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Efficiency of ultraviolet light for disinfection of fertile broiler eggs

[*Eficiência da luz ultravioleta para desinfecção de ovos férteis de matrizes de frangos de corte*]

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ABSTRACT

This study aimed to evaluate the effectiveness of ultraviolet light in reducing bacterial load of eggshells and the impact of experimental disinfection on hatching, embryo mortality, and time-borne distribution using broiler breeder hens of different ages (38, 42, and 48 weeks old). Fertile eggs were subjected to different exposure periods (5, 7, and 9 minutes) of UV light (**UV-C**) with a 254 nm wavelength. For controls, eggs disinfected with paraformaldehyde (5.3 g/m^3) and eggs not disinfected (**NC**). After subjection to disinfection protocols, the eggs were placed into sterile plastic bags containing 20 mL of peptone saline solution (0.1% m:v) and massaged for 1 minute to release the bacterial load. Aliquots of this solution were incubated in specific medium for bacterial growth for 48 hours at 37ºC for subsequent CFU counts. To evaluate the effects of disinfection on production, eggs previously disinfected by UV-C (9 min) and paraformaldehyde and NC eggs were candled between incubation days 10 and 13 and at the end of the incubation period to assess embryonic mortality. Hatchability distribution was performed every 8 hours. The 9 minutes 254nm UV-C light exposure was able to disinfect viable eggs and matched the effectiveness of the paraformaldehyde technique.

Keywords: Hatchability, paraformaldehyde, microbiological evaluation, incubation, embryo mortality

RESUMO

Objetivou-se avaliar a eficácia da luz ultravioleta na redução da carga bacteriana de cascas de ovos e o impacto na eclosão e na mortalidade embrionária observando-se a idade das matrizes (38, 42 e 48 semanas). Os ovos foram submetidos a diferentes períodos de exposição (cinco, sete e nove minutos) à luz UV (UV-C) com comprimento de onda de 254nm. Os controles foram ovos desinfetados com paraformaldeído (5,3g/m³) e ovos não desinfetados (NC). Após a desinfecção, os ovos foram colocados em sacos plásticos estéreis contendo 20mL de solução salina peptonada (0,1% m:v) e massageados por um (1) minuto para descolamento das bactérias. Alíquotas dessa solução foram incubadas em meio para crescimento bacteriano por 48 horas a 37ºC e contagem de UFC. Para avaliar os efeitos da desinfecção, ovos previamente desinfetados por UV-C (nove minutos) e ovos com paraformaldeído e NC foram submetidos à ovoscopia entre os dias 10 e 13 de incubação e ao final do período de incubação, para avaliação da mortalidade embrionária. A distribuição da eclodibilidade foi realizada a cada oito horas. A exposição à luz UV-C de 25nm de nove minutos desinfetou os ovos férteis e coincidiu com a eficácia do paraformaldeído.

Palavras-chave: eclodibilidade, parafolmaldeído, avaliação microbiológica, incubação, mortalidade embrionária

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INTRODUCTION

The disinfection of fertile eggs is a crucial part of sanitary and pathogen control programs in the poultry production chain, and the efficiency of the disinfection is one of the factors determining the success of incubation (Campos, 2000). When appropriate management is applied, a batch of brood stocks can maintain an average hatching rate of 83% throughout their life.

Among the possible factors influencing the hatching rate are the quality and level of contamination on the incubated egg (Heier and Jarp, 2001). The shell and cuticle structural integrity and cleanliness play a key role in the egg's ability to protect its internal contents and, simultaneously, meet the demands of the embryo (Narushin and Romanov, 2005). Therefore, cuticle removal by egg disinfection procedures leads to internal water loss and reduction in the hatching percentage of eggs (Peebles *et al*., 1987, 1998).

Formaldehyde is probably the most commonly used chemical in egg disinfection protocols worldwide. In the presence of an oxidizing source and small amounts of water, it tends to polymerize spontaneously and, thus, form solid paraformaldehyde (Ladeira *et al*., 2012) that covers the egg shell in fumigation process. Despite its ability to control salmonellosis (Gradel *et al*., 2004) and other bacteria (Keïta *et al*., 2016), the regulation of formaldehyde is the subject of much discussions, as it has been implicated in the carcinogenesis of nasopharyngeal areas, brain, pancreas, and blood (Nielsen and Wolkoff, 2010). The use of formaldehyde near hatchlings can cause ultra- and micro-structural changes in the chicks trachea and lungs, commonly causing ciliary membrane rupture, ciliary agglutination, epithelial desquamation, and heterophil infiltration (Freitas, 2007), with the latter leading to considerable losses in productivity.

