

Disinfection of Hatching Eggs With Chemical and Natural Agents: Effects on Hatchability and sex Ratio in Laying Hens

Key words

hatching eggs;
hydrogen peroxide;
Ecocid® S;
embryonic mortality;
chick sex ratio

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Abstract: In this study, the effects of six disinfectants (Ecocid® S, ethanol, formaldehyde, propolis, salt and hydrogen peroxide) and distilled water (as a negative control) applied to hatching eggs on hatchability, embryonic development, chick quality and the sex ratio of the resulting chicks were investigated. In addition, an untreated control group was included to establish baseline values without any application. Hydrogen peroxide achieved the highest hatchability (79.4% of eggs set; 85.7% of eggs fertilised), followed by formaldehyde, Ecocid® S (Krka, d.d. Novo mesto, Slovenia) and ethanol. Compared to these four treatments, which formed a statistically homogeneous group ($p > 0.05$), the groups with salt and propolis had a significantly lower hatchability ($p < 0.05$). Propolis significantly reduced the hatchability (16.2 %; $p < 0.05$) and egg weight loss (7.6 %; $p < 0.05$), probably due to pore blockage and impaired gas exchange. A significantly higher ratio of early to late dead embryos (ED/LD) was observed in the propolis group (0.49) compared to all other experimental groups (0.05–0.24; $p < 0.05$). A significant effect on the sex ratio was found ($p < 0.05$): the propolis and distilled water groups showed a male-biased ratio (1.20), while the salt group showed a female-biased trend (0.78). Although no significant differences were found among three concentrations of Ecocid® S (0.5%, 1.0%, 2.0%) and hydrogen peroxide (3.0%, 5.0%, 7.0%) in terms of hatchability or sex ratio ($p > 0.05$), the control group had a significantly higher ED/LD ratio (1.26) than all Ecocid® S and hydrogen peroxide treated groups (0.11–0.39; $p < 0.05$). The study confirms the efficacy and safety of hydrogen peroxide and Ecocid® S and emphasises the importance of considering sex ratio effects when evaluating the embryotoxicity of disinfectants in hatching poultry.

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Introduction

Effective hygiene protocols in commercial hatcheries are crucial to ensure optimal hatching performance. Hatching eggs are particularly susceptible to microbial contamination from both the hen's reproductive tract and environmental sources such as contaminated litter, faeces and handling equipment (1). Despite natural protective barriers such as the cuticle and shell membranes, pathogenic microorganisms could enter the egg shell, leading to increased embryonic mortality and lower chick quality (2). To minimise these risks, the hatching eggs are routinely disinfected before incubation. Formaldehyde fumigation is still the industry standard in large hatcheries as it has a broad spectrum

of antimicrobial effects and is convenient. However, formaldehyde is a known carcinogen and respiratory irritant that jeopardises the health of hatchery personnel and raises environmental safety concerns (3, 4). These concerns have led to the search for safer and environmentally friendly alternatives such as hydrogen peroxide, which has been shown to effectively reduce microbial load without compromising hatchability and degrades to harmless by-products (5, 6). Natural substances such as propolis have also attracted attention due to their antimicrobial properties; however, their effects on embryonic development and viability are still insufficiently studied (7, 8). The aim

of the study was to investigate the effects of chemical and natural disinfectants – hydrogen peroxide, Ecocid® S, ethanol, salt and propolis – on important hatching parameters in laying hens. Particular attention was paid to the possible influence of their treatments on the sex ratio of hatched chicks, a parameter that has been largely ignored in previous disinfection research. The study contributes to the ongoing development of safer, formaldehyde-free disinfection protocols for use in commercial hatcheries.

Materials and methods

Ethical statement

All trials were conducted in a registered hatchery of the Department of Animal Science, Biotechnical Faculty, University of Ljubljana, according to standard procedures based on best practices in commercial hatcheries. According to Directive 2010/63/EU on the protection of animals used for scientific purposes (European Parliament and Council of the European Union, 2010), research carried out as part of a normal production cycle is classified as non-experimental agricultural practices (Chapter I, Article 1, point 5) and therefore does not require ethics committee approval. The procedures used in this study have not gone beyond standard commercial practices in commercial hatcheries and have not caused additional pain, suffering or lasting harm to the animals.

