Use of reactive oxygen species (ozone, hydrogen peroxide) for disinfection of hatching eggs

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ABSTRACT The sample consisted of 480 hatching eggs of Japanese quails and was divided into 4 groups. Before the transfer to the incubator, the first group was not disinfected (negative control). In the second group, eggs were disinfected by means of formaldehyde fumigation (positive control). In the third and fourth group, reactive oxygen forms were used for disinfection—perhydrol ($\mathbf{H_2O_2}$) and ozone ($\mathbf{O_3}$), respectively. Eggs were incubated under standard conditions. On the 14th D, eggs were candled, and proportions of fertilized eggs and died embryos were calculated. In addition, samples were collected for microbiological examination. After 17.5 D, the results of the whole hatching were evaluated. Chicks were reared for 14 D. Their survivability and body weight gain were recorded. Disinfection by means of reactive oxygen forms did not prove to be more effective in reducing the number of bacterial colonies on the shell. Reduced hatching and significantly increased mortality in the O_3 group may indicate the negative impact of this gas on developing embryos. The results of hatching from eggs disinfected with H_2O_2 did not differ from those obtained in control groups. The biggest chicks were obtained from O_3 disinfected eggs. However, during rearing, their growth did not match the one observed for birds in the remaining groups. Chicks hatched from eggs disinfected with H_2O_2 were characterized by the largest survivability. Disinfection with reactive oxygen forms did not significantly improve the hygiene of hatching eggs, hatching performance, and quality of hatched chicks. Hydrogen peroxide, whose application offered satisfactory hatching results, may be the recommended disinfectant. On the other hand, O_3 appears to be undesirable because of its negative impact on bird embryos.

Key words: ozone, hydrogen peroxide, disinfection, hatching egg

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INTRODUCTION

The high quality of newly hatched chicks is considered as the basis for effective poultry production. Their health and survivability are influenced by many factors, such as genotype, age, and health of the parent stock or the laying hens' nutrition. However, the most crucial element is the quality of eggs intended for hatching. Natural microflora, specific to the environmental conditions prevailing in the hen house, is present on the surface of egg shells. Its quantitative and qualitative composition depends upon the birds' rearing system (De Reu et al., 2006). The microbiological analysis of the egg surface indicates the presence of a number of microorganisms such as *Escherichia coli*, *Salmonella*, *Streptococcus*, *Staphylococcus*, and *Yersinia* (Jones et al., 2004; Musgrove et al., 2008). The presence of bacteria from the genera of *Micrococcus*, *Achromobacter*, *Aerobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Cytophaga*, *Flavobacterium*, *Pseudomonas*, *Aeromonas*, *Proteus*, *Sarcina*, and *Serratia* (Mayes and Takeballi, 1983) has also been reported.

During incubation, microclimate conditions foster the growth of microorganisms. This may have an adverse impact on hatching results. Therefore, disinfection of both the eggs and the incubator chamber is necessary. The basic method of disinfection is fumigation with formaldehyde vapors. However, despite the relatively low price and high efficiency, formaldehyde may have a

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toxic and carcinogenic impact. As a consequence, when working with this preparation, special care should be taken (Ledoux, 2002). That is why effective alternatives proving high efficiency while maintaining the safety of employees and the lack of negative influence upon hatching results are being searched for.

Among the alternative methods of disinfection, UV C radiation is mentioned most frequently (Al-Shammari et al., 2015). In addition, preparations applied directly on the egg shell, such as colloidal silver (Batkowska et al., 2017), substances of natural origin, for example, propolis (Aygun et al., 2012; Batkowska et al., 2018a), or plant extracts such as thyme (Stahl-Biskup and Laakso, 1990) and cinnamon (Ulucay and Yildirim, 2010), alicine (Copur et al., 2011), oregano oil (Yildirim et al., 2003), or red grapefruit juice (Batkowska et al., 2018b) are offered.

