloid. Interf. Sci. **275**, 177-182.

SONG, H.Y., K.K. KO, L.H. OH, B.T. LEE, 2006: Fabrication of silver nanoparticles and their antimicrobial mechanisms. Europ. Cells & Mat. J. **11**, 50- 58.

THURMAN, R.B., C.P. GERBA, 1989: The molecular mechanisms of copper and silver ion disinfection of bacteria and viruses. Crit. Rev. Env. Contr. **18**, 295-315.

TURBLIN, V., 2011: Good quality chicks from disinfected eggs. World Poult. **27**, 1-3.

UNDERWOOD, C., A.W. VAN EPS, 2012: Nanomedicine and veterinary science: The reality and the practicality. Review Vet. J. **193**, 12-23.

WHISTLER, P.E., B.W. SHELDON, 1989: Comparison of Ozone and Formaldehyde as poultry hatchery disinfectants as an alternative hatching egg disinfectant. Poult. Sci. **68**, 1345-1350.

YILDIRIM, I., M. ÖZSAN, R. YETISIR, 2003: The use of oregano (*Origanum vulgare* L) essential oil as alternative hatching egg disinfectant versus formaldehyde fumigation in quails (*Coturnix coturnix japonica*) eggs. Revue Méd. Vét. **154 (5)**, 367-370.

Correspondence: Justyna Batkowska PhD, Institute of Biological Basis of Animal Production, University of Life Sciences in Lublin, 13 Akademicka St, 20–950 Lublin, Poland; e-mail: justyna.batkowska@up.lublin.pl