Alternatives to formaldehyde have been explored in recent decades, and new protocols for disinfecting hatching eggs are being developed, such as the use of UV light (Chavez *et al*., 2002; Coufal *et al*., 2003; Gottselig *et al*., 2016). UV light acts by damaging the DNA of microorganisms (Kielbassa *et al*., 1997) and, despite the varied UV spectrum, UV disinfection generally provides the best results at a wavelength of 254 nm (Maclean *et al*., 2014). However, disinfection protocols using UV are not standardized and there are some unclear questions in its use as the exposing time, efficiency and the impact on hatching, embryonic mortality and birth distribution. For example, Chavez *et al*. (2002) reported a significant bacterial reduction after 60 seconds of exposure to UV-C light, whereas Coufal *et al*. (2003) found it was necessary to expose the eggs to UV for more than 4 minutes. Moreover, the result of decontaminating with ultraviolet light is improved when combined with hydrogen peroxide (Wells *et al*., 2010; Al-Ajeeli *et al*., 2016; Gottselig *et al*., 2016) and impaired in the presence of organic matter (Reu *et al*., 2006).

The objective of this work was to compare the disinfection efficiency of protocols using paraformaldehyde to those using different 254 nm UV exposure times in reducing the bacterial contamination of fertile eggshells, as well as to investigate the impacts of these disinfection protocols on hatching, embryonic mortality, and the on-time birth distribution.

MATERIAL AND METHODS

The eggs used in the present study were randomly collected during the first day's collection from heavy-breeding Hubbard great grandmother's whit hens of 38, 42, and 48 weeks old. Only eggs qualified as "eggshell cleaned and not washed" were selected. The experiment was carried out under the certificate of approval issued by the Animal Ethics Committee of the University of Brasilia (CEUA-UnB nº164039/2015).

Each treatment was performed with 35 eggs, each egg being a repetition. The eggs were randomly assigned to one of five treatment groups: fumigation procedure with paraformaldehyde 5.3 g/m³ (PC) inside the egg supply farm; different exposure times of 254 nm UV-C for 5, 7, and 9 minutes (UV-C5, UV-C7, and UV-C9, respectively); and a control group without disinfection (NC) to analyze the primary contamination of collected eggs.

The paraformaldehyde fumigation process took 30 minutes and was carried out 1 hour 30 minutes after the start of the collection. The procedure was performed at a temperature of 27°C and 75% relative humidity for the air inside the fumigator.

The eggs subjected to the NC and UV-C5, UV-C7, and UV-C9 treatments were transported in a hermetically sealed sterile box to the ornithopathology laboratory of the University of Brasília before the eggs belonging to the PC group to facilitate the simultaneous application of treatments.

UV-C light disinfection protocols were started at the same time as the fumigation protocol on the farm, allowing all eggs to be disinfected between 1 hour 30 min to 2 hours after collection. A laminar flow cabinet – ESCO® Optimair (Hatboro, Pennsylvania, USA) - was used with a UV-C lamp of wavelength 254 nm positioned above and at a distance of 50 cm from the eggs. The intensity of UV light at the time of exposure was measured in quintuplet using a photometer (Field Max II®, Coherent, California, USA). All 35 eggs of each treatment were placed on the rollers of a scrolling machine built in the laboratory to rotate them around their main axis (pole to egg pole) thus, the entire surface was exposed to UV light (Fig.1). The rollers and all apparatus inside the flow cabinet were sterilized between treatment applications using a protocol of cleaning completely the interior of cabinet with application of NaClOH 10% (v/v), then ethanol 70% (v/v) followed by UV disinfection for 5 min.

After this procedure, each egg was deposited in a sterile Whirl-Pak® plastic bag (LAS do Brasil, Goiás, Brazil) containing 20 ml of 0.1% peptone saline solution and massaged for 1 minute to obtain the shell surface microbiota. From the resulting solution, a 1 ml aliquot was taken and diluted in 9 ml of 0.1% peptone saline. A 1 ml aliquot of the resulting dilution was seeded into a sterile Petri dish and 20 ml of standard Plate Count Agar (Casein-peptone Dextrose Yeast Agar) was added for subsequent bacterial counting. After 30 minutes of rest for agar cooling, the plates were placed in an oven at 37 °C for 48 hours for subsequent CFU counting according to the MAPA protocol (Brazil, 2003). The reduction in the amount of CFU for each treatment was based on the difference between the mean CFU of the treatment in question and the mean CFU observed for the NC treatment.

Figure 1. An illustration of a pilot study of the scrolling machine used to rotate eggs. Font: personal archive.