The search for alternative disinfection methods also includes reactive oxygen forms such as ozone ($\mathbf{O_3}$) and perhydrol ($\mathbf{H_2O_2}$). The biocidal properties of O_3 were observed over 100 yr ago in the aspect of combating wound infections (Stokeer, 1916). They result directly from its chemical structure. O_3 is one of the strongest oxidants, and the mechanism of action includes both the bacterial cell membrane degradation and the peroxidation of cell components (Bocci, 2006). O_3 is used as a disinfectant for drinking, industrial, and refrigeration water and in wastewater treatment (Macauley et al., 2006). Its antimicrobial activity exceeds that of chlorine 50 times at a much shorter time of action than sodium hypochlorite. Hydrogen peroxide (H_2O_2) is a very strong oxidant which forms free radicals exerting a destructive impact upon cell membranes. As a result, it has found a wide application as a biocide. However, the mechanism of the action of this substance is not fully understood. It is believed that the underlying mechanism is the reaction of Fenton to produce free hydroxyl radicals (Linley et al., 2012).

The aim of the study was to evaluate the use of reactive oxygen forms (O_3, H_2O_2) as alternative methods for the disinfection of hatching eggs.

MATERIAL AND METHODS

The test material consisted of 480 hatching eggs of Japanese quail. Before incubation, the eggs were divided into 4 groups of 120 (4 replicates per group) and disinfected outlined in the diagram in Table 1. The eggs were incubated in a Jarson incubator (Jarson, Gostyń, Poland) hatching apparatus under the following conditions:

- setter compartment: 1 to 14.5 D, temperature 37.6°C to 38.0°C, humidity 50 to 65%, the position of the trays with eggs was changed every 2 h by 180°C;
- hatcher compartment: 15 to 17.5 D, temperature 37.0°C to 37.5°C, humidity 75 to 80%.

The candling of eggs was performed on the 14th D of incubation to assess the number of fertilized eggs and dead embryos. The fertility of eggs as well as the embryos' mortality in all eggs were verified upon the eggs opening and in the course of biological analyses. Subsequently, egg samples for microbiological analyses were taken. After that, 5 eggs from each group were placed in sterile containers containing 50 mL of phosphate buffered saline with 100 µL of TWEN 80. Containers were left on the stirrer for 1 h. Serial dilutions of samples in phosphate buffered saline were placed on sterile substrates to obtain total bacteria count, total mesophilic aerobic bacteria, coliforms, hemolytic bacteria, *Salmonella* sp., *Staphylococcus* sp., yeast-like fungi, and molds (Gentry and Quarles, 1972; Jones et al., 2002; ISO 4833-1:2013, 2013; ISO 21528-1:2017, 2017). After the incubation period, colonies were counted, and the result was expressed as colony forming unit (CFU)/1 mL of egg liquid. Microscopic examinations, Gram staining, and biochemical tests of bioMerieux API (BioMerieux SSC Europe Sp. z o.o.) (Fassatiova, 1983; Watanabe, 2002; Özcelik, 2007) were performed to identify bacterial species. Substrates used during the analyses are presented in Table 2.

After candling, eggs were transferred to the hatching compartment. After the incubation (17.5 D), hatching results were analyzed, and the proportions of fertilized and hatched eggs and the periodic mortality of embryos were calculated. The eggs were weighed before the incubation, during the transfer (divided into alive, dead, and infertile), and at the end of incubation (unhatched). On this basis, water conductivity of the shell was calculated (mg H_2O/day/mmHg) according to the formula of Christensen et al. (2001) calculated on the initial weight of the egg. The obtained chicks were raised in the cage system for 14 D. Their survivability and body weight gain were recorded.

The data were analyzed using the statistical package SPSS 20.0 PL (IBM Corp., 2011). Normality of data was evaluated with Kolmogorov-Smirnov test.

Table 1. Design of the experiment.

Group	n	Factor (disinfection method)
NC	120	Not disinfected
PC	120	Disinfected by fumigation with formalin and permanganate
H_2O_2	120	Disinfected with 30% hydrogen peroxide by spraying
O_3	120	Disinfected by ozone (O_3, 4.2 mg O_3/h, 5 min)

Table 2. Incubation conditions used in the microbial analysis.

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

Subsequently, a one-way ANOVA with Tukey's post-hoc test was carried out. The number of bacteria CFUs was verified using nonparametrical χ^2 test.

