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THE EFFECT OF EXPOSING BROILER HATCHING EGGS TO DIFFERENT PERIODS OF OZONE DISSOLVEDIN WATERON SOM HATCHING CHARACTERS, PRODUCTIVE PERFORMANCE OF HATVHED CHICKS

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Abstract:
The study conducted at the poultry breeding farm of the Animal Production Department, Faculty of Agriculture, University of Basra, aimed to investigate the impact of dipping broiler hatching eggs in ozone dissolved in water at varying durations on hatching qualities and the productive performance of hatched chicks. A total of 1,080 broiler hatching eggs Rose 308 were incubated, and six treatments were implemented with varying ozone exposure times. Hatching Ratio and Chick Weight: The results revealed a significant improvement ($P < 0.05$) in hatching ratio and chick weight for eggs treated with ozone compared to control treatments. The fourth treatment, with a two-minute ozone exposure, demonstrated the highest hatching ratio (89.73%) and chick weight (46.31 g), outperforming other treatments and controls.

Late-Stage Embryo Characteristics: Different ozone exposure periods significantly influenced the weights of late-stage dead embryos, late-stage embryo mortality, and total embryo mortality. The second and fourth treatments exhibited lower weights for dead embryos (39.98 g and 39.05 g, respectively) compared to the third and fifth treatments. The fifth treatment showed a significant increase in late-stage embryo mortality (6.09%) and total embryo mortality (11.00%).Load on Egg Shells**:** Ozone treatment at a concentration of 400 mg/s effectively reduced microbial colonies on the eggshell surface. The fourth treatment demonstrated superior microbial reduction, indicating the efficacy of ozone in breaking down microbial cells on the eggshell surface during incubation.

Keywords: ozone, broiler hatching eggs, hatchability, chick's performance. Microbial.

Hatching Time: No significant differences were observed in hatching times among the treatments, ranging from 487.74 to 488.53 hours. This indicates that ozone exposure did not significantly impact the time of hatching.

Vital Qualities of Hatched Chicks: Observations of external vital qualities of one-day-old chicks indicated significant differences ($P < 0.05$). The second, fourth, and sixth ozone treatments demonstrated increased chick weights compared to control treatments.

Key findings: significant improvement in hatching ratios and chick weights with ozone treatment. The study reveals that ozone exposure positively influenced hatching qualities, reduced microbial load on eggshells, and enhanced chick performance. These insights contribute to optimizing poultry hatchery practices for improved efficiency and sustainability.

INTRODUCTION

Increasing the quality and the ability to hatch of chicks is a crucial first step towards improving the efficiency of broiler production. Lowering the amount of microbiological contamination on eggshells could help lower the incidence of bacterial infections in developing embryos and recently fledged chicks. Through imperfections such as holes or crack in the shell, bacteria can damage the embryo's growth and hinder ability to hatch. And have a deleterious impact on the chick post-hatching (Williams, *et al*.1968). Emerged chicks may also get infected after coming into contact with polluted eggshells and the incubator supplies According to Cason *et al*. (1994), bacteria such as Salmonella aggressive E. coli and Streptococcus are spread by infected chicks to additional chicks in the developing flock (Venkitanarayanan *et al*., 1999).

These microbes could be detrimental to the health and productivity of flocks. Because of the salmonella problem in poultry, disinfection technologies are necessary (Zeinab *et al*.2022). Ozone, a powerful oxidizing agent,

(Bocci, 2006) has been extensively studied for its antimicrobial efficacy in water and disinfection (Perry *et al.* 2008). Its ability to eliminate various pathogens and contaminants raises intriguing possibilities for its application in the realm of poultry incubation. Despite the growing interest in ozone as a treatment for broiler hatching eggs, there remains a significant gap in understanding the nuanced effects of different exposure periods and concentrations on key hatching qualities and the subsequent performance of hatched chicks.

The investigation's outcomes hold promise not only for hatchery management but also for broader implications in terms of improving the health and performance of broiler flocks. As concerns regarding antimicrobial resistance and sustainable production practices continue to escalate, the exploration of alternative, eco-friendly interventions such as ozone treatment underscores the necessity of continuous scientific inquiry in the pursuit of innovative and responsible poultry management strategies.