For the hatching assessment, embryonic mortality, and on-time birth distribution, eggs classified as "eggshell cleaned and not washed" were randomly removed from the first collection of the day and disinfected by the PC, UV-C9, or NC treatment protocols. Then 35 eggs from PC and UV-C9 were marked with a pen and randomly distributed in two incubators. Eggs from NC (n=35) were positioned in other incubator, separately, in order to avoid microbial cross-contamination. Prior to incubation, the eggs were kept (in previously sterilized boxes) at 20 °C for 2

days. Temperature and humidity sensor accompanied the eggs during incubation and, following the manufacturer's recommendation, the incubators were adjusted to maintain a temperature of 37.7 °C and relative humidity of around 62% during the first 18 days of incubation. After that, during the preparation for hatching eggs, the roller incubators were removed and replaced by wire mesh to provide stability for hatchlings. In this second incubation phase, the incubator temperature was adjusted to 33 °C and humidity remained unchanged.

Between the 10th and 13th day of incubation, the eggs were submitted to ovoscopy with a dimmable flashlight to identify and discard infertile and unviable eggs. Egg hatching was monitored every 8 hours. The number of chicks hatched at each interval was recorded to identify the temporal distribution of hatching and to evaluate possible interference caused by the disinfection processes.

Data were statistically analyzed using SAS® software (v9.4, Cary, North Carolina, USA). Data on decontamination, embryonic mortality and temporal distribution of birth were assessed for normality by the Shapiro-Wilk test. Then, data related to decontamination and temporal distribution of birth were subjected to analysis of variance with subsequent comparison of means by the Tukey test at a 5% significance level. Data related to embryonic mortality was subject to a survival analysis using categoric data as described by Stokes *et al*. (2003) and PROC GENMOD in SAS® software. Data from hatchability was used only for descriptive purposes.

RESULTS AND DISCUSSION

Although the nominal intensity of the UV-C lamp used in this experiment was 8 mW/cm^2, this value refers to the UV intensity at zero distance to the lamp. In the present study, the intensity of UV light at the time of egg exposure within the laminar flow cabinet was 232 ± 5.05 µW/cm^2. This difference between nominal power and effective power is due the distance between light source and eggs that decreases light power in a log scale according to distance travelled by light. However, the consulted literature mentioned the use of UV light intensities (for the same purpose) of the order of 6.55 mW/cm^2 (Chavez *et al*., 2002), 7 mW/cm^2 (Coufal *et al*., 2003), and 7.5 mW/cm^2 (Chavez *et al*., 2002), but these papers do not specify whether this refers to the nominal or effective power at the exposure distance of the eggs. Therefore, it is inferred that values of about 28 times higher than the power described here are relative to the nominal power of the UV lamps used. In addition, high power UV lights are expensive and difficult to obtain and attempts to reduce the distance between light source and eggs in order to improve substantially the UV effective power could cause increase in egg temperature (as a secondary effect of UV light) and creation of shadows between the eggs that could only be overcome adding more light sources or reducing drastically the quantity of eggs disinfected each round. Both solutions are impractical and makes disinfection using UV light too expensive. To date, Coufal *et al*. (2003) needed 6 UV lamps in order to overcome the problem with shadow (in their words: "this system was employed to minimize shadows on the eggs…") and even that way, in their conclusion, they state UV disinfection was below their expectations.

Despite low UV power at egg level, there was a difference ($P < 0.0001$) in the amount of CFU between the treatment without disinfection (NC) and the other treatments (Table 1), which indicates effective exposure of the shell surface to UV-C and corroborates the hypothesis that egg disinfection by UV-C is a viable method for reducing the shell surface microbiota.

However, exposure of the entire egg surface to UV-C is one of the key factors in the technique's efficiency. Coufal *et al*. (2003), who used UV-C as a disinfection method to reduce the amount of *Salmonella typhimurium*, *Escherichia coli*, and aerobic microorganisms on eggshells, explained that their results fell short of expectations because they did not completely expose the eggshells to UV-C, even using six UV lamps. Therefore, when using UV-C as a disinfection method, it is important to consider that radiation only has an effect on exposed surfaces and is ineffective in shady places or areas covered by UV-C opaque surfaces. Moreover, even surfaces transparent to viable light (like glass) attenuate UV radiation and only certain substances, such as air, water, and polyethylene, facilitate the action of UV-C rays (Coufal *et al*., 2003), depending on the thickness of the structure being irradiated by UV light. Moreover, according to Bachmann (1975), direct exposure to UV light is required for the

effectiveness sanitizer. Therefore, bacteria within the pores of the shell, for example, are sheltered from the direct action of UV-C for at least part of the exposure time.

