Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v46i1.65262

Efficiency of ozone compared to commercial sanitizers for hatching eggs from older breeders

Leticia Cardoso Souza, Joel Lima da Silva Junior, João Paulo Ferreira Rufino*, Sebastião Corrêa de Nazaré, and Marco Antonio de Freitas Mendonça

Faculdade de Ciências Agrárias, Universidade Federal do Amazonas, Av. General Rodrigo Octávio Jordão Ramos, 6200, 69077-000, Manaus, Amazonas, Brazil. *Author for correspondence. E-mail: joaopaulorufino@live.com

ABSTRACT. This study aimed to evaluate the potential of ozone as a sanitizer compared to commercial sanitizers for hatching eggs stored in hatching machines using different turning systems. The eggs (n = 120) were distributed in a completely randomized design using a factorial scheme (6x2) where the treatments were constituted by different sanitizers applied (non-treated eggs, Ozone 1.6 mg L^{-1}, Ozone 3.2 mg L^{-1}, *Cyphenothrin*, UVC, and paraformaldehyde) and two turning systems (vertical and horizontal) with 10 eggs each, with the egg considered as a replicate. Data collected were subjected to the Tukey test at 0.05. We observed a very similar performance in the incubation yield results of the hatching machines with different turning systems, where the hatching percentage of eggs stored in the hatching machine using vertical turning presented better ($p < 0.05$) results. Comparing ozone to other sanitizers, we observed that paraformaldehyde and UVC provided better ($p < 0.05$) hatching percentage. However, both ozone concentrations used also presented good hatching percentage results. Chicks from treated eggs, except those from eggs treated with the highest ozone concentration (3.2 mg L^{-1}), were heavier ($p < 0.05$) at hatch. Chicks from eggs treated with the low concentration of ozone (1.6 mg L^{-1}) presented, in several scenarios, higher ($p < 0.05$) weight at hatch than chicks from eggs treated with sanitizers commonly used, especially paraformaldehyde and UVC. Conclusively, ozone can be used as a sanitizer to treat eggs from older breeders, presenting potential to replace commonly used sanitizers stored in hatching machines using both vertical and horizontal turning systems.

Keywords: chicks; incubation; paraformaldehyde; poultry; sanitization.

Received on October 1, 2022.
Accepted on January 12, 2024.

Introduction

The biosecurity of fertile eggs involves their sanitation from the moment they are collected in the nest to their storage in the hatching machine, aiming to maintain the embryo's security against potential microorganisms that could compromise its development (Oliveira, Santos, Nascimento, & Rodrigues, 2020a). Disinfection procedures for fertile eggs are crucial in preventing the spread of bacteria, especially from broiler breeder flocks to their offspring (Spickler, Buhr, Cox, Bourassa, & Rigsby, 2011; Clímaco et al., 2018). The embryo yolk sac and neonatal chicks are primarily at risk of contamination in the laying environment due to bacteria that can colonize the eggshell and enter the egg through its pores (Clímaco et al., 2018). Therefore, efficient disinfection can reduce egg contamination and, consequently, enhance the incubation capacity, quality, growth, and performance of the chicks (Araújo & Albino, 2011; Oliveira et al., 2020a; Oliveira, Santos, Rodrigues, & Nascimento, 2020b).

Hatching eggs in the poultry industry are commonly sanitized using paraformaldehyde fumigation to reduce potentially pathogenic microorganisms (Rui, Angrimani, Cruz, Machado, & Lopes, 2011; Kusstatscher, Cernava, Liebminger, & Berg, 2017). However, it is proven that this product causes adverse effects in the embryos and is harmful to the health of the farm and hatchery professionals (Zeweil, Rizk, Bekhet, & Ahmed, 2015; Kusstatscher et al., 2017). It is also important to mention that, for several years, disinfection of hatching eggs has been considered a problem for the poultry industry due to the absence of an economical, effective, and safe alternative to formaldehyde fumigation (Clímaco et al., 2018).

In this scenario, research is necessary to ensure effective methods for fertile egg sanitization, considering the high probability of eggshell contamination after laying, as well as bird health care, poultry litter quality, and the correct egg collection procedures (Wells, Coufal, Parker, & McDaniel, 2010). Currently, among the available alternative technologies, ozone is a promising one, which has proven its efficacy and usefulness over the years with its widespread application in the treatment of water and food (Miller, Silva, & Brandão, 2013). Studies have reported the ozone potential to combat microbial contaminations because of its very rapid action and strong oxidative characteristics, presenting fast auto-decomposes into molecular oxygen and leaving no hazardous halogenated compounds (Pandiselvam, Chandrasekar, & Thirupathi, 2017; Pandiselvam et al., 2019).

