Scientific Annals of Polish Society of Animal Production - Vol. 13 (2017), No 2, 25-35

Assessment of the effect of selected substances used for disinfection of hatching eggs on hatching results in chickens

Klaudia Korowiecka, Magdalena Trela, Barbara Tombarkiewicz, Krzysztof Pawlak, Jerzy W. Niedziółka, Magdalena Swadźba, Marcin W. Lis#

University of Agriculture in Krakow, Faculty of Animal Sciences, Institute of Veterinary Science, Department of Veterinary Science, Animal Reproduction and Animal Welfare, al. Mickiewicza 24/28, 30-059 Kraków; #e-mail: rzlis@cyf-kr.edu.pl

The aim of the study was to investigate whether egg disinfectants have a toxic effect on the tissues of the developing chicken embryo. The basic active ingredients of the disinfectants tested were quaternary ammonium compounds (Amino-Steril); stabilized peracetic acid and hydrogen peroxide (Oxydion); glutaraldehyde, didecyldimethylammonium chloride, quaternary ammonium compounds and benzyl-C12-C16-alkyldimethyl (Viron FF); and stabilized hydrogen peroxide (Hydro-Clean). The tests were performed on hatching eggs from Ross 308 parent stock. The potential adverse effects of aqueous solutions of the disinfectants were tested in vitro using the Hen's Egg Test – Chorioallantoic Membrane (HET-CAM). The results were confirmed in in vivo tests by analysing the hatchability of disinfected eggs. In the in vitro tests, aqueous solutions of the disinfectants with concentrations of 1%, 0.5%, 0.25% and 0.125% were spotted onto previously prepared chorioallantoic membranes of live eight-day-old chicken embryos (n = 8 embryos/disinfectant/concentration). The toxicity of the substances was assessed on the basis of the occurrence of hyperaemia, haemorrhage, and coagulation of the blood vessels of the membrane after 0.5, 2 and 5 minutes, using the 21-point Luepke scale. The in vivo testing consisted of two experiments conducted under production conditions, using eggs from flocks in the peak (37th week of life) and the final (54th week) laying periods. The eggs were sprayed with a 1% aqueous solution of disinfectant (400 eggs/disinfectant/experiment) about 2 hours before incubation. Hatching results, the stage of embryonic development at the time of death and any cases of infection were evaluated. The HET-CAM tests showed that the 1% solutions of the disinfectants induced strong (Hydro-Clean), moderate (Oxydion and Amino-Steril) and weak (Viron FF) reactions, while the 0.125% concentration produced a weak reaction or none. Analysis of hatching results showed that they were not affected by the disinfectants. However, in the case of laying hens in

their final production period, spraying with aqueous solutions of each agent reduced losses due to early embryo mortality. In conclusion, the disinfectants tested can be safely used in poultry hatcheries.

KEY WORDS: Hen's Egg Test – Chorioallantoic Membrane / disinfection / hatching eggs / hatchability

The task of the poultry hatchery is to provide the manufacturer with healthy chicks of full value. This depends both on the quality of the reproductive stock, i.e. its genetic value, health status, and nutritional status, and on housing conditions, handling of hatching eggs before setting, and the incubation technology itself. At each of these stages, however, strict adherence to the principles of biosafety is of key importance [5]. It is commonly believed that the microorganisms found on the shell, *Salmonella enteritidis*, *Escherichia coli*, *Enterobacter*, and also moulds and yeasts [1], may be a factor significantly reducing hatching capacity and may also cause deaths in chicks in the first few days after hatching [3]. A treatment effectively preventing the development of microorganisms on the shell is disinfection of the eggs. It is recommended that hatching eggs should be disinfected twice: first immediately after egg collection and then before incubation. The most common methods are fogging with formalin vapour, ultraviolet irradiation and spraying with a disinfectant [6].

A good disinfectant should be effective against micro-organisms, resistant to adverse environmental conditions and relatively inexpensive. It should also be odourless, easily biodegradable and non-toxic to living organisms [14].