In the present study, the reduction in microbial load on the shell surface by UV-C disinfection occurred in a time-dependent manner, such that the longer the exposure time to UV-C the lower the CFU count. Therefore, the UV-C5 treatment resulted in higher CFU values, but they were not significantly different to the UV-C7 treatment, in turn, UV-C7 resulted in a higher (but not significantly) microbial load to the UV-C9 treatment. However, the microbial load measured after the UV-C5 and UV-C7 treatments were considerably higher ($P > 0.05$) than those measured after in the PC treatment. On the other hand, UV-C9 treatment showed a similar efficacy to paraformaldehyde treatment, as the bacterial load obtained from the shell after UV-C exposure was comparable to the microbial load obtained from the PC treatment (Table 1).

Therefore, these results highlight the value of developing of a new disinfection tool based on the use of UV light to control bacteria during the incubation of embryonated eggs. Above all, this new tool has advantages because, as proposed earlier in the paper, it is less harmful to hatchery house employees and probably has a positive effect on the viability of new-born birds compared to paraformaldehyde as UV is fundamentally a physical agent with a recent history of use and its unknown (and to date, until now, nonexistent) residual effects, meanwhile paraformaldehyde has a residual effect several days after fumigation (Ladeira *et al*., 2012). However, the longevity of hatched birds was not evaluated by this study.

Table 1. Mean CFU (log10 CFU/egg) found in all treatments

Treatment	NC	UV-C			PC
		5 min	7 min	9 min	
Mean	3.26^{a}	2.58^{bc}	2.25^{cd}	2.00^{de}	1.52^{e}
SD	0.51	0.80	0.63	0.67	1.05

Different letters (a, b, c, d, e) in the same line indicate that values differ from each other by the Tukey test ($P < 0.05$). NC: undisinfected control; PC: paraformaldehyde disinfected control; UV-C: ultraviolet light; SD = standard deviation.

The reduction in the amount of CFU in our experiment (1.26 $\log_{10}$ CFU/egg in the UV-C9 treatment) was smaller, i.e., less efficient, than that described by Wells *et al*. (2010), who reported a reduction of 2.07 $\log_{10}$ CFU/egg after 8 minutes of exposure to UV light. This may be due to differences in the initial bacterial load of the egg, which could not be measured, because the procedure to obtain such information would be destructive and would make the quantifications after the proposed treatments unfeasible. Bacterial load could only be estimated on the basis of the control group that was set up solely for this purpose. Therefore, the measurement of initial bacterial load was not included in the present study.

However, the time of exposure to UV-C was determinant in the CFU count reduction. A direct relationship ($P < 0.0001$) was observed between the exposure time and reduction in bacterial load, such that the UV-C9 treatment had the best results: 1.26 $\log_{10}$ CFU/egg mean (Figure 2) bacterial load reduction and, in absolute terms, the treatment resulted in a residual bacterial load of 2.0 ± 0.67 $\log_{10}$ CFU/egg.