MATERIALS AND METHODS

The research project was conducted in the poultry breeding farm of the animal production department, Faculty of Agriculture, University of Basra, from January 8, 2021 to March 3, 2021. 1080 broiler hatching eggs of rose 308 were incubated in order to determine the impact of dipping broiler hatching eggs for varying periods of time in ozone dissolved in water the hatching qualities and productive performance of hatched chicks in this manner. Based on the examined equations below, 180 fertilized eggs and three duplicates for every 60 fertilized eggs were divided among six Treatments every Treatment, after the eggs had been weighed:

1. The first treatment (Control 1): the hatching eggs are dipped in stiller water (DW-1Min). 2. The second treatment: by dipping the hatching eggs with dissolved ozone in (DW-1Min). 3. The third treatment (Control 2): The hatching eggs are dipped in (DW-2Min). 4. Fourth treatment: dipping hatching eggs with dissolved ozone in (DW-2Min). 5. The fifth treatment (Control 3): the hatching eggs are dipped in (DW-5Min). 6. The sixth treatment: the hatching eggs are dipped in ozone dissolved in (DW-5Min). After completing the sterilization process and returning the eggs to the incubators, which were set to a temperature of 37.5 degrees Celsius and a humidity of 65%, the stirring was automatic every hour for 24 hours, and the following measurements were taken:

The proportion of hatched chicks (Hatchability), chick weight, total mortality chicks, the percentage of late-stage dead embryos LSDE%, the average weights of the late-stage dead chicks, WLSDE g and the hatching time, The vital qualities of hatched chicks according to the method of characterization by Tona, (2004) Samples of eggs were taken before dipping (control) and after dipping (ozone treatment) in order to conduct a total count of bacteria on egg shell.

Statistical analysis

ANOVA was performed to determine the significant differences among the treatment groups. Post-hoc Turkey's test was conducted for pairwise comparisons. The significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

The effect of using different periods of water-dissolved ozone at a concentration of 400 mg / s for broiler hatching eggs on the hatchability and the weight of hatched chicks

Table (1) shows the effect of using different periods of water-dissolved ozone at a concentration of 400 mg / s for broiler hatching eggs on the hatchability and the weight of hatched chicks. It is noted from the table that there is a significant improvement ($P<0.05$) in the hatching ratio and the weight of hatched chicks for Treatments that have been treated with ozone for different periods compared to the control treatment for each of them if the fourth Treatment is morally superior ($P<0.05$) to the control treatment and to the rest of the Treatments if the hatching ratio and the (89.73±0.254 %) , (46.31±0.30) g, respectively compared to the control Treatment (80.44 ±0.131 , 80.37±0.332, 81.43±0.974) % , (42.22±0.22 , 41.77±0.22 , 43.16±0.42) GC respectively and the rest of the ozone coefficients (the second Treatment and the sixth Treatment (84.58±0.376) , 86.34±0.723) % , (43.12±0.42 , 43.52±0.24) g, respectively, and this may be due to the effect of ozone sterilization time, which significantly affects the microbial content, as will be shown in the following table (2) if ozone breaks down microbes on the surface of the Shell, which leads to an increase in the hatching rate and a decrease in the number of dead embryos, therefore, the growth of embryos inside the eggs will improve as a result of a decrease in the microbial effect on vital growth processes inside the embryo body (Fasenko *et al.*, 2009) and these results are consistent with what they pointed out (Fuhrmann *et al.*, 2010) . While (Melo *et al.* 2019) use of disinfectants, including ozone at a concentration of (5-15) ppm for 30 minutes and at a humidity of 70% in hatching eggs of chickens from the Cobb 500 herd at the age of 70 weeks, did not have a negative or positive effect on the total hatching percentage of fertilized eggs or on the weight of hatched chicks. the reason for this was attributed to the great correlation between these measurements and the breed, the age of the herd and the period of storage of eggs, and since the eggs were collected from one source and under the same storage conditions, they disappeared variations , However, the concentration at 30.03% had a negative effect on the hatching rate, and this discrepancy in the results or effects may be due to the formula or dilution used for the and the period of exposure of the eggs to ozone , which affects these readings (Gholami-Ahangaran *et al.*, 2016). In compared to the effects of no treatment or water misting, hatchability was significantly reduced (26.5 to 37.5%) after ozonation (3.03% ozone by weight, 2 h). Whistler and Sheldon (1989).

This could suggest that O_3 has a distinct effect on embryo development, perhaps as a result of its high shell penetration. The distribution of embryo mortality in both hatching stages showed intriguing results based on the sanitizing periods.

Table 1. The effect of using different periods of water-dissolved ozone at a concentration of 400 mg / s for broiler hatching eggs on the hatchability and weight of hatched chicks (mean ± standard error).