Structure of the study

The study was conducted in two phases, Trial A and Trial B. Trial A served as an initial screening of different disinfectants, while trial B focussed on the dose–response of the two most promising agents. In trial A, 3.600 hatching eggs were divided into eight treatment groups: 3% hydrogen peroxide (H₂O₂), 0.5% Ecocid® S (active ingredient potassium peroxymonosulphate - KHSO₅), 96% ethanol (C₂H₆O), 10% salt (NaCl), 7% ethanolic propolis solution, formaldehyde (CH₂O - standard fumigation), distilled water and an untreated control group. Each group consisted of 450 eggs, divided into three replicates of 150 eggs in each. Based on the results of trial A, three concentrations of hydrogen peroxide (3%, 5%, 7%) and Ecocid® S (0.5%, 1%, 2%) were tested in trial B on a new batch of 3.150 eggs, with the group structure and replicates remaining unchanged.

Selection of disinfectants, disinfection procedure and application method

Disinfectants were selected based on practical and biological criteria relevant to commercial hatchery conditions, including antimicrobial efficacy, availability, health risk to personnel, minimal corrosiveness to hatchery equipment and affordability. Hydrogen peroxide and Ecocid® S were selected for their strong antimicrobial activity and low toxicity. Ethanol, salt and propolis were used as natural antimicrobial agents. Formaldehyde served as a conventional reference and distilled water was used as a negative control. In order to determine reference values under untreated conditions, a control group not exposed to any disinfectant was

included in the experimental design. The day before incubation, the eggs were acclimatised at 24.0°C. Disinfection was then carried out with solutions preheated to 37.8°C to prevent the penetration of disinfectants into the eggs. Disinfection was carried out immediately before incubation. Formaldehyde was applied by fumigation with 2 ml formalin and 1 g KMnO₄ per m³ for 20 minutes. All other treatments were carried out with a hand sprayer to ensure complete surface coverage while minimising handling and possible damage to the shell.

Treatment of hatching eggs

In both trials, the eggs came from parent flocks of Prelux-G hybrids, a Slovenian layer cross. In trial A, eggs were collected over 12 days from a 60-week-old flock and in trial B over 6 days from a 38week-old flock. Only clean, uniformly sized eggs with intact shells were selected. The eggs were stored at 12°C and 75% relative humidity and turned daily to maintain the viability of the embryos. To exclude possible age-related effects on hatching performance, eggs were randomly assigned to treatment groups to ensure an even age distribution in the groups.

Incubation management and evaluation of hatching parameters

The incubation lasted 21 days in automated Petersime incubators (Petersime, Zulte, Belgium). To avoid possible cross-contamination, the eggs of the control group were incubated separately from those that had been fumigated or sprayed with disinfectants. The eggs were incubated for 18 days at 37.8°C and 60% relative humidity on setter trays with the pointed end facing downward. They were then weighed and placed horizontally in hatcher baskets and incubated for the last three days at 37.2°C and 75% humidity. Eggs from one replicate per treatment were placed in individual separation grids to allow for separate monitoring of incubation results. After hatching (day 21), the live chicks were counted, weighed and sexed on the basis of early/late feathering. Hatchability was assessed in two ways: as the proportion of hatched chicks in relation to the total number of eggs set (hatchability 1) and as the proportion of hatched chicks in relation to the number of fertilised eggs (hatchability 2). The weight loss of the eggs was calculated as the percentage difference between the initial weight and the weight on day 18. Fertility was assessed by macroscopic examination of unhatched eggs, which were opened and categorised as infertile, early dead (≤ 9 days) or late dead (≥ 10 days) according to the criteria described by (9). To analyse the eggshell thickness, fragments from three shell regions (blunt end, equator, pointed end) were measured with a micrometre (TSS, York, England) and the mean thickness calculated.

Statistical analysis

The statistical analyses were performed using the statistical software package SAS/STAT, version 9.4 (SAS Institute Inc., Cary, NC, USA). Two types of variables were included: continuous and nominal. The MIXED method (PROC MIXED) was used to analyse continuous variables (values in g, mm or %), with treatment (disinfectant) included in the model as a fixed effect and setter

tray/hatcher basket as a random effect. Nominal variables (e.g. ratio/number of fertilised/unfertilised eggs, early/late dead embryos, male/female chicks) were analysed using the LOGISTIC method (PROC LOGISTIC). The differences were considered significant at $p < 0.05$.