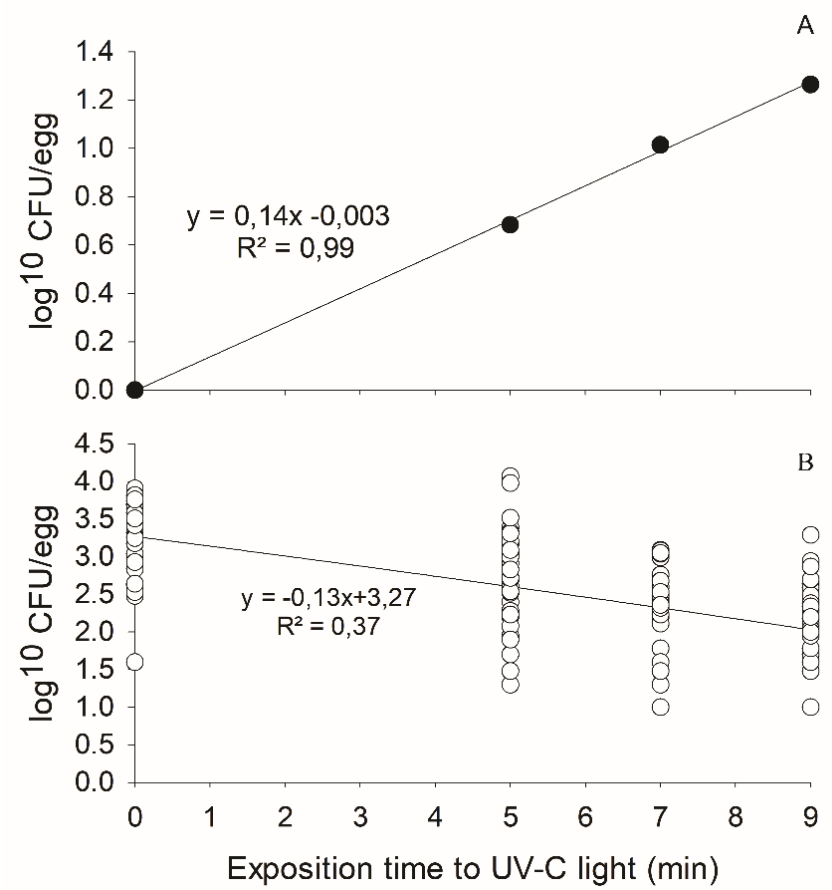

Figure 2: Exposure time (minutes) to ultraviolet light (UV-C5, UV-C7, UV-C9) and its effect on CFU count reduction (A) and total bacterial count (B).

Another important factor modifying the reduction in CFU efficiency by UV light is the light intensity used. Coufal *et al*. (2003), working with a light intensity of 7.5mW/cm², observed a reduction of 3 log_{10} CFU/egg with only 48 seconds of UV-C exposure with a treatment involving egg rotation. Chavez *et al*. (2002), also using a scrolling system and a combination of lamps with intensities of 7.5mW/cm² and 6.55 mW/cm², observed a reduction of 2 log_{10} CFU/egg after 30 seconds of exposure and between 2 and 3 log_{10} CFU/egg (depending on lamp combination) after 60 seconds of exposure. Although, in the present work, the lamp used provided a light intensity of 232 ± 5.05 µW/cm², lower than the intensity described in the works by Chavez *et al*. (2002) and Coufal *et al*. (2003), other factors such as friction time and friction intensity/vigor during the peptone water massage to obtain the residual microbiota from the shell may influence the bacterial load count. Unfortunately, there is no consensus in the literature regarding the most appropriate friction time for egg disinfection. As for friction intensity, the standardization of this variable is complex and sometimes unfeasible, as it involves manual work and invariably differs from person to person. However, as long as there are no changes in the methodology used at this stage of the sample preparation process, the friction intensity/vigor variation can be minimized by allocating this stage of the experiment to a single person (as in this study) and to a standard friction time (1 minute).

In general, when evaluating the results of previous studies, and considering the results of this work, when the friction time is shorter (close to 1 min) the result is more efficient (Chavez *et al*., 2002; Coufal *et al*., 2003) than in protocols where embryonated eggs have been manipulated longer (Wells *et al*., 2010; Al-Ajeeli *et al*., 2016; Gottselig *et al*., 2016).

The number of eggs used here was small and unable to detect subtle but potential important differences between treatments in terms of hatchability. Thus, prior to validating or attesting the safety of this methodology for disinfection purposes it is essential to carry out tests with a much higher number of eggs. Therefore, hatchability results are only descriptive and are not intended to compare the methods used (Figure 3). Despite this, the importance of effective disinfection of fertile eggs cannot be neglected: the disinfection process prior to incubation is fundamental to biosecurity inside the hatchery house and for the subsequent housing of chicks on the farm (Scott *et al*., 1993; Coufal *et al*., 2003).