RESULTS

Table 3 presents the results of microbiological analysis of samples taken from the egg shell depending on the type of egg disinfection method. The varying values of bacteria on the shell in each of the studied groups are visible. The smallest number of bacteria was detected in the negative group (1.21 CFU). In the positive control group (disinfected by means of formalin fumigation), a greater number of bacteria were observed than in the negative group. Egg shells in both experimental groups showed a general increase in microflora. Both experimental groups differed in a statistically significant manner from the group in which the eggs were not subjected to any sanitizing treatment.

Also in Table 3, bacterial species isolated from the egg shell are shown as a percentage of their total number of colony, depending on the egg disinfection method. The number of colonies of all bacterial species significantly depended on the experimental group ($P < 0.05$). In the positive group, mostly *Staphylococcus* Spp. bacteria (86%) were isolated. However, no unidentified microorganisms were found. This may indicate not only the effectiveness of the traditional disinfection method but also the preferential conditions for this group of microorganisms. In the next study, involving groups H_2O_2 and O_3, the biggest species diversity of microorganisms was recorded—only *Corynobacterium* Spp. was not found.

Despite the similar action mechanism of reactive oxygen species, H_2O_2 has been shown to create preferential conditions for *Kucuria kristinae* and *Micrococcus* Spp. development, while these species were not identified in the O_3 group. A reversal tendency was observed in relation to *Staphylococcus* bacteria, whose more numerous colonies were isolated from eggs disinfected with O_3 in comparison to H_2O_2. In the NC group, the biggest number of unidentified bacteria (amounting to 36.36%) were isolated. Owing to such a high frequency, this fact requires further research.

Table 4 presents the hatching results of Japanese quail depending on the eggs' disinfection method. The percentage of fertilized eggs oscillated around 89.8 to 85.8%. Hatching results depended significantly on the disinfection method used ($P < 0.05$). Attention is drawn to the low percentage of hatched chicks in the final O_3 group. Full quality chicks were obtained only from 64% of fertilized eggs. On the other hand, in the remaining groups, chicks hatched from 80% of eggs. The mortality of embryos in the group treated with O_3 was considerably higher than that in the remaining groups, both in relation to the set and fertile eggs. This may indicate a negative impact of O_3 on embryo development, possibly resulting from its significant permeability through the shell. Interesting results were recorded in the distribution of embryo mortality in both phases of hatching depending on the disinfection method. In the control groups and in the group disinfected with O_3 group, the largest number of embryos died in the second phase of incubation. The situation differed in H_2O_2 group where over 94% of embryos (in relation to all dead) died in the first phase and only 5.6% in the second phase (in the hatching compartment).

Relatively large permeability of O_3 through shells and membranes can also be demonstrated by water conductivity. High values of eggshell conductance, especially calculated for fertile eggs, in which the embryo develops properly, mean significant losses of water during egg incubation, which in turn leads to a smaller body weight of hatched chicks, which cannot be compensated during rearing (Wyatt et al., 1985). In the present study, it was shown that most of the water per day of incubation was reduced in eggs from the O_3 group disinfected with

Table 3. Bacteria counts and their identification on the eggshell of Japanese quail depending on disinfection method.

	Groups					
Trait	NC	PC	H_2O_2	O_3		SEM
Total number of bacteria[1]	1.21[a]	1.37[a,b]	1.44[b]	1.54[b]		0.031
Identified bacteria species[2]					Total	χ^2 (P value)
Bordetella Sp.	9.09				1.20	0.000
Corynebacterium propinquum				19.15	5.42	0.000
Corynebacterium Spp.		2.00			0.60	0.296
Kocuria kristinae	9.09		53.19		16.27	0.000
Micrococcus Spp.	22.73		27.66		10.84	0.000
Salmonella Spp.				12.77	3.61	0.000
Staphylococcus aureus	22.73	10.00	10.64		9.04	0.000
Staphylococcus sciuri				23.40	6.63	0.000
Staphylococcus Spp.		86.00	4.26	21.28	33.13	0.000
Streptococcus Spp.		2.00	2.13	19.15	6.63	0.000
Nonindentified	36.36		2.13	4.26	6.63	0.000

[a,b]Lowercase alphabets within rows (for groups) mean significant difference at $P < 0.05$.
Abbreviations: H_2O_2, hydrogen peroxide; NC, negative control; O_3, ozone; PC, positive control.
[1]– Log10 CFU/1 mL of liquid from egg.
[2]– % of total isolates.