Formaldehyde, which is commonly used for disinfection of eggs, is highly toxic, an irritant, and carcinogenic; it is also slowly biodegradable and therefore harmful to the natural environment [16, 20]. For this reason, it is increasingly being replaced in poultry production by disinfectants based on chemical compounds such as hydrogen peroxide, ammonium compounds, peracetic acid and aldehydes (excluding formaldehyde). It should be noted, however, that it has never been conclusively demonstrated whether preparations based on these compounds are completely safe for the bird embryo.

One of the methods used to evaluate the irritant effect of chemical substances, as alternatives to the Draize test, is the Hen's Egg Test – Chorioallantoic Membrane (HET CAM) developed by Luepke [10]. The model of the chorioallantoic membrane of the chicken egg is successfully used to study biological processes such as gas exchange and substance transport, as well as to assess the toxicity of chemical substances and the virulence of microbes [18].

It therefore seemed interesting to evaluate the potential adverse effects of four disinfectants in vitro using the Hen's Egg Test – Chorioallantoic Membrane and to verify these results in vivo based on analysis of hatching results.

Material and methods

The main active ingredients in the disinfectants used in the study are as follows:

– quaternary ammonium compounds (Amino-Steril)

– stabilized peracetic acid and hydrogen peroxide (Oxydion)

– glutaraldehyde, didecyldimethylammonium chloride, quaternary ammonium compounds and benzyl-C12-C16-alkyldimethyl (Viron FF)

– stabilized hydrogen peroxide (Hydro-Clean)

All disinfectants were manufactured by DDD-1 (Poland).

The potential harmful effects of the disinfectants were assessed in two stages: in vitro testing by HET-CAM and in vivo.

Hatching eggs from parent stock of Ross 308 meat chickens were incubated in Masalles 65 incubators at a temperature (T) of 37.8°C and 50% relative humidity (RH) for 8 days. Then the eggs were candled, and unfertilized and damaged eggs and those containing dead embryos were discarded. In the remaining eggs an opening was made above the air chamber, with a diameter of about 20 mm, through which the internal egg shell membrane (IESM) was moistened with 0.9% NaCl and placed in the incubator for 20 minutes. Then the eggs were removed from the incubator, the remaining physiological fluid was pipetted, and the IESM was removed, exposing the chorioallantoic membrane (CAM). Only embryos with intact chorioallantoic membranes were used in the test.

A 200 µl volume of an aqueous solution of the test product was spotted on the chorioallantoic membrane (CAM) prepared in this manner. Solutions of Amino-Steril, Oxydion, Hydro-Clean and Viron FF were analysed at concentrations of 1%, 0.5%, 0.25% and 0.125% (n = 8 embryos/disinfectant/concentration). A 21-point scale was used in the toxicity assessment (0-0.9 points - no reaction, 1.0-4.9 - weak reaction, 5.0-8.9 - moderate reaction, 9.0-21 points - strong reaction) based on the degree of hyperaemia, haemorrhage and coagulation of the blood vessels of the membrane after 0.5, 2 and 5 minutes [10]. The observations were made at room temperature using a magnifying glass.

In the in vivo evaluation we used hatching eggs from Ross 308 (Aviagen®) meat chickens from the same breeding farm, kept in a litter system. Two experiments were conducted, using eggs from flocks in different laying stages:

– peak period (38th week of life), egg weight of 62.1 ±3.42 g (mean ±SD)

– final period (54th week of life), egg weight of 70.6 ±7.38 g

Before setting the eggs were stored for 72 h in an egg storage room (T=18 ±1°C, RH=70 ±2%). In order not to interfere with the results of the experiment, the eggs for the experiment were not disinfected immediately after collection.

About 2 hours prior to incubation, the hatching eggs were divided into 6 experimental groups composed of 8 hatching trays with a capacity of 50 eggs (n = 8/group, 8 trays × 50 eggs = 400 eggs/group). Using a hand sprayer, a large drop of one of the following was

applied to the surface of the egg shells (about 20 ml/egg): pure water (blank sample) or a 1% aqueous solution of Amino-Steril, Oxydion, Viron FF, or Hydro-Clean. Eggs from the control group were not sprayed. The temperature of the water and solutions was about 20°C. The egg trays were left to dry for about 30 minutes and then placed randomly on one egg setter. The same procedure was used in each replicate of the experiment.