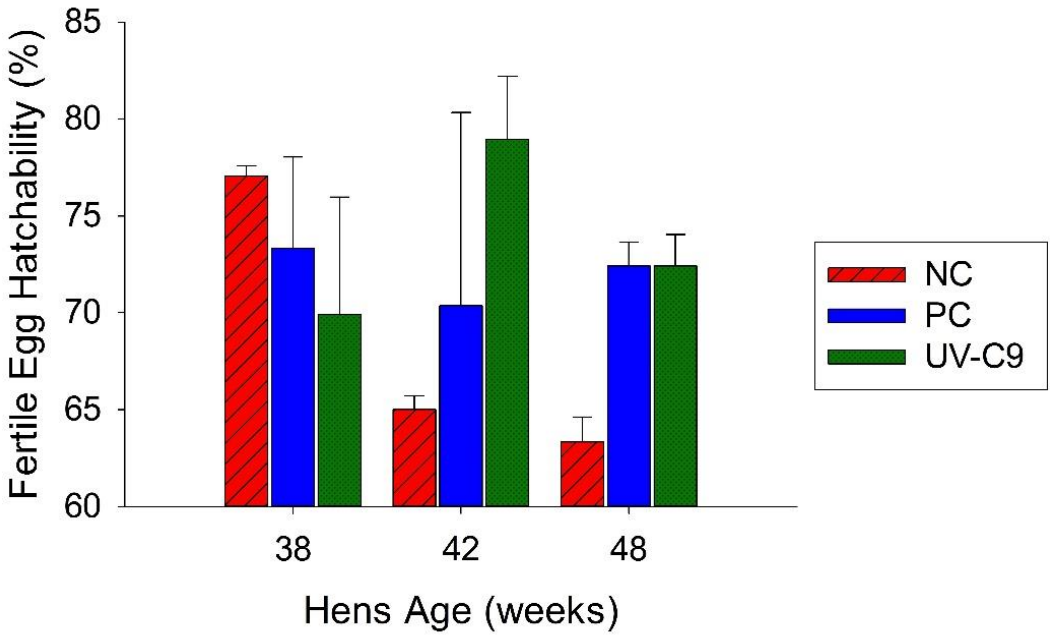

Figure 3. Hatchability according to the age of the breeding broiler (38, 42, or 48 weeks) and disinfection method.

The observed embryonic mortality was smaller ($P<0.0001$) on initial and intermediate periods than late period (Figure 4). The UV-C9 treatment presented a distribution of births similar to that found in eggs that did not undergo any disinfection protocol (NC), regardless of the age of the broiler breeder hens used (38, 42, and 48 weeks). They reached peaks of 31.78% (UV-C9) and 32.59% (NC) of births within the 482 to 490 hours incubation interval corresponding to a period of 20.08 to 20.42 days, while the PC treatment showed a significant difference in the distribution of births compared to NC and UV-C9, with hatching peaks (maximum 24.63%) between 494 and 502 hours of incubation (20.58 to 20.91 days) (Figure 5). Although the interaction between several factors during incubation influences embryonic development and, consequently, incubation time (Boleli *et al*., 2016), temperature and maintenance of moisture during this period are the most influential factors on embryo development (Meijerhof, 2009). Because the temperature conditions were uniform for all experimental treatments, we can infer that a factor related to paraformaldehyde treatment increased the incubation time of these eggs. Since the incubation period was shorter in eggs subjected to UV disinfection, the viability of birds from eggs treated with this technique is an important aspect for future study.

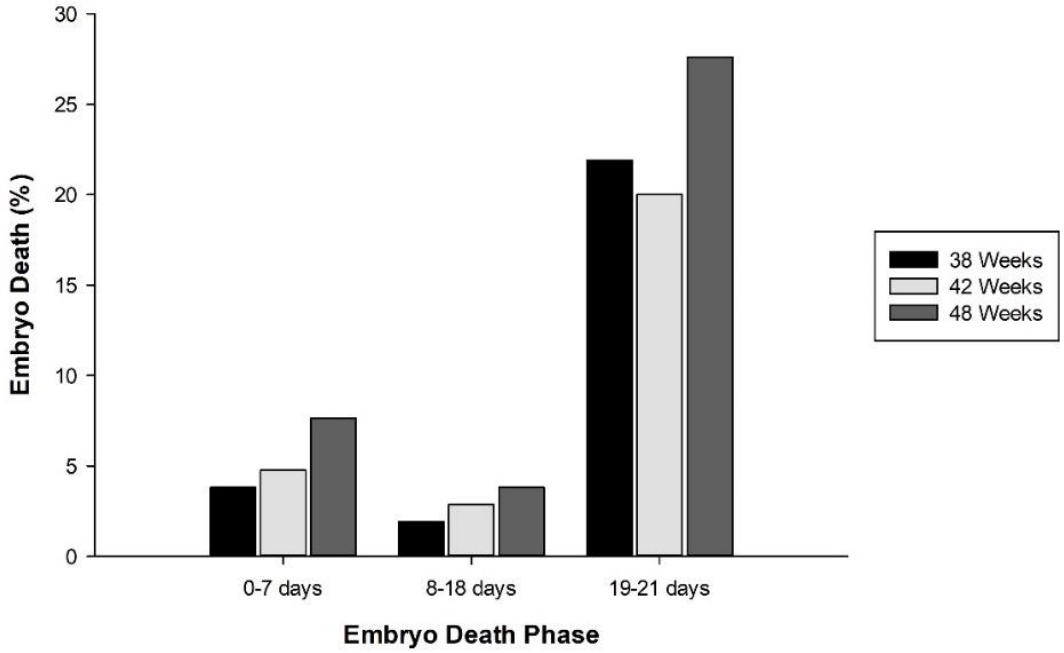

Figure 4. Initial (0 to 7 days), intermediate (8 to 18 days), and late (19 to 21 days) embryonic mortality phases (P < 0.05).