Table 4. Hatchability traits and eggshell conductance of Japanese quail depending on disinfection method.

Traits	Treatment				
	NC	PC	H_2O_2	O_3	SEM
Fertility	88.84	83.80	84.17	84.15	2.234
Hatchability					
Set eggs	77.62	73.94	72.17	61.54	2.078
Fertile eggs	80.91^{b}	78.23^{b}	82.50^{b}	64.32^{a}	2.252
Mortality 0–14 D					
Set eggs	1.3^{a}	3.7^{a}	11.4^{b}	8.1^{b}	1.007
Fertile eggs	1.6^{a}	5.5^{b}	13.5^{c}	15.2^{b,c}	1.641
Mortality 15–17.5 D					
Set eggs	11.8^{b}	11.2^{b}	0.6^{a}	15.5^{c}	1.432
Fertile eggs	15.6^{b}	16.3^{b}	1.0^{a}	20.5^{b}	1.952
Crippled chicks (% of hatched chicks)	0.00	0.00	0.00	0.00	-
Eggshell conductance constant – mgH_2O/day/g of egg weight					
Fertile eggs (15th D)	1.39^{a}	2.51^{a}	1.78^{a}	4.27^{b}	0.462
Unfertile eggs (15th D)	2.82	2.67	2.33	2.82	0.286
Dead embryos (15th D)	2.76^{a}	5.18^{b}	2.02^{a}	2.38^{a}	0.277
Unhatched	4.16^{a,b}	4.79^{b}	2.18^{a}	2.77^{a,b}	0.324
Chicks	6.72^{b}	6.82^{b}	6.05^{a,b}	4.98^{a}	0.204

$^{a-c}$Lowercase alphabets mean differences between mean values for treatments are significant at $P < 0.05$.

Abbreviations: H_2O_2, hydrogen peroxide; NC, negative control; O_3, ozone; PC, positive control.

O_3, and the least from those which were not treated with any sanitization (NC). Formaldehyde and H_2O_2 did not differentiate the value of this parameter. Moreover, chicks hatched in the O_3 group lost most of the body weight in relation to the initial weight of eggs.

Table 5 shows the mass of eggs and the mass of hatched and reared chicks as well as their survivability in particular groups depending on the disinfection method used. The weight of eggs in all groups on the first day of incubation was similar and ranged from 9.6 to 10.4 g. However, in the body mass of day-old chicks, statistically significant differences between the groups occurred. The smallest chicks hatched from nondisinfected eggs and those disinfected with formaldehyde vapors. Significantly larger ones hatched from eggs treated with H_2O_2 and O_3. The largest difference amounted to over 13% (PC vs. O_3). The observations conducted in the subsequent rearing period seem to be significant. The first 3 groups covered by the studies are convergent in terms of body weight. In group IV, weight decreased. The difference in body weight of 14-day-old chicks between the lightest and heaviest was 11.6% (NC vs. O_3) and was statistically significant ($P < 0.05$). Confirmation of previous observations is illustrated in the proportion of newly hatched chicks in the initial egg weight depending on the method of egg disinfection. The largest share was observed in eggs disinfected with O_3, over 72% (In here too, the initial weight of the hatchling was the largest.). The lowest percentage share was found in eggs disinfected with formaldehyde vapor, 60.94%. The difference in the chick proportion in the initial egg mass between the highest and the lowest value amounted to approximately 11%.

Between the first and seventh day of rearing, the highest survival rate was obtained for chicks hatched from eggs disinfected with H_2O_2, 93.59%, while the lowest in those treated with formaldehyde fumigation, 76.14%. In the following week (between 8 and 14 D), there was

Table 5. Results of Japanese quail chicks up to 14th D of their life depending on disinfection method.