Even at very low concentrations, ozone can exhibit a remarkable sanitizing capacity (Pandiselvam et al., 2019). Ozone has the unique ability to destroy a wide range of microorganisms, even at relatively low concentrations, thus effectively meeting the global demand for sustainable sanitization (Miller et al., 2013; Pandiselvam et al., 2019). Another notable advantage of using ozone for sanitization is its potential for on-site generation. Ozone generators can efficiently produce ozone using oxygen as the source gas (Nakamura, Oya, Hanamoto, & Nagashio, 2017). This on-site generation eliminates the need for storing hazardous chemicals, an essential safety consideration (Pandiselvam, Thirupathi, & Anandakumar, 2015). In addition to these benefits, ozone treatment boasts an energy-efficient profile. Compared to other sanitization methods like radiation, microwave, and thermal treatment, the energy input required for ozone treatment is significantly lower (Khadre, Yousef, & Kim, 2001). This energy efficiency contributes to both cost savings and reduced environmental impact.

The applications of ozone as a sanitizer in the food industry and other agroindustry areas have been extensively reviewed in the literature (Berrang, Cox, Frank, Burh, & Bailey, 2000; Braun, Fernandez, & Fuhrmann, 2011; Karaca & Velioglu, 2014; Yüceer, Aday, & Caner, 2016; Glowacz & Rees, 2016; Gonçalves, 2016). However, this study seeks to focus on its applications as a sanitizer for hatching eggs, aiming to enhance their incubation performance in comparison to other commonly employed sanitizers within the poultry industry. By exploring the unique benefits and capabilities of ozone in this specialized context, this study endeavors to shed light on its suitability and effectiveness in optimizing the incubation process (Clímaco et al., 2018). The poultry industry relies on the success of hatching eggs, making it imperative to identify the most efficient and sustainable sanitization methods for this crucial stage of production (Zeweil et al., 2015; Clímaco et al., 2018). Considering the above, the objective of this study was to evaluate the potential of ozone as a sanitizer compared to commercial sanitizers for hatching eggs stored in hatching machines using different turning systems.

Material and methods

This study was conducted in the Poultry Sector, College of Agrarian Sciences, Federal University of Amazonas, Manaus (State of Amazonas), Brazil. The experimental procedures were approved by the Ethics Committee in Use of Animals of Federal University of Amazonas, Manaus, State of Amazonas, Brazil. Standard size eggs were obtained from Rhode Island Red breeders (n = 64; 62 wks-of-age; average body weight of 2.05±0.12) housed in an aviary with a density of 1 bird per m^2, feeding 115 g $bird^{-1}$ day^{-1} of balanced diets (requirements according to Rostagno et al., 2017) and water *ad libitum*. Breeders presented previous attested fertility.

The eggs (n = 120; 52.15±2.52) were distributed using a completely randomized design in a factorial arrangement (6x2) where the treatments were constituted by different sanitizers applied (non-treated eggs, ozonated water at a concentration of 1.6 mg L^{-1}, ozonated water at a concentration of 3.2 mg L^{-1}, *Cyphenothrin*, UVC, and paraformaldehyde) and two turning systems in the hatching machine (vertical egg turning and horizontal egg turning) with 10 eggs each, with the egg considered as a replicate. All treated eggs were exposed for 60 min. to their respective sanitizer. Ozonated water, *Cyphenothrin*, and paraformaldehyde were applied using fumigation (Rui et al., 2011; Clímaco et al., 2018). Ozonated water was produced using an ozone generator model OP 22 (Interozone©, Brazil), adjusted according to the proposed concentrations of ozone in the water (1.6 and 3.2 mg L^{-1}) (Braun et al., 2011; Miller et al., 2013; Nakamura et al., 2017). *Cyphenothrin* (currently used in the hatchery) was activated through a chemical reaction by combustion to carry out the fumigation process (Oliveira et al., 2020b). Paraformaldehyde was used for sanitization at a concentration of 6 g m^{-3} (Nielsen & Wolkoff, 2010). Product burning, fumigation, and gas exhaust occurred in a hermetically sealed chamber. The relative humidity and temperature in the chamber were maintained at 70% and 30°C, respectively. UVC light disinfection was performed using a laminar flow cabinet – ESCO® Optimair (Hatboro, Pennsylvania, USA) with a UVC lamp with a wavelength of 254 nm positioned above the eggs at a distance of 50 cm (Branco, Dallago, & Bernal, 2021).

After sanitizer treatments' application at room temperature, the eggs from each treatment were separated into two groups. One group (n = 60) was stored in a hatching machine (Barbaresco & Prado©, Brazil) with 2 months old, a capacity for 200 eggs, and a vertical turning system. The other group (n = 60) was stored in a hatching machine (Galinha Choca©, Brazil) with 6 years old, a capacity for 200 eggs, and a horizontal turning system. From 1 to 17 days of the incubation period, the relative humidity and temperature of each hatching machine were set at 65% and 37.5°C, respectively. From 18 to 21 days of the incubation period, the relative humidity and temperature of each hatching machine were adjusted to 75% and 36.5°C, respectively. Every 7 days, the eggs were removed from the hatching machine and subjected to an ovoscopy procedure, which lasted a short period (< 30 min.), to analyze the presence of embryonic mortality and to record the corresponding period of this mortality.