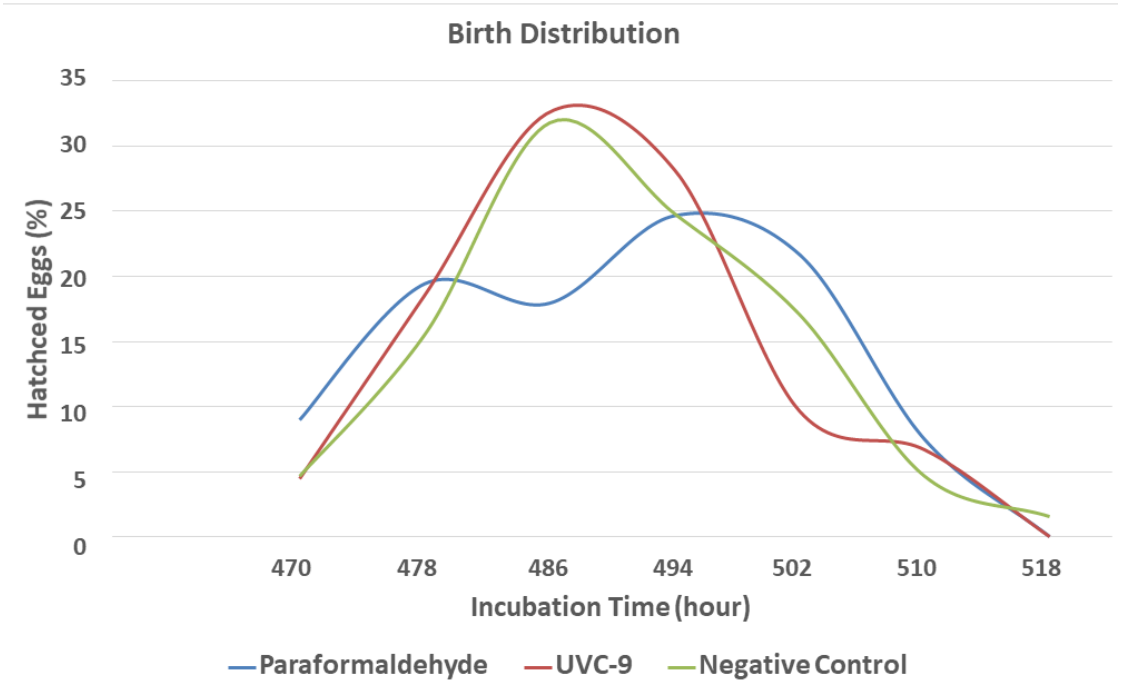

Figure 5. Distribution of births according to the disinfection treatments PC, NC, and UV-C 9 (P < 0.05).

Under industrial hatchery house conditions, the total incubation time is predefined, with most births concentrated over a very short period of time, unlike the results obtained in the PC treatment that did not show optimal birth grouping. This can influence the quality of day-

old birds, as early born birds need to remain longer in the incubator and may dehydrate. Although unavoidable, the difference in the number of dehydrated and newly hatched chicks is smaller when there is a larger volume of hatchlings within a shorter time frame, as found in NC and UV-C9.

CONCLUSIONS

Ultraviolet light was effective in reducing the bacterial load present on eggshells when the eggs were exposed for 9 minutes. Therefore, this technology may be a new tool for the disinfection of hatching eggs and could replace traditional methods such as paraformaldehyde. However, there is a need to evaluate and adjust the optimum UV intensity and exposure time to comply with hatchery house biosecurity standards, while taking operating costs into consideration.

BIOETHICS AND ANIMAL ETHICS COMMITTEE APPROVAL

This research was conducted in compliance with the ethical requirements of CONCEA – National Council for Control of Animal Experimentation with approval issued by CEUA-UnB No. 164039/2015.

REFERENCES

AL-AJEELI, M.N.; TAYLOR, T.M.; ALVARADO, C.Z. *et al*. Comparison of eggshell surface sanitization technologies and impacts on consumer acceptability. *Poult. Sci*., v.5, p.1191-1197, 2016.

BACHMANN, R. Sterelization by intense ultraviolet radiation. *Brown Boveri Rev.*, v.62, p.206-209, 1975.

BOLELI, I.C.; MORITA, V.S.; MATOS JR, J.B. *et al*. Poultry egg incubation: integrating and optimizing production efficiency. *Braz. J. Poult. Sci*., v.18, p.1-16, 2016.

BRASIL. Ministério da Agricultura Pecuária e Abastecimento - MAPA. Instrução Normativa nº 62. (*Dispõe sobre os métodos analíticos oficiais para análises microbiológicas para controle de produtos de origem animal e água*). 2003.

CAMPOS, E. *Avicultura*: razões, fatos e divergências. Belo Horizonte: FEP-MVZ, 2000. 311p.