The eggs were incubated under production conditions in Reform-Poldrob multi-level incubators at 37.8°C and RH 51% (days E1-E18). After being transferred to a Jartom hatching compartment, the microclimatic parameters were varied according to the hatching stage (the temperature was gradually reduced from the initial 37.8°C and RH 55% to 37.2°C and RH 60% during the internal pipping phase, 37.0°C and RH 70% when the chick was leaving the shell, to 36.5°C and RH 60% while the chicks dried off).

All eggs culled during candling on the 10th day of incubation (E10) and those remaining after hatching in the hatching baskets underwent pathological embryo analysis, during which fertilization was confirmed and the developmental stage of the embryo was determined according to the key proposed by Borzemska [2]. In addition, any infections, faulty positioning of the embryo in the egg, or developmental deformities were noted.

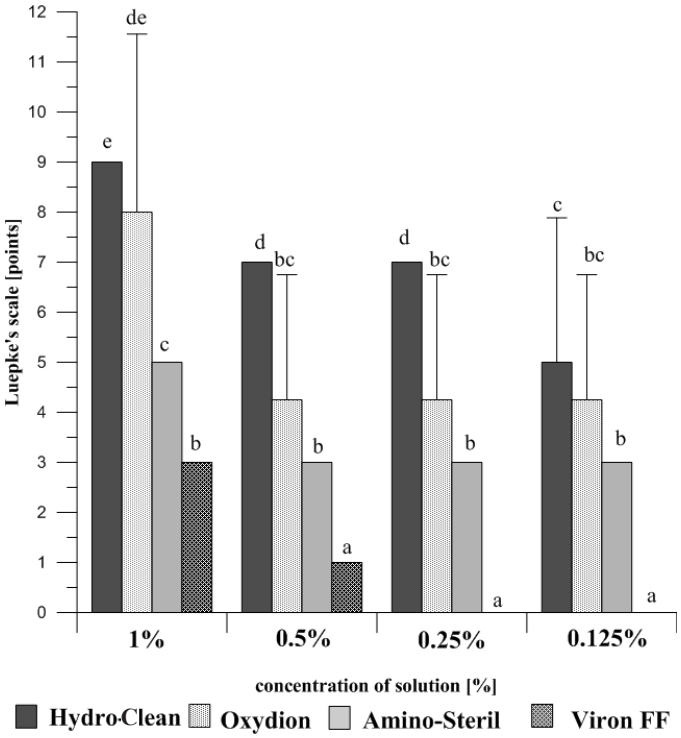
The HET-CAM results were analysed by two-way analysis of variance, taking into account the type and concentration of disinfectant, and differences between groups were determined by the Holm-Sidak test.

The effects of spraying with a given disinfectant on hatching results were compared to the number of eggs actually fertilized and analysed by one-way analysis of variance, with each group comprising 8 trays (n=8). The differences between groups were determined by the Tukey test, or in the absence of normal distribution, by the Holm-Sidak test. Statistical calculations were performed using SigmaStat 3.5 software (USA).

Results and discussion

The HET-CAM test revealed significant differences in the toxicity of the disinfectants (Figure 1). The toxicity of aqueous 1% solutions (recommended for disinfection by the manufacturer) was strong in the case of Hydro-Clean (9 pts), moderate for Oxydion and Amino-Steril (8 and 5 pts, respectively) and weak for Viron FF (3 pts), according to the Luepke scale [10]. As the concentration of the active substance was reduced, its toxicity decreased ($P \leq 0.05$). No reaction was noted at the 0.25% concentration for Viron FF, while a moderate reaction was observed for the other disinfectants even at a concentration of 0.125% (Figure 1).