Immediately post-hatch, all hatched chicks were counted to calculate the hatching rate (hatched chicks per eggs incubated multiplied by 100). The non-hatched eggs were opened to evaluate the embryonic mortality stage, being classified as intermediary embryonic mortality (dead embryos between 8 and 14 days of incubation) and late embryonic mortality (dead embryos between 15 and 21 days of incubation with no pecking in the eggshell). Hatched chicks from each treatment were weighed. Chicks' weight data were related to their respective eggs' weight to calculate the chick/egg ratio.

Before performing data statistical analysis, all data were tested for normality and transformed if necessary. All data were analyzed by one-way ANOVA using the R software (version 4.1.3). All commands were performed according to Logan (2010). Tukey's honestly significant difference test was used to test the significant differences among the mean values and compare the efficiency of ozone as a sanitizer in relation to commercial sanitizers for hatching eggs stored in hatching machines using different turning systems. The results are presented as means, and the significance level for differences was set at $p < 0.05$.

Results and discussion

In the results obtained in this study (as presented in Table 1), we observed remarkably similar performance in the incubation yields of hatching machines utilizing different turning systems, with significant differences noted only in hatching percentages, where hatching machines employing vertical turning demonstrated a slightly superior outcome ($p < 0.05$). Nonetheless, the observed difference was minimal, less than 10%. This suggests that even in a relatively small proportion, the direction of egg turning in the hatching machine can indeed exert an influence on the hatching percentage of the eggs, in alignment with the findings of Yadav et al. (2021).

Table 1. Incubation yields of eggs from older breeders treated using ozone compared to commercial sanitizers and stored into hatching machines using different turning systems.

Factors[1]	Variables[2]				
	HT, %	IEM, %	LEM, %	CW, g	CER
TS					
Horizontal	31.66^{b}	16.67	83.33	45.20	0.75
Vertical	38.33^{a}	16.67	83.33	45.02	0.75
ST					
Non-treated	25.00^{c}	75.00^{a}	25.00^{c}	41.41^{b}	0.69^{bc}
Ozone 1.6 mg L^{-1}	35.00^{b}	25.00^{b}	75.00^{b}	47.16^{b}	0.78^{b}
Ozone 3.2 mg L^{-1}	40.00^{ab}	0.00^{c}	100.00^{a}	38.62^{c}	0.64^{c}
Cyphenothrin	20.00^{c}	0.00^{c}	100.00^{a}	57.00^{a}	0.95^{a}
UVC	45.00^{a}	0.00^{c}	100.00^{a}	43.50^{b}	0.72^{b}
Paraformaldehyde	45.00^{a}	0.00^{c}	100.00^{a}	43.00^{b}	0.71^{b}
Effect	p-value				
TS[3]	0.05*	0.76^{ns}	0.76^{ns}	0.25^{ns}	0.24^{ns}
ST[4]	0.01*	0.01*	0.01*	0.01*	0.01*
TS x ST[5]	0.05*	0.36^{ns}	0.36^{ns}	0.02*	0.02*
CV[6], %	13.93	22.84	15.68	18.28	18.22

[1]TS – Turning systems. ST – Sanitizer treatment. [2]HT – Hatching. IEM – Intermediary Embryonic Mortality. LEM – Late Embryonic Mortality. CW – Chick Weight. CER – Chick-Egg Relation. [3]Averages followed by lowercase letters in the column demonstrate a significant effect of TS on the variables analyzed by Tukey's test at 0.05 significance. ns – not significant. [4]Averages followed by lowercase letters in the column demonstrate a significant effect of ST on the variables analyzed by Tukey's test at 0.05 significance. [5]p-value above 0.05 demonstrates a direct influence of one factor on the result of the other and vice versa. ns – not significant. [6]CV – Coefficient of variation.

Understanding the implications of this subtle difference in hatching percentages can have practical implications for hatcheries aiming to maximize their yield (Tona, Onagbesan, Bruggeman, Mertens, & Decuypere, 2005; Elibol & Braket, 2006; Boleli, Morita, Matos Jr, Thimotheo, & Almeida, 2016). While the influence of turning systems on hatching performance might not be considerable, the accumulated effect over a large-scale hatchery operation can be of economic significance (Tona et al., 2005; Oliveira et al., 2020b). It underscores the importance of fine-tuning incubation processes to achieve the highest possible hatch rates. Further research into the underlying mechanisms behind this phenomenon, as well as the potential for more sophisticated turning systems, could offer valuable insights into optimizing hatchery operations and ensuring consistent, high-quality chick production (Ramli, Lim, Wahab, & Zin, 2015; Oliveira et al., 2020b).