Results

Trial A

Significant differences ($p < 0.05$) were found between the treatment groups in terms of hatchability, embryonic mortality, egg

weight loss, eggshell thickness and sex ratio. Hydrogen peroxide achieved the highest hatchability rate, both for total eggs set (79.4%) and fertilised eggs (85.7%), followed by formaldehyde (77.2% and 83.3%) and Ecocid® S (76.1% and 81.9%) (Figure 1). These three treatments formed a statistically homogeneous group, well ahead of ethanol (70.0% and 75.2%) and the control group (75.1% and 81.5%), although they did not always reach significance. In contrast, propolis (16.2% and 20.8%) had a significantly lower hatchability compared to all other treatments ($p < 0.05$), while salt (36.1% and 39.2%) also resulted in a significantly lower hatchability than Ecocid® S, formaldehyde, hydrogen peroxide, ethanol and the control ($p < 0.05$), but was not significantly different from distilled water (Figure 1).

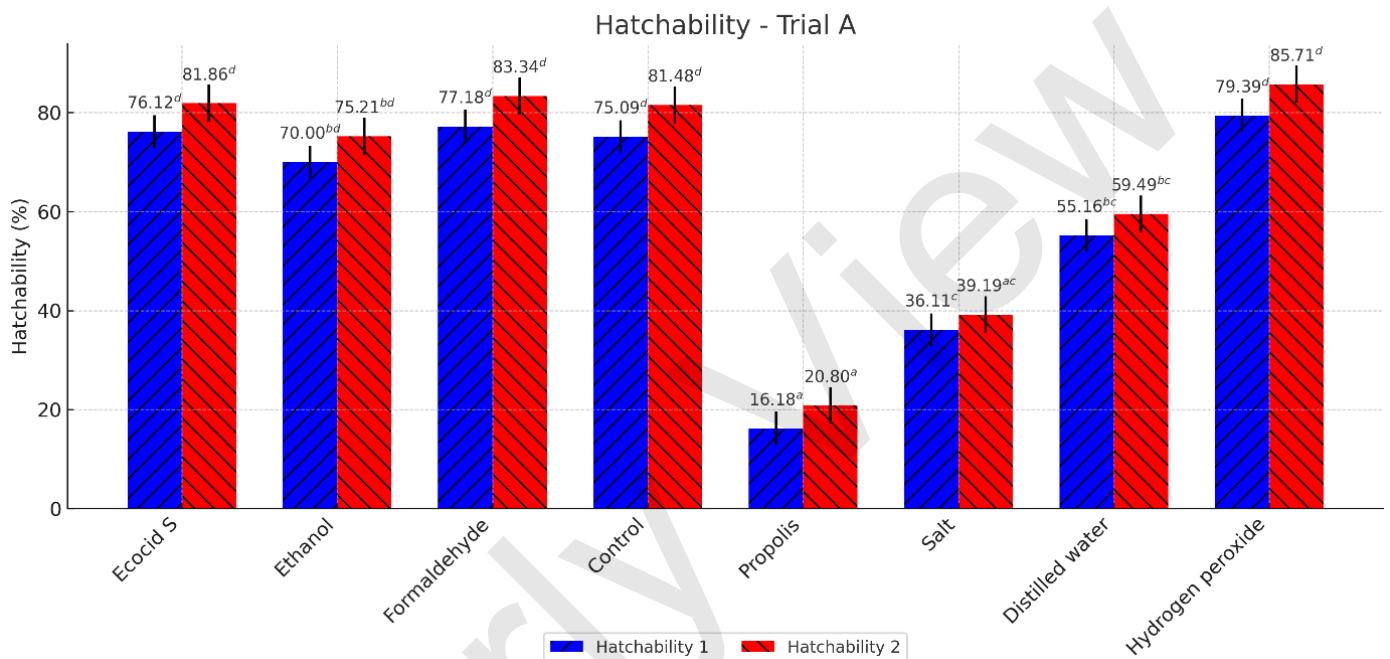


Figure 1: Hatching results after treatment of the hatching eggs with various chemical and natural disinfectants (Hatchability 1 = proportion of hatched chicks in relation to the eggs set; Hatchability 2 = proportion of hatched chicks in relation to the fertilised eggs; a,b,c,d. Different superscripts within the same hatchability group indicate statistical significance at $p < 0.05$)