Bearing in mind the objectivity of the analysis and interpretation of the results obtained in successive in vivo experiments, it should be noted that the fertilization rate of the hatching eggs from the flock in the peak laying period was 97.1 ± 2.29% (mean ± SD),

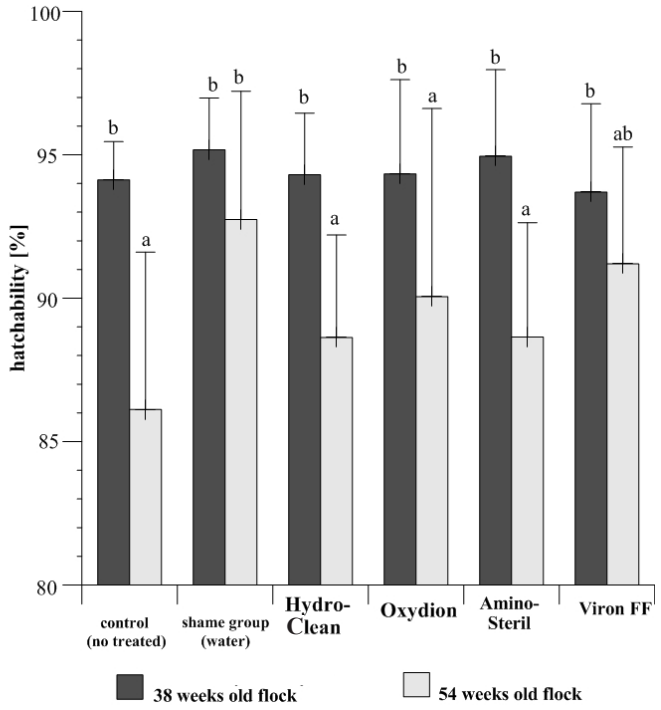


a, b – values marked with different letters differ significantly (P≤0.05)

Fig. 1. Toxicity assessment by Hen's Egg Test – Chorioallantoic Membrane of 1%, 0.5%, 0.25% and 0.125% aqueous solutions of disinfectants: Amino-Steril, Oxydion, Hydro-Clean and Viron FF (n = 8 embryos/disinfectant/concentration). Results presented in Luepke's 21-point scale (0-0.9 points – no reaction, 1.0-4.9 points – weak reaction, 5.0-8.9 points – moderate reaction, 9-21 points – strong reaction) based on the degree of hyperaemia, haemorrhage and coagulation of the blood vessels of the membrane after 0.5, 2 and 5 minutes

which was 12.7 percentage points (pp) higher than for the eggs from the final production period (P≤0.05). However, the detailed pathological analysis of the embryos revealed that the biological value of the eggs (proportion of fertilized eggs) was similar in the two groups (P>0.05, Tables 1 and 2) and did not skew the evaluation of the primary experimental factor (disinfectant).

Hatching rates for fertilized eggs from the flock in the peak laying period was higher than for the flock at the final stage of production. In the control group, the difference was 8.6 pp (P≤0.05), while in the experimental groups it ranged from 1.8 pp (group disinfected with Viron FF; P>0.05) to 5.8 pp (group disinfected with Amino-Steril P≤0.05) (Fig. 2, Tables 1 and 2). At the same time, the final hatching results of eggs from the flock at 54 weeks of age treated with aqueous solutions of the disinfectants were from 2.5 pp (Amino-Steril and Hydro Clean) to 6.6 pp (pure water) higher than in the untreated group (Table 2). This was due to the reduction in losses resulting from early embryo mortality in the groups sprayed with water or with aqueous solutions of Amino-Steril, Viron FF, Hydro-Clean and Oxydion, as compared to the control group: differences of 3.1, 1.8, 2.1, 1.8 and 2.3-times, respectively (P≤0.05, Table 2). This effect was not observed in the case of spraying of hatching eggs from the flock in the peak laying period (P>0.05, Table 1).



a, b – values marked with different letters differ significantly (P≤0.05)

Fig. 2. Hatchability of fertilized eggs from parent stock of Ross 308 broiler breeders at the age of 38 and 54 weeks depending on the disinfectant used