Treatment	Hatchability%	Weight of hatched chick's g
T1	80.44 ±0.131^{d}	42.22±0.221^{b}
T2	84.58±0.376^{c}	43.12±0.423^{b}
T3	80.37±0.332^{d}	41.77±0.222^{c}
T4	89.73±0.254 ^{a}	46.31±0.304^{a}
T5	81.43±0.974^{d}	43.16±0.426^{b}
T6	86.34±0.723^{ab}	43.52±0.248^{b}

Where T1 represents the control treatment where the eggs are immersed in DW-1Min, T2 represents the second treatment where the eggs are exposed to the dissolved Ozone in DW-1Min, and T3 represents the third treatment control treatment where the eggs are immersed in DW-2Min. T4 represents the fourth treatment, where the eggs are exposed to dissolved Ozone in DW-2Min; T5 represents the fifth treatment control, where the eggs are immersed in DW-5Min. T6 represents the sixth treatment, where the eggs are exposed to the Ozone dissolved in DW-5Min. The lowercase letters (a, b, c, d) are used to indicate significant differences between treatments at the level of $P < 0.05$.

The effect of using different periods of water-dissolved ozone at a concentration of 400 mg/s for hatching eggs in broilers on the average weights of dead embryos during the late stage of embryonic development, the percentage of dead embryos during the late period of embryonic development, and the percentage of total deaths:

Table (2) shows the effect of using different periods of water-dissolved ozone at a concentration of 400 mg/s for hatching eggs of broilers on the average weights of dead embryos during the late stage of embryonic development, the percentage of dead embryos during the late period, and the percentage of total embryo mortality as Data analysis revealed that the percentage of hatching in them with ozone in DW and for different periods compared to the other treatments and control showed noticeable variations at ($P<0.05$). A decrease in the weights of the dead embryos was observed for the second and fourth treatments, reaching (39.98±1.432, (39.05±2.943) g, compared to the third and fifth treatments, reaching (40.81±0.432, 42.11±2.442) g, respectively, and the first treatment and the sixth treatment, where higher values were recorded, reaching (41.44±1.645, 42.09±3.711) g, The above table also notes that there are significant differences at ($P<0.05$) in the percentage of embryos lost during the late period and the percentage of total embryo mortality.

The percentage of delayed embryo mortality in the fifth Treatment increased significantly compared to the rest of the Treatments and control Treatments, recording a value of 6.09±1.291) %, while the second and fourth Treatments did not record any late embryo mortality it was 0.00±0.00) % for both Treatments compared to the first and third and the sixth, having reached (1.12±0.442, 2.64±0.022, 3.28±1.025) %, respectively.

At the same time, the fifth Treatment recorded the highest percentage of total embryo deaths, where a value was recorded (11.00±3.728) compared to the rest of the Treatments and control Treatments, while the second and fourth Treatments were the lowest in the total percentage of embryo deaths, which was recorded statistically, reaching (2.41±3.215, 2.01±4.645) %, while the first, third, and sixth (control) Treatments recorded the following percentages (5.06±2.312, 6.81±2.118, 8.49±1.363) %. The percentage of dead embryos is influenced by many factors, including the weight of the egg and how clean it is (Melo etal.2019), and as is known, there is a direct relationship between the hatching rate and the content of the outer shell of microbes or rot. As noted (de Reu et al., 2014), the hatching rate in chicken eggs decreases as a result of the penetration of rot and germs into the shell membrane or as a result of their crossing by the mother hen through the oviduct in a way called vertical infection from mother to chicks, causing early and late embryo deaths. Therefore, we note that the hatching rate and the percentage of deaths vary between the Treatments subjected to sterilization by ozone and control Treatments, where O_3 inhibits the growth of microbes on the surface of the shell (De Reu et al., 2006), where O_3 oxidizes and replaces the cell walls of those contaminated microorganisms (Bocci, 2006), and it also effectively reduces the growth of germs on the surface of the shell without leaving accumulated side effects, which is classified as environmentally friendly by (Braun *et al*. 2011, Wells *et al*., 2011).

Table 2 effect of using different periods of water-dissolved ozone at a concentration of 400 mg/s for hatching eggs in broilers on the average weights of dead embryos during the late stage of embryonic development, the percentage of dead embryos during the late period of embryonic development, and the percentage of total mortality (mean ± standard error).