Parameter	Treatment				
	NC	PC	H_2O_2	O_3	SEM
Egg weight (g)	9.59	9.69	10.38	9.98	0.119
BW proportion in egg weight (%)	63.09^{a}	60.94^{a}	65.79^{a}	72.02^{b}	1.153
BW of 1-day-old chick (g)	6.05^{b}	5.89^{b}	6.80^{b}	7.23^{b}	0.099
BW of 7-day-old chick (g)	24.14^{b,c}	24.43^{c}	22.80^{a}	16.91^{b}	0.411
BW of 14-day-old chick (g)	45.53^{b}	44.08^{b}	44.80^{b}	40.24^{a}	0.480
Survivability of birds (%)					χ^2 (P value)
From 1–7 D of rearing	88.37	76.14	93.59	90.00	0.026
From 7–14 D of rearing	94.19	90.91	98.72	95.71	0.193
Total	82.56	67.05	92.31	85.71	0.005

$^{a-c}$Lowercase alphabets mean differences between mean values for treatments are significant at $P < 0.05$.

Abbreviations: BW, body weight; H_2O_2, hydrogen peroxide; NC, negative control; O_3, ozone; PC, positive control.

a generally higher survivability of chicks than in the previous period. Similar to the first week, the highest survivability was observed in group (H_2O_2) chicks, and the lowest in the PC group. The difference amounted to 8%. In the whole rearing period, chicks' mortality considerably depended on the group and hatching eggs' disinfection method.

DISCUSSION

When assessing the effectiveness of disinfection with reactive oxygen species, attention should be drawn to the significant microbiological cleanliness of eggs. According to literature data, the standard number of bacterial colonies on eggs may vary from 4.0 to 4.5 log CFU/egg aerobic bacteria in cage rearing system and up to almost 6.0 in aviary (De Reu et al., 2008). The total number of bacteria found in these studies in the group not subjected to disinfection is lower than that presented by Nowaczewski et al. (2013) for quail eggs disinfected with ethanol solution. Also, in other works, the presented data indicate a much stronger microbiological contamination of hatching eggs (Aygun et al., 2012; Vilela et al., 2012).

The development of *Stapyloccocus* bacteria on egg shells disinfected with formalin fumigation is noteworthy. *Staphylococcus aureus* is the most pathogenic species among *staphylococci*. It is characterized by high ability to acquire resistance to antibiotics, disinfectants, and antiseptics, and at the same time, it belongs to the class of most commonly occurring microorganisms. These properties classify it at the top of the list of microorganisms that pose a threat to human and animal health (Rosenstein and Götz, 2013). It seems that the traditional disinfection method may create preferential conditions for the development of this bacteria.

In the H_2O_2 group, the increase of *Kocuria kristinae* and *Micrococcus* Spp. was noted. These bacteria are very common on human skin and may have been "dragged." In addition, this species belong to micrococci, which do not produce toxins. As a consequence, it is considered to be nonpathogenic (Lakshmikantha et al., 2015). However, the obtained results are somewhat contradictory to the data presented by Bailey et al. (1996) who demonstrated statistically significant efficacy of both O_3 and H_2O_2 used as hatching egg disinfectants, in relation to the total number of bacteria, in particular to the *Enterobacteriaceae* and *Salmonella* genera.

The egg shell surface is the habitat of many bacterial communities interacting with each other and undergoing a dynamic change in the number of colonies (Grizard et al., 2015). The observed differences in the identified microflora present on the egg shell surface should be primarily attributed to the presence of microorganisms competing for the habitat. The native microflora of the shell surface and the interdependence among its species via the competition for nutrients, accompanied by the low activity of water, effectively inhibit the development of other microorganisms. This is due to the limited capacity of the nondisinfected shell to receive an additional number of bacterial cells. The elimination of the native microflora creates conditions for shell surface colonization by species manifesting a higher tolerance for adverse environmental conditions (Tomczyk et al., 2018). The survivability of microorganisms is strictly correlated with their ability to survive and adapt to environmental changes. Gram-positive bacteria were the dominant flora of egg shells. These bacteria can tolerate dry and harsh environmental conditions and are ubiquitous, which is the main reason for their presence on the egg shell surface (Chaemsanit et al., 2015).