Table 1

Results of pathological embryo analyses of eggs laid by 38-week-old hens from the parent stock of Ross 308 broiler breeders, disinfected before incubation by spraying with a 1% solution of Amino-Steril, Viron FF, Hydro-Clean or Oxydion

Specification		Control (not desinfected)		Blank (sprayed with water)		Amino-Steril		Viron FF		Hydro-Clean		Oxydion	
		mean	±SD	mean	±SD	mean	±SD	mean	±SD	mean	±SD	mean	±SD
Set eggs	(number of eggs)	50.0	±0.00	50.0	±0.00	50.0	±0.00	50.0	±0.00	50.0	±0.00	50.0	±0.00
Cracked eggs		0.5	±0.71	1.0	±0.00	0.5	±0.71	0.0	±0.00	0.8	±0.75	1.0	±0.00
Unfertilized eggs		0.9	±0.83	0.8	±0.71	0.6	±0.74	0.5	±0.93	1.3	±1.28	0.9	±0.83
Fertilized eggs		**48.5**	**±0.53**	**48.8**	**±1.04**	**49.0**	**±0.53**	**48.9**	**±1.25**	**47.9**	**±1.73**	**48.5**	**±1.41**
Dead embryos	beetwen E1-E6	2.3	±1.72	2.8	±2.16	1.5	±1.79	3.0	±2.40	2.1	±1.51	3.1	±3.52
	beetwen E7-E17	0.3	±0.71	0.5	±0.94	1.0	±1.07	1.3	±1.49	1.3	±1.54	0.8	±1.07
	beetwen E18-E21	1.5	±1.81	1.5	±1.78	2.3	±1.72	1.3	±2.38	2.6	±1.86	1.6	±1.86
	Total	**4.1**	**±2.17**	**4.8**	**±1.81**	**4.8**	**±1.86**	**5.5**	**±3.33**	**5.9**	**±2.50**	**5.4**	**±3.58**
Malformations	(percentage from ferilized eggs)	0.3	±0.71	0.0	±0.00	0.3	±0.71	0.3	±0.71	1.1	±2.32	0.5	±0.93
Conaminated eggs		1.0	±1.08	0.5	±0.94	0.5	±0.93	1.3	±2.38	0.5	±1.47	0.5	±0.97
Hatched chicks		**94.7**	**±2.12**	**95.2**	**±1.81**	**94.4**	**±2.56**	**93.0**	**±3.84**	**92.5**	**±3.02**	**93.5**	**±4.00**

Table 2

Results of pathological embryo analyses of eggs laid by 54-week-old hens from the parent stock of Ross 308 broiler breeders, disinfected before incubation by spraying with a 1% solution of Amino-Steril, Viron FF, Hydro-Clean or Oxydion

Specification		Control (not desinfected)		Blank (sprayed with water)		Amino-Steril		Viron FF		Hydro-Clean		Oxydion	
		mean	±SD	mean	±SD	mean	±SD	mean	±SD	mean	±SD	mean	±SD
Set eggs	(number of eggs)	50.0	±0.00	50.0	±0.00	50.0	±0.00	50.0	±0.00	50.0	±0.00	50.0	±0.00
Cracked eggs		0.2	±0.41	0.3	±0.46	0.1	±0.35	0.3	±0.46	0.0	±0.00	0.4	±0.52
Unfertilized eggs		7.7	±1.75	6.6	±1.30	6.6	±2.56	7.1	±3.04	8.3	±1.98	6.9	±1.13
Fertilized eggs		**41.7**	**±1.86**	**43.0**	**±1.51**	**42.5**	**±2.33**	**42.3**	**±3.06**	**41.4**	**±2.07**	**42.4**	**±1.19**
Dead embryos	beetwen E1-E6	8.0	±4.92[b]	2.6	±1.45[a]	4.4	±2.05[a]	3.9	±2.13[a]	4.5	±2.93[a]	3.5	±4.00[a]
	beetwen E7-E17	1.2	±1.95	1.7	±2.69	1.8	±2.42	1.4	±2.08	2.1	±1.48	1.4	±1.75
	beetwen E18-E21	2.8	±4.51	2.1	±1.99	2.6	±1.95	2.3	±1.72	2.4	±2.21	4.1	±2.10
	Total	**12.0**	**±7.23[b]**	**6.4**	**±4.00[a]**	**8.8**	**±3.54[ab]**	**7.7**	**±3.79[ab]**	**9.0**	**±2.81**	**9.1**	**±6.54[ab]**
Malformations	(percentage from ferilized eggs)	0.8	±1.20	0.9	±1.22	0.9	±1.18	0.3	±0.75	1.5	±1.22	0.0	±0.00
Conaminated eggs		1.1	±1.91	0.3	±0.80	1.7	±1.57	0.9	±1.18	0.9	±1.25	0.9	±1.21
Hatched chicks		**86.1**	**±5.49**	**92.7**	**±4.47**	**88.6**	**±3.99**	**91.2**	**±4.08**	**88.6**	**±3.58**	**90.1**	**±6.56**