Table 1: Effects of different disinfectant treatments on egg weight loss, embryo mortality, chick sex ratio, chick body weight and eggshell thickness

| Treatment | Egg weight loss ^a (%) (LSM ± SE) | Ratio ED/LD ^b | Ratio M/F ^c | Chick weight ^d (%) (LSM ± SE) | Eggshell thickness (mm) (LSM ± SE) |
|-------------------|---|--------------------------|------------------------|--|------------------------------------|
| Ecocid® S | 12.03 ± 0.249 ^c | 0.19 ^c | 0.97 ^{ab} | 66.48 ± 0.507 ^{ab} | 0.334 ± 0.002 ^{abc} |
| Ethanol | 10.60 ± 0.249 ^b | 0.10 ^{bc} | 0.96 ^{ab} | 67.18 ± 0.523 ^b | 0.340 ± 0.003 ^{ab} |
| Formaldehyde | 11.80 ± 0.249 ^c | 0.19 ^c | 0.94 ^{ab} | 64.61 ± 0.497 ^a | 0.324 ± 0.002 ^c |
| Control | 11.90 ± 0.249 ^c | 0.24 ^c | 0.98 ^{ab} | 66.09 ± 0.480 ^{ab} | 0.322 ± 0.002 ^c |
| Propolis | 7.57 ± 0.249 ^a | 0.49 ^a | 1.20 ^b | 67.68 ± 0.790 ^b | 0.346 ± 0.004 ^a |
| Salt | 11.30 ± 0.249 ^{bc} | 0.05 ^b | 0.78 ^a | 65.58 ± 0.670 ^{ab} | 0.329 ± 0.003 ^{abc} |
| Distilled water | 11.69 ± 0.249 ^c | 0.07 ^b | 1.20 ^b | 66.89 ± 0.585 ^{ab} | 0.330 ± 0.003 ^{abc} |
| Hydrogen peroxide | 11.63 ± 0.249 ^{bc} | 0.22 ^c | 1.00 ^{ab} | 67.05 ± 0.468 ^b | 0.329 ± 0.002 ^{bc} |

^aWeight loss of the eggs up to the 18th day of incubation, expressed as a percentage of the initial egg weight. ^bThe ratio between early-dead (≤ 9 days) and late-dead (≥ 10 days) embryos; this parameter reflects early embryonic vulnerability and is commonly associated with microbial contamination. ^cThe ratio of male to female chicks. ^dChick weight expressed as a percentage of initial egg weight. LSM = least square means, SE = standard error. Different letters (a, b, c, d) within the columns indicate significant differences ($p < 0.05$)