Comparing the effectiveness of ozone to other sanitizers applied (Table 2), it becomes evident that both paraformaldehyde and UVC treatments significantly outperformed ($p < 0.05$) in terms of hatching percentages. Nevertheless, it is noteworthy that both concentrations of ozone used also yielded commendable hatching percentage results. Conversely, *Cyphenothrin* and non-treated eggs displayed notably lower hatching percentages, underscoring the efficacy of the sanitization methods employed.

In the context of the interaction between the factors influencing hatching percentage outcomes (Table 2), the previously observed patterns are reaffirmed, with the most favorable ($p < 0.05$) hatching percentages occurring in eggs treated with paraformaldehyde and UVC and stored in hatching machines utilizing a vertical turning system. Intriguingly, eggs subjected to ozone treatment exhibited higher ($p < 0.05$) hatching percentages when stored in hatching machines with a horizontal turning system. These findings shed light on the multifaceted relationship between sanitization methods, turning systems, and their combined impact on hatching performance, emphasizing the need for tailored approaches to maximize hatch rates and optimize poultry production (Rui et al., 2011; Clímaco et al., 2018). Further investigation into the specific mechanisms underlying these trends could offer valuable insights for hatchery management and egg hatchability enhancement (Rui et al., 2011; Clímaco et al., 2018; Oliveira et al., 2020a; 2020b).

The hatching percentage results observed in this study, while notably lower compared to the ideal percentages recommended by the literature (Araújo et al., 2016; Boleli et al., 2016; Silva, Pereira, Salgado, Ramos, & Freitas, 2017; Oliveira et al., 2020b), can be attributed to the relatively advanced age of the breeders used to produce the eggs examined. It is well-established that the reproductive system of breeders experiences a natural wear and decline in performance as they age, which subsequently leads to the development of more fragile embryos as they approach the latter stages of their productive period, typically occurring around 70 to 80 weeks of age, as corroborated by the findings of Francisco et al. (2012), Araújo et al. (2016) and Silva et al. (2017). Understanding the influence of breeder age on hatching outcomes is vital in realistic production settings, where variations in breeder age and reproductive performance can have a considerable impact on overall hatchability rates (Ramli et al., 2015; Araújo et al., 2016; Silva et al., 2017). Further research to delineate the interplay of breeder age, egg quality, and hatchability is essential for optimizing poultry production and ensuring consistent hatching success (Ramli et al., 2015; Araújo et al., 2016; Silva et al., 2017; Clímaco et al., 2018; Oliveira et al., 2020b).

The fact of paraformaldehyde has presented better results are according to the literature, where paraformaldehyde is commonly pointed out as the main used chemical in egg disinfection protocols worldwide (Branco et al., 2021) justly due to its ability to control salmonellosis (Gradel, Jørgensen, Andersen, & Corry, 2004) and other bacteria (Keïta et al., 2016) which, consequently, provide better incubation performance (Ladeira et al., 2012; Yadav et al., 2021). However, the regulation of paraformaldehyde is the subject of much discussions, especially due to its application has been related to the carcinogenesis of nasopharyngeal areas, brain, pancreas, and blood (Cadirci, 2009; Nielsen & Wolkoff, 2010).

Table 2. Interaction between the hatching machine turning systems (TS) and sanitizer treatments (ST) on the hatching results of eggs from older breeders.

Factors	TS[2]	
ST[1]	Horizontal	Vertical
Non-treated	20.00^{Cb}	30.00^{Ba}
Ozone 1.6 mg L^{-1}	40.00^{Ba}	30.00^{Bb}
Ozone 3.2 mg L^{-1}	50.00^{Aa}	30.00^{Bb}
Cyphenothrin	20.00^{Ca}	20.00^{Ca}
UVC	30.00^{Bb}	60.00^{Aa}
Paraformaldehyde	30.00^{Bb}	60.00^{Aa}

[1]Means followed by capital letters (columns) show a significant difference ($p < 0.05$) between the different ST. [2]Means followed by lowercase letters (lines) show a significant difference ($p < 0.05$) between the different TS.

The UV light has disappointed as a good alternative to paraformaldehyde replacement in recent decades (Gottselig, Dunn-Horrocks, Woodring, Coufal, & Duong, 2016), presenting results proximal to the paraformaldehyde application (Maclean, McKenzie, Anderson, Gettinby, & MacGregor, 2014; Gottselig et al., 2016), such as observed in this study. 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 (Branco et al., 2021). The low efficiency of *Cyphenothrin* in hatching percentage results may be associated to the fact that it is only an insecticide/pesticide (Mendis, Tennakoon, & Jayasinghe, 2018; Leong et al., 2020; Boukouvala & Kavallieratos, 2020), with no real effect on the microbiota that commonly cause problems to hatch eggs, which is basically of bacterial origin (Kizerwetter-Świda & Binek, 2008; Hameed, Akram, & Anjum, 2014; Clímaco et al., 2018; Nogueira et al., 2019). Therefore, their effect is minimal or none to avoid their action on the embryos.