a, b – values in rows marked with different letters differ significantly ($P \leq 0.05$)

The disinfectants did not affect the frequency of developmental deformities (P>0.05). However, in the eggs from the flock at the end of the production period, developmental defects occurred 2.4 times more often than at peak laying (Tables 1 and 2).

The analyses showed no effect of the disinfectant or the reproductive stage on the frequency of changes in the egg contents due to bacteria or fungi. Such changes were noted in 0.5-1.3% of eggs (P≥0.05, Tables 1 and 2).

The results show that the sensitivity of the embryos to the disinfectant varies depending on the age of the flock. This phenomenon may indicate a decline in the biological value of the egg as the flock 'ages' [7]. In the experiment, in the flock at the peak of the laying period the disinfectants had no significant effect on hatchability. In the flock at the end of the production cycle, in the group of eggs sprayed with pure water or aqueous solutions of Amino-Steril, Viron FF, Hydro-Clean and Oxydion, the hatching rate was 2.5-6.6 pp higher than in the group of untreated eggs. This was primarily due to the reduced embryo mortality between days E1 and E6 (the first peak in mortality). A similar phenomenon has been observed by other authors [8, 11, 17], but they did not attempt to explain it. Particularly surprising is the a significant improvement in the hatching results of eggs sprayed with pure water, as contact with water is thought to be conducive to the development of pathogens on the shell and their penetration through the shell membranes [4].

The high hatchability of the chicks in the experimental groups demonstrates that the disinfectants were not toxic to the embryos, and the low frequency of developmental defects also rules out teratogenic effects. This is confirmed by the HET-CAM results. The 1% aqueous solutions of the disinfectants were found to have weak (Viron FF) or moderate (Amino-Steril and Oxydion) toxicity. Only Hydro-Clean had a strong irritant effect on the blood vessels of the chorioallantoic membrane. This is probably due to the strong haemolytic effect of hydrogen peroxide, which is the main active ingredient of this disinfectant [12, 19].

Pathological embryo analysis showed no differences between groups in the number of eggs whose contents indicated infection with pathogenic microbes. This suggests that the isolated infections resulting in the death of some embryos were the result of transovarial (vertical) infections, due to the health status of the laying hen [9, 13]. These infections, unlike horizontal infections, cannot be linked to the effectiveness of the disinfectants. It should also be noted that when prophylactic principles are complied with on the farm and at the hatchery, cases of horizontal infection in the incubator occur only sporadically [4, 15].

The results obtained indicate that disinfectants based on hydrogen peroxide (Hydro--Clean), ammonium compounds (Amino-Steril), peracetic acid (Oxydion) and aldehydes (Viron FF) are safe for embryos.