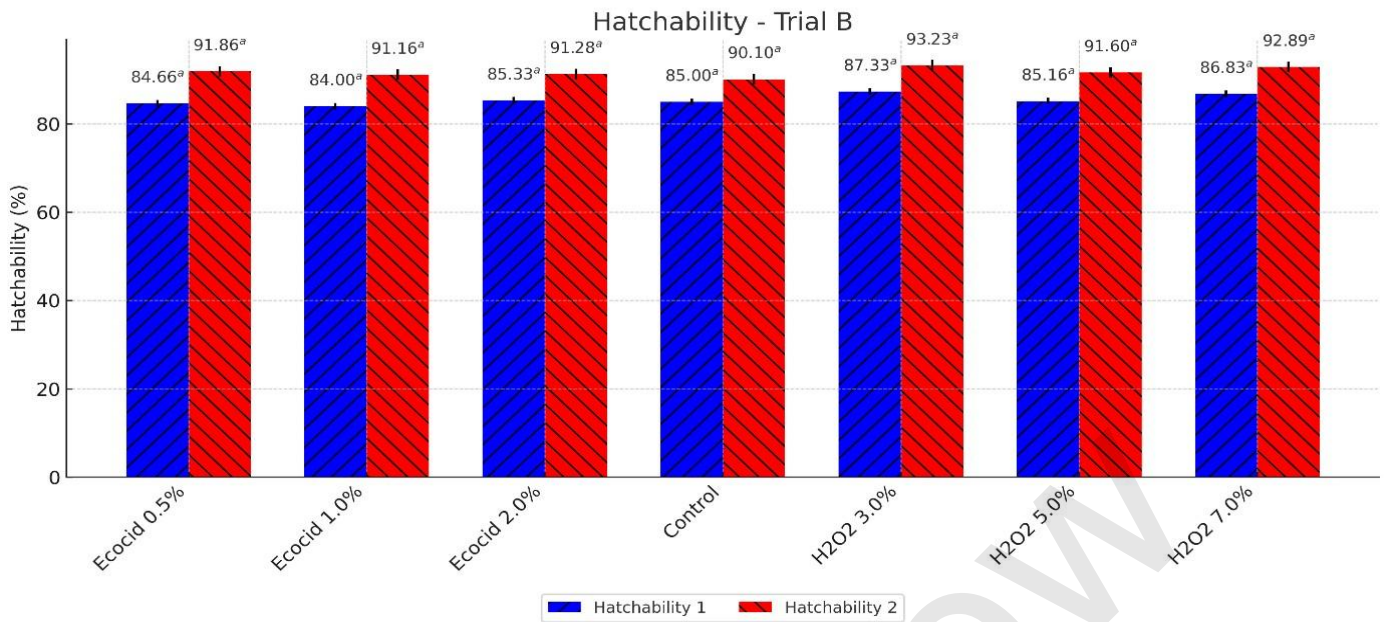


Figure 2: Hatchability performance of hatching eggs treated with different concentrations of hydrogen peroxide and Ecocid® S (Hatchability 1 = percentage of hatched chicks in relation to the eggs set; Hatchability 2 = percentage of hatched chicks in relation to the fertilised eggs; aThe same superscript within the same hatchability group indicates no statistically significant difference ($p > 0.05$))

Table 2: Effects of different concentrations of Ecocid® S and hydrogen peroxide on egg weight loss, embryonic mortality, chick sex ratio, chick body weight and eggshell thickness

| Treatment | Egg weight loss ^a (%) (LSM ± SE) | Ratio ED/LD ^b | Ratio M/F ^c | Chick weight (%) ^d (LSM ± SE) | Eggshell thickness (mm) (LSM ± SE) |
|------------------------|--|--------------------------|------------------------|---|---------------------------------------|
| Ecocid® S 0.5% | 12.07 ± 0.161 ^a | 0.22 ^a | 1.10 ^a | 66.89 ± 0.278 ^a | 0.332 ± 0.003 ^a |
| Ecocid® S 1.0% | 11.76 ± 0.161 ^a | 0.11 ^a | 1.00 ^a | 67.05 ± 0.275 ^a | 0.338 ± 0.003 ^a |
| Ecocid® S 2.0% | 11.66 ± 0.161 ^a | 0.23 ^a | 1.14 ^a | 66.46 ± 0.279 ^a | 0.333 ± 0.003 ^a |
| Control | 11.65 ± 0.161 ^a | 1.26 ^b | 1.00 ^a | 66.82 ± 0.275 ^a | 0.340 ± 0.003 ^a |
| Hydrogen peroxide 3.0% | 11.91 ± 0.161 ^a | 0.21 ^a | 0.95 ^a | 67.95 ± 0.278 ^a | 0.338 ± 0.003 ^a |
| Hydrogen peroxide 5.0% | 12.23 ± 0.161 ^a | 0.39 ^a | 0.98 ^a | 67.54 ± 0.277 ^a | 0.345 ± 0.003 ^a |

^aWeight loss of the eggs up to the 18th day of incubation, expressed as a percentage of the initial egg weight. ^bThe ratio between early-dead (≤ 9 days) and late-dead (≥ 10 days) embryos; this parameter reflects early embryonic vulnerability and is commonly associated with microbial contamination. ^cThe ratio of male to female chicks. ^dChick weight expressed as a percentage of initial egg weight. LSM = least square means, SE = standard error. Different letters (a, b) within the columns indicate significant differences ($p < 0.05$)