The good results presented by ozone-treated eggs, which were very close to eggs treated using paraformaldehyde and UVC, confirm the sanitizing potential of this technology, a point very reported in literature (Miller et al., 2013; Yüceer et al., 2016; Nakamura et al., 2017; Pandiselvam et al. 2017; Pandiselvam et al., 2019), in addition to propose another possible application of this technology. According to Karaca (2010) and Pandiselvam et al. (2019), due to its good results in several scenarios as sanitizer and ecologically correct approach (without residues), ozone technology is gradually replacing conventional sanitation and fumigation techniques including chlorine, steam or hot water, and pesticides (fumigation) like phosphine, aluminum phosphide, and methyl bromide. Moreover, a number of commercial food preservation industries have started using ozone technology.

In the results of embryonic mortality (Table 1), there was embryonic mortality only in the non-treated eggs and eggs treated with the minimal ozone concentration (1.6 mg L^{-1}), indicating that the minimum treatment of eggs can be sufficient to avoid ($p < 0.05$) embryonic mortality up to 14 days of incubation, regardless of the turning system used in the hatching machine. On the other hand, most of the embryonic mortality occurred in this study was concentrated ($p < 0.05$) in the final stage of the incubation period (late mortality). In addition to a possible effect of treatments, this mortality concentrated at this stage may be related to the advanced age of breeders used, which according to literature (Francisco et al., 2012; Araújo et al., 2016; Nowaczewski, Babuszkiewicz, & Kaczmrek, 2016; Silva et al., 2017) tends to weaken embryos in the final stage of the incubation period and, consequently, cause a considerably raise in the embryonic mortality at this period.

In examining the results related to the hatchlings (as summarized in Table 1), a noticeable trend emerged where chicks from treated eggs, with the exception of those subjected to the highest ozone concentration (3.2 mg L^{-1}), exhibited significantly higher hatch weights ($p < 0.05$). These findings align with the insights of Araújo and Albino (2011), Kusstatscher et al. (2017), and Oliveira et al. (2020a), who emphasize that even subtle and appropriately balanced pre-incubation treatments can substantially contribute to a cleaner and more hygienic environment for embryo development, thereby manifesting in superior hatchling outcomes. This underscores the crucial role of pre-incubation sanitation practices in optimizing hatchling quality and growth. Further research may delve into the specific mechanisms and parameters that influence these effects, allowing for the refinement of egg treatment strategies and their implications for overall poultry production efficiency and the poultry industry as a whole.

In the interaction between the factors affecting chick weight results (as outlined in Table 3), notable patterns emerge, highlighting the efficacy of low-concentration ozone treatment (1.6 mg L^{-1}). Chicks hatched from eggs treated with this ozone concentration consistently displayed higher hatch weights compared to chicks from eggs treated with commonly used sanitizers, particularly Paraformaldehyde and UVC ($p < 0.05$). These findings further underscore the potential of ozone in creating an environment conducive to optimal embryo development during their incubation period in the hatching machine (Yüceer et al., 2015; Nakamura et al., 2017; Pandiselvam et al., 2017; Pandiselvam et al., 2019). The observed positive impact on hatch weight speaks to the significance of environmental factors on chick development and hatchery outcomes. These insights may have practical implications for hatchery management and strategies aimed at enhancing the overall quality and growth potential of hatched chicks (Rui et al., 2011; Sgavioli et al., 2016; Clímaco et al., 2018; Oliveira et al., 2020a; 2020b). Further research is warranted to delve deeper into the underlying mechanisms behind these effects and to explore potential strategies for maximizing chick weight and overall poultry production efficiency.

Table 3. Interaction between the hatching machine turning systems (TS) and sanitizer treatments (ST) on the chick weight (CW) and chick-egg relation (CER) results of chick from older breeders.

CW		
Factors	TS[2]	
ST[1]	Horizontal	Vertical
Non-treated	38.50^{Cb}	44.30^{Ba}
Ozone 1.6 mg L^{-1}	43.00^{Bb}	51.33^{Aa}
Ozone 3.2 mg L^{-1}	41.75^{Ba}	35.50^{Cb}
Cyphenothrin	56.50^{Ab}	57.50^{Aa}
UVC	51.75^{Aa}	35.25^{Cb}
Paraformaldehyde	39.75^{Cb}	46.25^{Ba}
CPO		
Factors	TS[2]	
ST[1]	Horizontal	Vertical
Non-treated	0.64^{Cb}	0.74^{Ba}
Ozone 1.6 mg L^{-1}	0.71^{BCb}	0.86^{Ba}
Ozone 3.2 mg L^{-1}	0.70^{BCa}	0.59^{Cb}
Cyphenothrin	0.94^{Ab}	0.96^{Aa}
UVC	0.86^{Ba}	0.58^{Cb}
Paraformaldehyde	0.66^{Cb}	0.77^{Ba}

[1]Means followed by capital letters (columns) show a significant difference ($p < 0.05$) between the different ST. [2]Means followed by lowercase letters (lines) show a significant difference ($p < 0.05$) between the different TS.