Not very high rates of fertility in all groups could result from the origin of eggs which were obtained from the pure breed of birds and maintained for many generations in a closed population, which could increase the herd's inbreeding, thereby reducing the percentage of fertile eggs (Sittmann et al., 1966). As for the other parameters of hatching, Fuhrmann et al. (2010) reported that the O_3 treatment of hatching eggs significantly modifies their composition that it reduces vitamin A and E content in the egg yolk and changes its fatty acid profile, which, at high doses, can completely prevent the development of embryos. Surai et al. (2016) recognize vitamin E in egg yolk as the basic antioxidant component, while O_3 has strong oxidizing properties. These dependencies are not confirmed by other studies on the O_3 use (Hrnčár et al., 2012), in which the hatching results of "Oravka chicken" from the group disinfected with O_3 did not differ from those obtained in the group disinfected traditionally by means of the fumigation with formalin and potassium permanganate. However, the results of our own studies do not confirm the observations of the indicated authors because of the use of reactive oxygen species as a disinfecting factor contributing to the reduction of hatchability.

Sander and Wilson (1999) showed significant efficacy of H_2O_2 applied in the form of an aerosol as a disinfectant for hatching eggs. However, they indicated a significant loss of moisture (egg mass) during incubation but without the decrease of hatchability. The use of this substance did not affect the production outcome of obtained birds (body weight, feed intake, and feed conversion ratio). It did however limit the number of absorbed yolk sac in 42-day-old broilers. Chicks' body weight after hatching is considered to be the main indicator of their quality and potential of future production outcome such as final body weight or performance of the breast muscle (Molenaar et al., 2008; Petek et al., 2010). Theoretically, chicks with the highest initial body weight should maintain this advantage in relation to smaller birds during further rearing (Michalczuk et al., 2011). The relationships observed in these studies are not consistent with those presented in the available literature, while the inverse relationship is best seen in the O_3 group. Some studies explain the perinatal variability of body weight with the variation of the chicks in terms of absorbing the yolk sac (Joseph et al., 2006). However, birds with unabsorbed yolk sacs were noted in each group, possibly due to excessive humidity or weight loss during the

incubation. In addition, the results of water conductivity of the shell do not indicate this.

Japanese quail hatching eggs manifested a significantly higher microbial purity than data presented in the available literature. However, better effectiveness of disinfection with reactive oxygen species (H_2O_2, O_3) in reducing the number of bacterial colonies on the shell was not proved. In addition, the fact that both innovative methods of disinfection created preferential conditions for the development of selected species of microorganisms was not acknowledged.

A significant deterioration of the hatching results in the group disinfected with O_3, reduced hatchability, and significantly increased mortality may indicate a negative impact of this gas on the developing embryos. At the same time, O_3 contributed to a greater loss of egg mass during the incubation. The results of hatching from eggs disinfected with H_2O_2 did not differ from those obtained in the control groups.

The largest and potentially the best quality chicks were obtained from eggs disinfected with O_3. However, during the rearing, they grew significantly worse than birds in the group that was disinfected with H_2O_2 or in control groups. The highest survivability of chicks during the first 2 wk of their life was recorded among birds hatched from H_2O_2-disinfected eggs.

The use of alternative methods, in relation to the traditional formaldehyde fumigation, to disinfect hatching eggs with reactive oxygen species does not significantly contribute to the improvement of the hygienic condition of eggs, hatching results, and the quality of the hatched chicks. Alternatively, the recommended substance may be H_2O_2, which allowed to obtain satisfactory incubation results. On the other hand, O_3 appears to be an undesirable substance because of its negative impact on living organisms (birds' embryos).

REFERENCES

Al-Shammari, K. I. A., J. Batkowska, and M. Gryzińska. 2015. Assessment of ultraviolet light effect in hatching eggs disinfection on hatchability traits of two breeds of quails and chickensActa. Sci. Pol. Zootech. 14:33–44.

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.

Bailey, J. S., R. J. Buhr, N. A. Cox, and M. E. Berrang. 1996. Effect of hatching cabinet sanitation treatments on Salmonella cross - contamination and hatchability of broiler eggs. Poult. Sci. 75:191–196.

Batkowska, J., K. I. Al-Shammari, M. Gryzinska, A. Brodacki, L. Wlazlo, and B. Nowakowicz – Debek. 2017. Effect of using colloidal silver in the disinfection of hatching eggs on some microbial, hatchability and performance traits in Japanese quail (*Coturnix cot. japonica*). Europ. Poult. Sci. 81 https://doi.org/10.1399/eps.2017.211.