Significant differences between treatments were also observed in egg weight loss ($p < 0.05$), an indicator of eggshell permeability and embryo gas exchange (Table 1). Propolis-treated eggs showed the lowest water loss (7.6%), indicating limited evaporation, probably due to the closure of the shell pores. Ethanol resulted in significantly lower weight loss (10.6%) compared to other treatments ($p < 0.05$) as well. In contrast, Ecocid® S (12.0%), formaldehyde (11.8%) and hydrogen peroxide (11.6%) kept egg weight loss within the optimal physiological range (Table 1). The ratio of early to late dead embryos (ED/LD) also differed significantly ($p < 0.05$) among groups. The propolis treatment had the highest ED/LD ratio (0.49), indicating increased early embryo mortality. In contrast, the Ecocid® S (0.19), formaldehyde (0.19), hydrogen peroxide (0.22) and control (0.24) groups had significantly lower ratios ($p < 0.05$) compared to propolis, indicating

more favourable conditions for early embryonic development (Table 1).

Significant differences were also found in the sex ratio (male:female). In the groups treated with propolis and distilled water, the sex ratio was in favour of the males (1.20), while in the salt-treated group the ratio was significantly shifted in favour of the females (0.78). These differences were significant ($p < 0.05$), suggesting that certain disinfectants or substances may influence sex ratio dynamics during embryogenesis. Other treatments including Ecocid® S, hydrogen peroxide, formaldehyde, and control produced balanced sex ratios close to 1:1 (Table 1). Regarding the weight of the chicks at hatching, the highest values - expressed as a percentage of the initial egg weight - were recorded in the propolis (67.7%) and ethanol (67.2%) groups, possibly due to lower water loss through evaporation. However,

these differences were not significant across most treatments ($p>0.05$), and their biological relevance may be limited by the low hatchability observed in these groups. The lowest chick weight was observed in the formaldehyde group (64.6%). Finally, significant differences in eggshell thickness were observed ($p<0.05$). The propolis group had the thickest shells (0.346 mm), which were significantly larger than those of the formaldehyde (0.324 mm), control (0.322 mm) and hydrogen peroxide (0.329 mm) groups ($p<0.05$). Other treatments, including Ecocid® S and ethanol, maintained eggshell thickness within the normal physiological range (0.334–0.340 mm) and supported structural integrity and proper gas exchange (Table 1).

Trial B

In the trial B, the hatchability and the associated development parameters were generally the same in all treatment groups (Figure 2, Table 2). The hatchability of total eggs set was between 84.0% and 87.3% and of fertilised eggs between 90.1% and 93.2%, with hydrogen peroxide 3.0% having the numerically highest values. However, no significant differences in hatchability were found between the individual treatment groups ($p>0.05$) (Figure 2). The only significant difference found in this trial was in the ratio ED/LD. The control group had a significantly higher ED/LD ratio (1.26) than all other groups treated with disinfectants, where the ratios were between 0.11 and 0.39 ($p<0.05$). This suggests that untreated eggs were more prone to early embryonic mortality, while all disinfectant treatments - regardless of concentration - supported more balanced embryonic development (Table 2). No significant differences were observed in the sex ratio (male:female) across groups, which remained within the expected biological range (0.92 to 1.14) ($p>0.05$). The data on egg weight loss, chick weight at hatching and eggshell thickness also showed no significant differences between the groups ($p>0.05$) in trial B (Table 2). The values for egg weight loss were between 11.65% and 12.23%, while chick weight was between 66.5% and 67.9% of the initial egg weight, indicating that water loss and chick development were the same in all treatments. Egg shell thickness was similar in all treatments (0.332 to 0.345 mm) (Table 2), indicating that neither Ecocid® S nor hydrogen peroxide had a negative effect on shell structure.