Conclusion

In conclusion, this study highlights the potential of ozone as an effective egg sanitizer, particularly in the context of eggs from older breeders. Ozone shows promise as a replacement for common sanitizers like paraformaldehyde and UVC, irrespective of the hatching machine's turning system. It consistently yielded favorable outcomes, enhancing hatching percentages and chick weights at hatch. This underscores ozone's role in improving hatchery efficiency and chick quality, indicating its relevance for more efficient poultry production practices.

References

Araújo, I. C. S., Leandro, N. S. M., Mesquita, M. A., Café, M. B., Mello, H. H. C., & Gonzales, E. (2016). Effect of incubator type and broiler breeder age on hatchability and chick quality. *Revista Brasileira de Ciência Avícola, 2*(special), 17-26. DOI: http://dx.doi.org/10.1590/1806-9061-2015-0146

Araújo, W. A. G., & Albino, L. F. T. (2011). Incubação comercial. Viçosa, MG: Transworld.

Berrang, M. E., Cox, N. A., Frank, J. E., Burh, R. J., & Bailey, J. S. (2000). Hatching egg sanitization for prevention or reduction of human enteropathogens: a review. *The Journal of Applied Poultry Research, 9*(2), 279-284. DOI: http://dx.doi.org/10.1093/japr/9.2.279

Boleli, I. C., Morita, V. S., Matos Jr, J. B., Thimotheo, M., & Almeida, V. R. (2016). Poultry egg incubation: integrating and optimizing production efficiency. *Brazilian Journal of Poultry Science, 18*(spe. 2), 1-16. DOI: http://dx.doi.org/10.1590/1806-9061-2016-0292

Boukouvala, M. C., & Kavallieratos, N. G. (2020). Effect of six insecticides on egg hatching and larval mortality of *Trogoderma granarium* Everts (Coleoptera: Dermestidae). *Insects, 11*(5), 263. DOI: http://dx.doi.org/10.3390/insects11050263

Branco, J. R. O., Dallago, B. S. L., & Bernal, F. E. M. (2021). Efficiency of ultraviolet light for disinfection of fertile broiler eggs. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 73*(5), 1137-1146. DOI: https://doi.org/10.1590/1678-4162-12279

Braun, P. G., Fernandez, N., & Fuhrmann, H. (2011). Investigations on the effect of ozone as a disinfectant of egg surfaces. *Ozone: Science & Engineering, 33*(5), 374-378. DOI: https://doi.org/10.1080/01919512.2011.589359

Cadirci, S. (2009). Disinfection of hatching eggs by formaldehyde fumigation – a review. *Archiv für Geflügelkunde, 73*(2), 116-123.

Clímaco, W. L. S., Melo, E. F., Vaz, D. P., Saldanha, M. M., Pinto, M. F. V. S., Fernandes, L. C. C., ... Lara, L. J. C. (2018). Eggshell microbiology and quality of hatching eggs subjected to different sanitizing procedures. *Pesquisa Agropecuária Brasileira, 53*(10), 1177-1183. DOI: http://dx.doi.org/10.1590/S0100-204X2018001000011

Elibol, O., & Braket, J. (2006). Effect of egg turning angle and frequency during incubation on hatchability and incidence of unhatched broiler embryos with head in the small end of the egg. *Poultry Science, 85*(8), 1433-1437. DOI: https://doi.org/10.1093/ps/85.8.1433

Francisco, N. S., Garcia, R. G., Caldara, F. R., Paz, I. C. L. A., Borille, R., Souza, K. M. R., ... Seno, L. O. (2012). Idade da matriz e tempo de estocagem dos ovos no desempenho e rendimento de carcaça de frangos de corte. *Agrarian, 5*(18), 393-401.

Glowacz, M., & Rees, D. (2016). The practicality of using ozone with fruit and vegetables. *Journal of the Science of Food and Agriculture, 96*(14), 4637-4643. DOI: http://dx.doi.org/10.1002/jsfa.7763

Gonçalves, A. A. (2016). Ozone as a safe and environmentally friendly tool for the seafood industry. *Journal of Aquatic Food Product Technology, 25*(2), 210-229. DOI: http://dx.doi.org/10.1080/10498850.2013.841785

Gottselig, S. M., Dunn-Horrocks, S. L., Woodring, K. S., Coufal, C. D., & Duong, T. (2016). Advanced oxidation process sanitization of eggshell surfaces. *Poultry Science, 95*(6), 1356-1362. DOI: https://doi.org/10.3382/ps/pev450

Gradel, K. O., Jørgensen, J. C., Andersen, J. S., & Corry, J. E. L. (2004). Monitoring the efficacy of steam and formaldehyde treatment of naturally *Salmonella*-infected layer houses. *Journal of Applied Microbiology, 96*(3), 613-622. DOI: https://doi.org/10.1111/j.1365-2672.2004.02198.x

Hameed, U., Akram, W., & Anjum, M. S. (2014). Effect of Salmonella on hatchability and fertility in laying hen, an assessment. *Veterinaria, 2*(2), 20-23.