Treatments	WLSD g	LSDE%	Total embryo mortality%
T1	41.44±1.645	1.12±0.442[b]	5.06 ±2.312[b]
T2	39.98±1.432	0.00±0.000[a]	2.41±3.215[a]
T3	40.81±0.432	2.64±0.022b[c]	6.81±2.118[b]
T4	39.05±2.943	0.00±0.000[a]	2.01±4.645[a]
T5	42.11±2.442	6.09±1.291[d]	11.00±3.728[d]
T6	42.09±3.711	3.28±1.025[c]	8.49±1.363b[c]

* Where T1 represents the control treatment where the eggs are immersed in DW-1Min, T2 represents the second treatment where the eggs are exposed to the dissolved Ozone in DW-1Min, and T3 represents the third treatment control treatment where the eggs are immersed in DW-2Min. T4 represents the fourth treatment, where the eggs are exposed to dissolved Ozone in DW-2Min; T5 represents the fifth treatment control, where the eggs are immersed in DW-5Min. T6 represents the sixth treatment, where the eggs are exposed to the Ozone dissolved in DW-5Min.LSDE refer to Percentage of dead embryos during the late period of embryonic development, WLSD refer to weights of dead embryos during the late stage of embryonic development. The lowercase letters (a, b, c, d) are used to indicate significant differences between treatments at the level of $P < 0.05$.

Utilizing this kind of sanitizer or a combination of sterilizing agents, including ozone with a concentration of (5–10) ppm for 20 minutes does not in any way affect the structure of the shell or the components of the egg prepared for hatching, which was collected from a flock of Cobb laying hens at the age of 42 weeks and therefore has no effect on embryonic development (Dos Santos Clímaco *et al*., 2018), but researchers Zeweil *et al*. (2015) have the opposite effect if they confirm in their study on the use of conventional chemical disinfectants in the disinfection of hatching chicken eggs that there is a negative impact on the development of embryos, leading to their death.

The researchers went with these results (Wlazlo *et al*., 2020), who reported an elevated in the percentage of halogens in the treatment of hatching quail eggs that were exposed to ozone by 4.2 mg O_3/h (5 min) at the age of 14 days of incubation period. As for Melo *et al*. (2019), they explained that there is no moral effect of using a set of disinfectants, including ozone, when exposed to hatching chicken eggs, on the quality of embryo Mortality due to the fact that this quality is significantly affected by the incubator temperature first, humidity, lack of important vitamins such as B group and vitamin D, and finally the cleanliness of eggs. Boleli (2013) found that now all eggs have been laid in the same incubator and fed from the same bush, so the differences were invisible, as they described in their study.

The effect of using different periods of ozone dissolved with water at a concentration of 400 mg / s for hatching eggs of broilers in the preparation of microbes on the surface of the shell during the incubation period.

The result of table 3 shows the effect of using different periods of water-dissolved ozone at a concentration of 400 mg / s for broiler hatching eggs in the preparation of microbes on the surface of the shell during the incubation period, noting from the table that there is a significant effect of the treatment of broiler hatching eggs with ozone gas contrasted to control, the fourth treatment was superior in the ability of (3.99 , 3.85, 2.68) ,(2.28 , 2.02) ×10 log, respectively, this indicates the ability of ozone to effectively break down colonies of microbial cells located on the surface of the egg shell in incubators, both depending on the time or period of exposure of eggs to ozone Higenyi *et al*. (2014) These findings align with those of the (Wells *et al*., 2011, Mattioli *et al*., 2020), who showed that the use of disinfectants, including ozone, led to a significant decrease in the number of microscopic germs present on the surfaces of the shells of hatching eggs, which can penetrate the Shell through the stomata into the egg, causing the death of developing embryos, Tests were conducted to determine ozone or Methanal (H2CO) might effectively eradicate microorganisms present on freshly laid, viable broiler eggs. Whistler and Sheldon (1989) reported that microbial counts were significantly lower ($P<0.05$) in water-misted and ozonated eggs (2.83% of mass) or formaldehyde-fumigated (threefold intensity) ova compared to control and water-misted eggs. However, it has also been demonstrated that the application of O_3 as a type of eggs disinfectant lowers bacteria counts; however, the impact on the potential for hatching needs to be reevaluated (Braun *et al*., 2011).

Table3. The effect of using different periods of ozone dissolved with water at a concentration of 400 mg / s for hatching eggs of broilers in the preparation of microbes on the surface of the shell during the incubation period

Treatments	Microbial counts LOG×10
T1	3.85[a]
T2	2.28[c]
T3	3.99[a]
T4	1.22[d]
T5	2.68[b]
T6	2.02[d]

Where T1 represents the control treatment where the eggs are immersed in DW-1Min, T2 represents the second treatment where the eggs are exposed to the dissolved Ozone in DW-1Min, and T3 represents the third treatment control treatment where the eggs are immersed in DW-2Min. T4 represents the fourth treatment, where the eggs are exposed to dissolved Ozone in DW-2Min; T5 represents the fifth treatment control, where the eggs are immersed in DW-5Min. T6 represents the sixth treatment, where the eggs are exposed to the Ozone dissolved in DW-5Min.The lowercase letters (a, b, c, d) are used to indicate significant differences between treatments at the level of $P < 0.05$.