Batkowska, J., K. I. Al-Shammari, L. Wlazlo, B. Nowakowicz-Debek, and M. Gryzinska. 2018a. Evaluation of propolis extract as a disinfectant of Japanese quail (*Coturnix coturnix japonica*) hatching eggs. Poult. Sci. 97:2372–2377.

Batkowska, J., L. Wlazlo, K. Drabik, B. Nowakowicz-Debek, K. I. Al-Shammari, and M. Gryzinska. 2018b. Evaluation of grapefruit juice (*Citrus paradisi*) as an alternative disinfectant for hatching eggs. Pak. J. Zool. 50:647–653.

Bocci, V. A. 2006. Scientific and medical aspects of ozone therapy. State of the art. Arch. Med. Res. 37:425–435.

Chaemsanit, S., A. Akbar, and A. K. Anal. 2015. Isolation of total aerobic and pathogenic bacteria from table eggs and its contents. Food Appl. Biosci. J. 3:1–9.

Christensen, V. L., J. L. Grimes, M. J. Wineland, and L. G. Bagley. 2001. Effects of turkey breeder hen age, strain, and length of the incubation period on survival of embryos and hatchlings. J. App. Poult. Res. 10:5–15.

Copur, G., M. Arslan, M. Baylan, and S. Canogullari. 2011. Use of allicin as an alternative hatching egg disinfectant versus formaldehyde fumigation in broiler hatching eggs. Biotechnol. Equip. 25:2494–2498.

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.

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.

Fassatiova, O. 1983. Grzyby Mikroskopowe W Mikrobiologii Technicznej. Wydawnictwo Naukowo - Techniczne, Warszawa, p. 255 (in Polish).

Fuhrmann, H., R. Nadine, A. Buchner, and P. Braun. 2010. The effect of gaseous ozone treatment on egg components. J. Sci. Food Agr. 90:593–598.

Gentry, R. F., and C. L. Quarles. 1972. The measurement of bacterial contamination on eggshells. Poult. Sci. 51:930–933.

Grizard, S., M. A. Versteegh, H. K. Ndithia, J. F. Salles, and B. I. Tieleman. 2015. Shifts in bacterial communities of eggshells and antimicrobial activities in eggs during incubation in a ground-nesting passerine. PLoS One 10:e0121716.

Hrnčár, C., S. Prachárová, and J. Bujko. 2012. The effect of disinfection of hatching eggs on hatchability of Oravka chickens. Sci. Pap. Anim. Sci. Biotechnol. 45:411–414.

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

Jones, D. R., K. E. Anderson, P. A. Curtis, and F. T. Jones. 2002. Microbial contamination in inoculated shell eggs, I. Effects of layer strain and hen age. Poult. Sci. 81:715–720.

Jones, D. R., M. T. Musgrove, and J. K. Northcutt. 2004. Variations in external and internal microbial populations in shell eggs during extended storage. J. Food Prot. 67:2657–2660.

Joseph, N. S., A. Lourens, and E. T. Moran. 2006. The effects of suboptimal eggshell temperature during incubation on broiler chick quality. Poult. Sci. 85:932–938.

Lakshmikantha, M., V. Devki, and C. Yogesh. 2015. Is *Kocuriakristinae* an upcoming pathogen? Int. J. Curr. Microbiol. App. Sci. 4:885–889.

Ledoux, L. 2002. Hatching egg sanitation beyond the myths. World Poult. J. 18:34–35.

Linley, E., S. P. Denyer, G. McDonnell, C. Simons, and J. Y. Maillard. 2012. Use of hydrogen peroxide as a biocide, new consideration of its mechanisms of biocidal action. J. Antimicrob. Chemother. 67:1589–1596.

Macauley, J. J., A. Quiang, C. D. Adams, R. Surampalli, and M. R. Mormile. 2006. Disinfection of swine wastewater using chlorine, ultraviolet light and ozone. Water Res. 40:2017–2026.

Mayes, F. J., and M. A. Takeballi. 1983. Microbial contamination of the hen's egg: a review. J. Food Prot. 46:1092–1098.