REFERENCES

1. AL-SHAMMARI K.I.A., BATKOWSKA J., GRYZIŃSKA M.M., 2015 – Assessment of ultraviolet light effect in hatching eggs disinfection on hatchability traits of two breeds of quails and chicken. ***Acta Scientiarum Polonorum Zootechnica*** 14, 33-44.
2. BORZEMSKA W.B., 1978 – Vademecum chorób drobiu. Państwowe Wydawnictwo Rolnicze i Leśne, Warszawa, 145.
3. COUFAL C.D., CHAVEZ C., KNAPE K.D., CAREY J.B., 2003 – Evaluation of a method of ultraviolet light sanitation of broiler hatching eggs**.** ***Poultry Science*** 82, 754-759.
4. COX N.A., BERRANG M.E., CASON J.A., 2000 – Salmonella penetration of egg shells and proliferation in broiler hatching eggs. ***Poultry Science*** 79, 1571-1574.
5. DROZD L., GONDEK M., SZKUCIK K., KNAGA S., ZIOMEK M., 2015 – Zanieczyszczenie mikrobiologiczne powierzchni jaj przepiórczych. ***Medycyna Weterynaryjna*** 71, 303-306.
6. DURMUS I., 2012 – Determining effects of use of various disinfecting materials on hatching results and total bacterial count. ***Asian Journal of Animal and Veterinary Advances*** 7, 739-744.
7. ELIBOL O., PEAK S.D., BRAKE J., 2002 – Effect of flock age, length of egg storage, and frequency of turning during storage on hatchability of broiler hatching eggs. ***Poultry Science*** 81, 945-950.
8. FASENKO G.M., O'DEA CHRISTOPHER E.E., MCMULLEN L.M. 2009 – Spraying hatching eggs with electrolyzed oxidizing water reduces eggshell microbial load without compromising broiler production parameters. ***Poultry Science*** 88, 1121-1127.
9. KĘDZIA R., LIS M.W., 2013 – Przyczyny śmiertelności piskląt brojlerów w początkowym okresie odchowu. ***Polskie Drobiarstwo*** 3, 2-7.
10. LUEPKE N.P., 1985 – Hen's egg chorioallantoic membrane test for irritation potential. ***Food and Chemical Toxicology*** 23, 287-291.
11. MAHARJAN P., COX S., GADDE U., CLARK F. D., BRAMWELL K., WATKINS S.E., 2017 – Evaluation of chlorine dioxide based product as a hatchery sanitizer. ***Poultry Science*** 96, 560-565.
12. MIZAK L., 2005 – Sporobójcza aktywność nadtlenku wodoru i kwasu nadoctowego wobec przetrwalników *Bacillus anthracis*. ***Medycyna Doświadczalna i Mikrobiologia*** 57, 437-422.
13. NEWELL D.G., FEARNLEY C., 2003 – Sources of campylobacter colonization in broiler chickens. ***Applied and Environmental Microbiology*** 69, 4343-4351.
14. OLESIAK P., STĘPNIAK L., 2012 – Skuteczność wybranych związków dezynfekcyjnych wobec przetrwalników Bacillus. ***Inżynieria i Ochrona Środowiska*** 15, 141-150.
15. PIJARSKA I., 2007 – Wpływ wybranych czynników zakaźnych na lęgi jaj kurzych. ***Polskie Drobiarstwo*** 12, 11-14.
16. RHOMBERG L.R., 2015 – Contrasting directions and directives on hazard identification for formaldehyde carcinogenicity. ***Regulatory Toxicology and Pharmacology*** 73, 829-833.
17. SHELDON B.W., BRAKE J., 1990 – Hydrogene peroxide as an alternative hatching egg disinfectant. ***Poultry Science*** 70, 1092-1098.
18. WALDES T.I., KREUTZER D., MOUSSY F., 2002 – The chick chorioallantoic membrane as a novel in vivo model for the testing of biomaterials. ***Journal of Biomedical Materials Research*** 62, 273-282.

19. WATT B.E., PROUDFOOT A.T., VALE J.A., 2004 – Hydrogen peroxide poisoning. ***Toxicological Reviews*** 23, 51-57.
20. WHISTLER P.E., SHELDON BW., 1989 – Bactericidal activity, eggshell conductance, and hatchability effects of ozone versus formaldehyde disinfection. ***Poultry Science*** 68, 1074-1077.