Karaca, H. (2010). Use of ozone in the citrus industry. *Ozone: Science & Engineering, 32*(2), 122-129. DOI: https://doi.org/10.1080/01919510903520605

Karaca, H., & Velioglu, Y. S. (2014). Effects of ozone treatments on microbial quality and some chemical properties of lettuce, spinach, and parsley. *Postharvest Biology and Technology, 88*, 46-53. DOI: https://doi.org/10.1016/j.postharvbio.2013.09.003

Keïta, A., Huneau-Salaün, A., Guillot, A., Galliot, P., Tavares, M., & Puterflam, J. (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. *Poultry Science, 95*(7), 1609-1616. DOI: https://doi.org/10.3382/ps/pew058

Khadre, M. A., Yousef, A. E., & Kim, J.-G. (2001). Microbiological aspects of ozone applications in food: a review. *Journal of Food Science, 66*(9), 1242-1252. DOI: https://doi.org/10.1111/j.1365-2621.2001.tb15196.x

Kizerwetter-Świda, M., & Binek, M. (2008). Bacterial microflora of the chicken embryos and newly hatched chicken. *Journal of Animal and Feed Sciences, 17*(2), 224-232. DOI: https://doi.org/10.22358/jafs/66602/2008

Kusstatscher, P., Cernava, T., Liebminger, S., & Berg, G. (2017). Replacing conventional decontamination of hatching eggs with a natural defense strategy based on antimicrobial, volatile pyrazines. *Scientific Reports, 7*, 13253. DOI: https://doi.org/10.1038/s41598-017-13579-7

Ladeira, C., Viegas, S., Carolino, E., Gomes, M., Prista, J., Gomes, M. C., & Brito, M. (2012). Exposição ocupacional a formaldeído: avaliação da exposição e efeitos genotóxicos. *Saúde & Tecnologia, 7*, 18-27.

Leong, X.-Y., Kim, D.-Y., Dang, K., Singham, G. V., Doggett, S. L., & Lee, C.-Y. (2020). Performance of commercial insecticide formulations against different developmental stages of insecticide-resistant tropical bed bugs (Hemiptera: Cimicidae). *Journal of Economic Entomology, 113*(1), 353-366. DOI: http://dx.doi.org/10.1093/jee/toz266

Logan, M. (2010). *Biostatistical design and analysis using R: a practical guide*. New Jersey, NJ: John Wiley & Sons Ltd.

Maclean, M., McKenzie, K., Anderson, J. G., Gettinby, G., & MacGregor, S. J. (2014). 405 nm light technology for the inactivation of pathogens and its potential role for environmental disinfection and infection control. *Journal of Hospital Infection, 88*(1), 1-11. DOI: https://doi.org/10.1016/j.jhin.2014.06.004

Mendis, J. C., Tennakoon, T. K., & Jayasinghe, C. D. (2018). Zebrafish embryo toxicity of a binary mixture of pyrethroid insecticides: d-Tetramethrin and Cyphenothrin. *Journal of Toxicology,* 4182694. DOI: http://dx.doi.org/10.1155/2018/4182694

Miller, F. A., Silva, C. L. M., & Brandão, T. R. S. (2013). A review on ozone-based treatments for fruit and vegetables preservation. *Food Engineering Reviews, 5*, 77-106. DOI: https://doi.org/10.1007/s12393-013-9064-5

Nakamura, H., Oya, M., Hanamoto, T., & Nagashio, D. (2017). Reviewing the 20 years of operation of ozonation facilities in hanshin water supply authority with respect to water quality improvements. *Ozone: Science & Engineering, 39*(6), 397-406. DOI: https://doi.org/10.1080/01919512.2017.1352413

Nielsen, G. D., & Wolkoff, P. (2010). Cancer effects of formaldehyde: a proposal for an indoor air guideline value. *Archives of Toxicology, 84*(6), 423-446. DOI: https://doi.org/10.1007/s00204-010-0549-1

Nogueira, W. C. L., Pena, A. C. S., Souza, C. N., Azevedo, I. L., Faria Filho, D. E., & Almeida, A. C. (2019). Disinfection of fertile eggs of free-range poultry with essential oils. *Revista Brasileira de Saúde e Produção Animal, 20*, e0822019. DOI: http://dx.doi.org/10.1590/S1519-9940200822019

Nowaczewski, S., Babuszkiewicz, M., & Kaczmrek, S. (2016). Effect of broiler breeders' age on eggshell temperature, embryo viability and hatchability parameters. *Annals of Animal Science, 16*(1), 235-243. DOI: https://doi.org/10.1515/aoas-2015-0081