Michalczuk, M., M. Stępińska, and M. Łukasiewicz. 2011. Effect of the initial body weight of Ross 308 chicken broilers on the rate of growth. Ann. Warsaw Univ. Life Sci. – SGGW. Anim. Sci. 49:121–125.

Molenaar, R., I. A. M. Reijrink, R. Meijerhof, and H. Van Den Brand. 2008. Relationship between hatchling length and weight on later productive performance in broilers. World Poult. Sci. J. 64:599–604.

Musgrove, M. T., J. K. Northcutt, D. R. Jones, N. A. Cox, and M. A. Harrison. 2008. Enterobacteriaceae and related organisms isolated from shell eggs collected during commercial processing. Poult. Sci. 87:1211–1218.

Nowaczewski, S., T. Szablewski, R. Cegielska-Radziejewska, and H. Kontecka. 2013. Microbiological response of Japanese quail eggs to disinfection and location in the setter during incubation. Folia Biol. (Krakow) 61:119–124.

Özcelik, B. 2007. Fungi/bactericidal and static effects of ultraviolet light in 254 and 354 nm wavelengths. Res. J. Microbiol. 2:42–49.

Petek, M., A. Orman, S. Dikmen, and F. Alpay. 2010. Physical chick parameters and effects on growth performance in broiler. Arch. Tierz. 53:108–115.

ISO 4833-1:2013. 2013. Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 degrees C by the pour plate technique. International Organization For Standardization, Geneva, Switzerland, pp. 1–9.

ISO 21528-1:2017. 2017. Microbiology of the food chain — Horizontal method for the detection and enumeration of Enterobacteriaceae — Part 1: Detection of Enterobacteriaceae. International Organization For Standardization, Geneva, Switzerland, pp. 1–17.

Rosenstein, R., and F. Götz. 2013. What distinguishes highly pathogenic *Staphylococci* from medium and non-pathogenic. Curr. Top. Microbiol. Immunol. 358:33–89.

Sander, J. E., and J. L. Wilson. 1999. Effect of hydrogen peroxide disinfection during incubation of chicken eggs on microbial levels and productivity. Avian Dis. 43:227–233.

Sittmann, K., H. Abplanalp, and R. A. Fraser. 1966. Inbreeding depression in Japanese quail. Genetics 54:371–397.

Stahl –Biskup, E., and I. Laakso. 1990. Essential oil polymorphism in finnish thymus species. Planta Med. 56:464–468.

Stokeer, G. 1916. The surgical uses of ozone. Lancet 2:611–614.

Surai, P. F., V. I. Fisinin, and F. Karadas. 2016. Antioxidant systems in chick embryo development. Part 1. Vitamin E, carotenoids and selenium. Anim. Nutr. 2:1–11.

Tomczyk, Ł., Ł. Stępień, M. Urbaniak, T. Szablewski, R. Cegielska-Radziejewska, and K. Stuper-Szablewska. 2018. Characterisation of the mycobiota on the shell surface of table eggs acquired from different egg-laying hen breeding systems. Toxins 10, 293. https://doi.org/10.3390/toxins10070293.

Ulucay, I. O., and I. Yildirim. 2010. Hatching traits of quail (*Coturnixcoturnix japonica*) eggs disinfected with carvacrol, cinnamaldehyde or thymol. J. Appl. Anim. Res. 38:139–142.

Vilela, C. O., G. D. Vargas, G. Fischer, S. Ladeira, R. O. de Faria, C. F. Nunes, M. de Lima, S. O. Hübner, P. Luz, L. G. Osório, and M. A. Anciuti. 2012. Propolis, a natural product as an alternative for disinfection of embryonated eggs for incubation. Arq. Inst. Biol. 79:161–167.

Watanabe, T. 2002. Pictorial Atlas of Soil and Seed Fungi, Morphologies of Species, 2nd ed. CRC Press, Boca Raton, Florida.

Wyatt, C. L., W.D. Weaver, Jr, and W. L. Beane. 1985. Influence of egg size, eggshell quality, and posthatch holding time on broiler performance. Poult. Sci. 64:2049–2055.

Yildirim, I., M. Ozsan, and 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*). Rev. Med. Vet. 154:367–370.