Discussion

The results indicated that hydrogen peroxide and Ecocid® S are associated with favourable trends in hatchability, early embryonic mortality and physiological parameters such as egg weight loss, shell thickness and chick sex ratio. These results are consistent with previous studies demonstrating the antimicrobial efficacy and embryo tolerance of hydrogen peroxide in poultry species (5, 10). Although Ecocid® S is commonly used for surface disinfection, it was evaluated here for the first time in the context of hatching egg disinfection and was found to be as effective as formaldehyde at all concentrations tested. Egg weight loss in the hydrogen peroxide and Ecocid® S groups remained within the optimal range of 11–14% (11), suggesting that these agents maintain shell porosity

and allow normal gas exchange during incubation. In contrast, propolis-treated eggs showed significantly lower weight loss and hatchability. This may be related to the formation of a physical layer on the eggshell surface, which could reduce shell permeability and impair gas exchange. Although direct microscopic confirmation of pore occlusion was not performed, the markedly reduced egg weight loss supports this assumption. In addition, the ethanolic formulation of propolis used in this study may have contributed to embryotoxic effects. Ethanol is known to affect embryonic development, and its presence in the propolis solution could have exacerbated negative outcomes. Therefore, both physical (reduced shell permeability) and chemical (ethanol-related toxicity) mechanisms should be considered when interpreting these results (12). Distilled water (negative control), while not as detrimental as propolis or salt, still resulted in reduced hatchability compared to the most effective treatments. This may be due to damage of the eggshell cuticle or increased microbial penetration when the eggshell surface is wetted (13). One of the most revealing results was the lower ED/LD ratio in all disinfectant-treated groups compared to the untreated control. This suggests that microbial contamination primarily affects early embryogenesis and that chemical disinfection could reduce this risk (2). Even a modest reduction in early embryo losses could significantly improve cumulative hatchery results on a commercial scale. A major strength of this study is the focus on the effects of disinfection on the sex ratio of chicks - an overlooked but important factor both in commercial poultry production and in research on sex-specific developmental sensitivity. While hydrogen peroxide and Ecocid® S had no significant effect on the sex ratio, the treatments with propolis, salt and distilled water showed deviations from the expected 1:1 ratio between males and females. Specifically, the propolis and water treatments resulted in a higher proportion of males, whereas the salt treatment produced more females. These findings should be interpreted with caution. The observed shifts may not necessarily reflect direct modulation of sex determination, but could instead result from sex-specific differences in embryonic survival. For example, male and female embryos may differ in their sensitivity to environmental stressors such as osmotic imbalance, oxygen availability, or chemical exposure during early development (14). In addition, methodological factors cannot be entirely excluded. Although sexing was performed using established feathering methods, minor classification errors may occur. Furthermore, selective early embryonic mortality prior to sex determination could bias the observed ratios. Therefore, the present results indicate a possible association between disinfection treatments and sex ratio outcomes, but do not allow definitive conclusions regarding underlying mechanisms. Further studies incorporating molecular sexing of embryos at different developmental stages would be required to clarify these effects. The main limitation of this study is the lack of a direct microbiological assessment of eggshell contamination before and after disinfection. Despite this limitation, the present study provides several indirect lines of evidence in favour of the antimicrobial efficacy of hydrogen peroxide and Ecocid® S. Early embryonic mortality is widely associated with bacterial penetration through the eggshell, particularly during the first days of incubation, when embryos are most

vulnerable. Therefore, the consistently lower ED/LD ratios observed in hydrogen peroxide and Ecocid® S treated groups may serve as a reliable proxy for reduced microbial load. In addition, improved hatchability of fertilised eggs and maintenance of normal physiological parameters (egg weight loss, shell integrity) further support the assumption of effective microbial control. Although indirect biological indicators of microbial suppression, such as early mortality and hatchability, are widely recognised (15, 16), it should be emphasised that they cannot fully substitute for direct microbiological quantification. Future studies should include microbiological sampling of eggshell surfaces before and after disinfection to validate these indirect findings and to better quantify the antimicrobial efficacy of the tested agents. It should also be noted that eggs used in Trial A and Trial B originated from flocks of different ages (60 vs. 38 weeks). Although eggs were randomly distributed among treatment groups within each trial to minimise bias, flock age is known to influence egg quality, shell characteristics, and hatchability. Therefore, comparisons between trials should be interpreted with caution, and the results of each trial should primarily be considered independently.