Oliveira, G. S., Santos, V. M., Nascimento, S. T., & Rodrigues, J. C. (2020a). Alternative sanitizers to paraformaldehyde for incubation of fertile eggs. *Poultry Science, 99*(4), 2001-2006. DOI: https://doi.org/10.1016/j.psj.2019.11.032

Oliveira, G. S., Santos, V. M., Rodrigues, J. C., & Nascimento, S. T. (2020b). Effects of different egg turning frequencies on incubation efficiency parameters. *Poultry Science, 99*(9), 4417-4420. DOI: https://doi.org/10.1016/j.psj.2020.05.045

Pandiselvam, R., Chandrasekar, V., & Thirupathi, V. (2017). Numerical simulation of ozone concentration profile and flow characteristics in paddy bulks. *Pest Management Science, 73*(8), 1698-1702. DOI: https://doi.org/10.1002/ps.4516

Pandiselvam, R., Subhashini, S., Priya, E. P. B., Kothakota, A., Ramesh, S. V., & Shahir, S. (2019). Ozone based food preservation: a promising green technology for enhanced food safety. *Ozone: Science & Engineering, 41*(1), 17-34. DOI: https://doi.org/10.1080/01919512.2018.1490636

Pandiselvam, R., Thirupathi, V., & Anandakumar, S. (2015). Reaction kinetics of ozone gas in paddy grains. *Journal of Food Process Engineering, 38*(6), 594-600. DOI: https://doi.org/10.1111/jfpe.12189

Ramli, M. B., Lim, H. P., Wahab, M. S., & Zin, M. F. M. (2015). Egg hatching incubator using conveyor rotation system. *Procedia Manufacturing, 2*, 527-531. DOI: https://doi.org/10.1016/j.promfg.2015.07.091

Rostagno, H. S., Albino, L. F. T., Hannas, M. I., Donzele, J. L., Sakomura, N. K., Perazzo, F. G., ... Brito, C. O. (2017). *Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais* (4a ed.). Viçosa, MG: UFV.

Rui, B. R., Angrimani, D. S. R., Cruz, L. V., Machado, T. L., & Lopes, H. C. (2011). Principais métodos de desinfecção e desinfetantes utilizados na avicultura: revisão de literatura. *Revista Científica Eletrônica de Medicina Veterinária, 9*(16), 1-14.

Sgavioli, S., Santos, E. T., Domingues, C. H. P., Quadros, T. C. O., Castiblanco, D. M. C., Andrade-Garcia, G. M., ... Baraldi-Artoni, S. M. (2016). Effect of high incubation temperature on the blood parameters of layer chicks. *Brazilian Journal of Poultry Science, 18*(spe. 2), 41-47. DOI: http://dx.doi.org/10.1590/1806-9061-2015-0095

Silva, G. F., Pereira, D. F., Salgado, D. D., Ramos, D. D., & Freitas, L. G. (2017). Incubation yield as a function of broiler breeder age. *Revista Brasileira de Engenharia de Biossistemas, 11*(3), 287-293. DOI: http://dx.doi.org/10.18011/bioeng2017v11n3p287-293

Spickler, J. L., Buhr, R. J., Cox, N. A., Bourassa, D. V., & Rigsby, L. L. (2011). Comparison between rinse and crush-and-rub sampling for aerobic bacteria recovery from broiler hatching eggs after sanitization. *Poultry Science, 90*(7), 1609-1615. DOI: http://dx.doi.org/10.3382/ps.2010-01305

Tona, K., Onagbesan, O., Bruggeman, V., Mertens, K., & Decuypere, E. (2005). Effects of turning duration during incubation on embryo growth, utilization of albumen, and stress regulation. *Poultry Science, 84*(2), 315-320. DOI: https://doi.org/10.1093/ps/84.2.315

Wells, J. B., Coufal, C. D., Parker, H. M., & McDaniel, C. D. (2010). Disinfection of eggshells using ultraviolet light and hydrogen peroxide independently and in combination. *Poultry Science, 89*(11), 2499-2505. DOI: http://dx.doi.org/10.3382/ps.2009-00604

Yadav, B. K., Pokharel, N., Khatiwada, D., Khanal, M., Bajracharya, T., & Dhakal, R. (2021). Design, fabrication, and performance analysis of an automatic horizontal egg incubator. *Journal of the Institute of Engineering, 16*(1), 77-85. DOI: https://doi.org/10.3126/jie.v16i1.36557

Yüceer, M., Aday, M. S., & Caner, C. (2016). Ozone treatment of shell eggs to preserve functional quality and enhance shelf life during storage. *Journal of the Science of Food and Agriculture, 96*(8), 2755-2763. DOI: http://dx.doi.org/10.1002/jsfa.7440

Zeweil, H. S., Rizk, R. E., Bekhet, G. M., & Ahmed, M. R. (2015). Comparing the effectiveness of egg disinfectants against bacteria and mitotic indices of developing chick embryos. *The Journal of Basic & Applied Zoology, 70*, 1-15. DOI: https://doi.org/10.1016/j.jobaz.2014.12.005