According to Braun *et al*. (2011), a high degree of Humidity has a favorable impact on O_3 sterilization. The fumi container's relative humidity ranged from Sixty five to seventy percentage as a result of the chemical and O_3 sterilization; however, these treatments were unable to appreciably reduce the number of egg bacteria exterior the chamber.

The effect of using different periods of water-dissolved ozone at a concentration of 400 mg / s for hatching eggs of broilers at the time of hatching chicks. .

The static analyzed in table 4 shows the effect of using different periods of ozone dissolved in water at a concentration of 400 mg/s for hatching eggs of broilers at the time of hatching chicks.as we if we observe that there is no noticeable effect of the use of ozone on the quality of hatching time (488.53, 487.74, 488.15, 487.90, 488.25, and 488.22)h .the moment of hatching provides a useful indication of the chicks' dispersion within the hatchery; therefore, it is best to minimize this range and limit the chicks' stay to prevent drying out.

These results contradict Shahein and Sedeek (2014) who stated that the shortest hatching time range for ozonized treats compared to the control as well, contrasting the results already cited (Mona, 2011), who found that chicks hatched from eggs treated with natural antiseptics had the quickest documented hatching times. Both trial situations' differing climates, breeds, and supervisory styles could have contributed to this discrepancy.

Table 4. The effect of using different periods of water-dissolved ozone at a concentration 400 mg / s for hatching eggs of broilers at the time of hatching chicks

Treatments	Hatching time/ Hour
T1	488.53
T2	487.74
T3	488.15
T4	487.90
T5	488.25
T6	488.22

Where T1 represents the control treatment where the eggs are immersed in DW-1Min, T2 represents the second treatment where the eggs are exposed to the dissolved Ozone in DW-1Min, and T3 represents the third treatment control treatment where the eggs are immersed in DW-2Min. T4 represents the fourth treatment, where the eggs are exposed to dissolved Ozone in DW-2Min; T5 represents the fifth treatment control, where the eggs are immersed in DW-5Min. T6 represents the sixth treatment, where the eggs are exposed to the Ozone dissolved in DW-5Min. The lowercase letters (a, b, c, d) are used to indicate significant differences between treatments at the level of $P < 0.05$.

The effect of using different periods of water-dissolved ozone at a concentration 400 mg/s for hatching eggs of broilers on the qualities of external vital broilers.

Table 5 shows the effect of using different periods of water-dissolved ozone at a concentration of 400 mg/s for hatching broiler eggs on the qualities of external vital broilers. The table below indicates the presence of significant differences ($P<0.05$) in the use of ozone gas on the vital qualities of hatched chicks at the age of one day, and through observation and notation of grades, only Tona documented the increase in the values of the second, fourth, and sixth treatments of disinfectants with gas by weight compared with the control coefficients. The idea that immersing or dipping hatching eggs in O_3 water would enhance the number and quality of emerged chicks at the moment when the embryo was extracted is supported by these findings. Similar to the ability to hatch, the findings, nevertheless, indicated that immersing or soaking hatching eggs in O_3 had no detrimental effects on chick quality (Fasenko *et al*., 2009).

Table 5. The effect of using different periods of water-dissolved ozone at a concentration of 400 mg/s for hatching

Treatments	Tona specifications/100 degrees
T1	92.19^{b}
T2	97.72^{a}
T3	91.31^{b}
T4	96.02^{a}
T5	90.91^{b}
T6	96.07^{a}

broiler eggs on the external vital qualities of broilers.

Where T1 represents the control treatment where the eggs are immersed in DW-1Min, T2 represents the second treatment where the eggs are exposed to the dissolved Ozone in DW-1Min, and T3 represents the third treatment control treatment where the eggs are immersed in DW-2Min. T4 represents the fourth treatment, where the eggs are exposed to dissolved Ozone in DW-2Min; T5 represents the fifth treatment control, where the eggs are immersed in DW-5Min. T6 represents the sixth treatment, where the eggs are exposed to the Ozone dissolved in DW-5Min. The lowercase letters (a, b, c, d) are used to indicate significant differences between treatments at the level of $P < 0.05$.

CONCLUSIONS

This study suggests that ozone treatment of broiler hatching eggs positively influences hatching ratio, chick weight, late-stage embryo characteristics, microbial load on eggshells, and vital qualities of hatched chicks. However, variations in effects were observed based on different ozone exposure periods, emphasizing the importance of optimizing ozone treatment conditions for enhanced hatchery performance. The findings contribute valuable insights to the application of ozone as a disinfectant in poultry hatchery management.

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