CHAVEZ, C.; KNAPE, K.D.; COUFAL, C.D. *et al*. Reduction of eggshell aerobic plate counts by ultraviolet irradiation. *Poult. Sci*., v.81, p.1132-1135, 2002.

COUFAL, C.D., CHAVEZ, C.; KNAPE, K.D. *et al*. Evaluation of a method of ultraviolet light sanitation of broiler hatching eggs. *Poult. Sci*., v.82, p.754-759, 2003.

FREITAS, A.G. Effect of fumigating hatcheries with formaldehyde on respiratory tract and performance of broiler chickens. 2007. 44p. Master Dissertation. Available in: https://repositorio.ufu.br/handle/123456789/12928. Accessed in: 21 Jun. 2021.

GOTTSELIG, S.M.; DUNN-HORROCKS, S.L.; WOODRING, K.S. *et al*. Advanced oxidation process sanitization of eggshell surfaces. *Poult. Sci*., v.95, p.1356-1362, 2016.

GRADEL, K.O.; JØRGENSEN, J.C.; ANDERSEN, J.S. *et al*. 2004. Monitoring the efficacy of steam and formaldehyde treatment of naturally Salmonella-infected layer houses. J. Appl. Microbiol., v.96, p.613-622, 2004.

HEIER, B.T.; JARP, J. An epidemiological study of the hatchability in broiler breeder flocks. *Poult. Sci*., v.80, p.1132-1138, 2001.

KEÏTA, A.; HUNEAU-SALAÜN, A.; GUILLOT, A. *et al*. 2016. A multi-pronged approach to the search for an alternative to formaldehyde as an egg disinfectant without affecting worker health, hatching, or broiler production parameters. *Poult. Sci*., v.95, p.1609-1616, 2016.

KIELBASSA, C.; ROZA, L.; EPE, B. Wavelength dependence of oxidative DNA damage induced by UV and visible light. *Carcinogenesis*, v.18, p.811-816, 1997.

LADEIRA, C.; VIEGAS, S.; CAROLINO, E. *et al*. Exposição ocupacional a formaldeído: avaliação da exposição e efeitos genotóxicos. *Saúde Tecnol*., v7, p.18-27, 2012.

MACLEAN, M.; MCKENZIE, K.; ANDERSON, J.G. *et al*. 405 nm light technology for the inactivation of pathogens and its potential role for environmental disinfection and infection control. *J. Hosp. Infect*., v.88, p.1-11, 2014.

MEIJERHOF, R. Incubation principles: what does the embryo expect from us? AUSTRALIAN POULTRY SCIENCE SYMPOSIUM, 20., 2009, Sydney. *Proceedings…* Sydney: Poultry Research Foundation, 2009. p.106-111.

NARUSHIN, V.G.; ROMANOV, M.N. Egg physical characteristics and hatchability. *Worlds. Poult. Sci*. *J*., v.58, p.297-303, 2005.

NIELSEN, G.D.; WOLKOFF, P. Cancer effects of formaldehyde: A proposal for an indoor air guideline value. Arch. Toxicol., v.84, p.423-446, 2010.

PEEBLES, E.D.; BRAKE, J.; GILDERSLEEVE, R.P. Effects of eggshell cuticle removal and incubation humidity on embryonic development and hatchability of broilers. *Poult. Sci*., v.66, p.834-840, 1987.

PEEBLES, E.D.; PANSKY, T.; DOYLE, S.M. *et al*. Effects of dietary fat and eggshell cuticle removal on egg water loss and embryo growth in broiler hatching eggs. *Poult. Sci*., v.77, p.1522-1530, 1998.

REU, K.; GRIJSPEERDT, K.; HERMAN, L. *et al*. The effect of a commercial UV disinfection system on the bacterial load of shell eggs. *Lett. Appl. Microbiol*., v.42, p.144-148, 2006.

SCOTT, T.A.; SWETNAM, C.; KINSMAN, R. Screening sanitizing agents and methods of application for hatching eggs III. Effect of concentration and exposure time on embryo viability. *J. Appl. Poult. Res*., v.2, p.12-78, 1993.

STOKES, M.E.; DAVIS, C.S.; KOCH, G.G 2003. Categorized time-to-event data. In: _____. Categorical data analysis using the SAS® system. 2.ed. Cary, NC: SAS Institute Inc., 2003. p.593-618.

WELLS, J.B.; COUFAL, C.D.; PARKER, H.M. *et al*. Disinfection of eggshells using ultraviolet light and hydrogen peroxide independently and in combination. *Poult. Sci*., v.89, p.2499-2505, 2010.