Conclusion

This study shows that disinfection treatments could influence not only the hatchability and survivability of embryos, but also the sex ratio of chicks — an aspect that is rarely addressed in hatchery research. Hydrogen peroxide and Ecocid® S were found to be safe and effective and resulted in a high hatchability, low early mortality and a balanced sex ratio, justifying their use as an alternative to formaldehyde. In contrast, propolis and salt significantly reduced the hatchability and altered the sex ratio, suggesting possible embryotoxic effects. Although the lack of microbiological data is a limitation, the consistent reduction in early mortality observed with hydrogen peroxide and Ecocid® S provide strong biological evidence for antimicrobial activity. In a combination with their low toxicity and easy spray application, these results favour their practical use in routine disinfection protocols. However, confirmation of antimicrobial efficacy through direct microbiological assessment remains an important objective for future research.

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Contributions of the authors: D.T. made a substantial contribution to the study design, performed the statistical analysis, was involved in the hatchery procedures and actively participated in the preparation, revision and editing of the manuscript. M.B. contributed to the study design, data collection and analysis and drafted the original version of the manuscript. M.S. was responsible for the overall study design, coordinated the project and

critically revised the manuscript for important intellectual content. All authors contributed to the final version of the manuscript and approved it for publication.

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Razkuževanje valilnih jajc s kemičnimi in naravnimi sredstvi: vplivi na valilnost in razmerje med spoloma pri nesnicah

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Izveček: V tej študiji so bili na valilnih jajcih kokoši preučeni učinki šestih razkužil (Ecocid® S, etanol, formaldehid, propolis, sol in vodikov peroksid) ter destilirane vode (kot negativne kontrole) na parametre valjenja, razvoj zarodkov, kakovost piščancev ter razmerje med spoloma ob izvalitvi. Vključena je bila tudi kontrolna skupina, v kateri jajca niso bila tretirana z nobenim razkužilom. Najvišja valilnost je bila dosežena v skupini z vodikovim peroksidom (79,4 % glede na vložena jajca; 85,7 % glede na oplojena jajca), sledile so skupine s formaldehidom, Ecocidom® S (Krka, d. d., Novo mesto, Slovenija) in etanolom. Te štiri skupine so tvorile statistično homogeno skupino ($p > 0,05$), medtem ko je bila valilnost v skupinah, tretiranih s soljo in propolisom, statistično značilno nižja ($p < 0,05$). V skupini s propolisom sta bili valilnost (16,2 %; $p < 0,05$) in izguba mase jajc (7,6 %; $p < 0,05$) značilno manjši, verjetno zaradi zamašitve por v lupini in otežene izmenjave plinov. V isti skupini je bilo opaženo najvišje razmerje med zgodaj in pozno zamrtimi zarodki (ZZ/PZ = 0,49) v primerjavi z vsemi drugimi skupinami (0,05–0,24; $p < 0,05$). Prav tako je bil ugotovljen značilen vpliv na razmerje med spoloma ($p < 0,05$): v skupinah, tretiranih s propolisom in destilirano vodo, je bilo razmerje premaknjeno v korist petelinčkov (1,20), medtem ko je bila v skupini s soljo izražena tendenca v korist jarčk (0,78). Med tremi koncentracijami Ecocida® S (0,5 %, 1,0 %, 2,0 %) in vodikovega peroksida (3,0 %, 5,0 %, 7,0 %) niso bile ugotovljene statistično značilne razlike v valilnosti ali razmerju med spoloma ($p > 0,05$), je pa bilo v kontrolni skupini ugotovljeno značilno višje razmerje ZZ/PZ (1,26) v primerjavi z vsemi skupinami, tretiranimi z Ecocidom® S in vodikovim peroksidom (0,11–0,39; $p < 0,05$). Z raziskavo sta bili potrjeni učinkovitost in varnost vodikovega peroksida ter Ecocida® S. Poleg tega je bilo poudarjeno, da je pri ocenjevanju embriotoksičnosti razkužil za valilna jajca treba upoštevati tudi njihov morebitni vpliv na razmerje med spoloma.

Ključne besede: valilna jajca; vodikov peroksid; Ecocid® S; embrionalna umrljivost; razmerje med spoloma piščancev