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Research Article

EFFECTS OF OZONE ON EGG SHELL MICROBIAL LOAD, HATCHING TRAITS AND CHICK PERFORMANCE IN QUAIL EGGS

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ABSTRACT

Objective: The aim of this study was to establish the effects of ozone on eggshell total aerobic mesophilic bacteria, egg weight loss, hatching traits, embryo deaths and chick performance.

Methods: A total of 825 fresh hatching quail eggs were randomly distributed into five groups. Treatment groups were formed as control group (benzalkonium cloride application; C), 1% ozone application (O1), 3% ozone application (O3), 5% ozone application (O5) and 7% ozone application (O7).

Results: There were no significant differences between the groups in terms of egg weight loss, hatching traits, embryo deaths, chick performance and livability. Ozone treatment had lower levels of total bacteria, especially in O7 group, than control eggs for the entire incubation period.

Conclusion: As a result, the application of ozone can be used as an alternative to natural chemical disinfectant without adversely affecting the incubation results and chick performance.

Key words: Ozone, hatchability, microbial load, hatching eggs, performance

INTRODUCTION

The egg is considered clean at the time of laying, it can be contaminated with microorganisms depending on the environmental conditions. Microorganisms passing through the pores of contaminated eggs can kill the embryo or adversely affect the chick performance [1, 2]. Therefore, it is necessary to sanitation hatching eggs for a successful incubation [3, 4].

Disinfectant solutions and formaldehyde gas are generally used for pre-incubation disinfection of hatching eggs. However, in recent years the use of solutions has been reduced due to the wetting of eggshells in the disinfection process of eggs and thus the possibility of microbial transmission. The use of formaldehyde gas in the form of fumigation is forbidden to be used as it harms the environment and the embryo. Ozone is gaseous at room temperature. It is colorless and has a characteristic odor [5, 6]. Ozone is a gas with very high oxidation power and the strongest known disinfectant [7]. Depending on its dosage, ozone completes its half-life shortly and disappears with its odor and taste, turning it into oxygen without leaving any residue. To our knowledge, studies on the effect of hatching eggs on shell microbiology are limited. Studies in this regard have different doses, different poultry of eggs and applications [8-10]. This study was conducted to determine the effect of pre-incubation ozone application on eggshell microbial load, hatching traits and chick performance in hatching eggs.

MATERIAL AND METHODS

Eggs and treatments

A total of 825 hatching quail eggs from a commercial farm in Konya were used in this study. The eggs were randomly distributed to 5 treatment groups Treatment groups were formed as control group (benzalkonium cloride application; C), 1% ozone application (O1), 3% ozone application (O3), 5% ozone application (O5) and 7% ozone application (O7).

Ozone generation equipment

Ozone gas is obtained using ozone generator (pro 400, Prodozon, Ankara, Turkey). The concentration of ozone was recorded with a portable ozone detector (Aeroqual,

series 205, Auckland, New Zealand). Treatment were performed in a custom-made chamber at 22 ± 2°C and 75% relative humidity.

Egg Weight Loss

A sample of 70 eggs from each group were individually weighed and numbered before the ozone application and on the 14th day of the incubation, and egg weight loss was calculated from these values. Unfertilized eggs and dead embryo eggs were not evaluated for egg weight loss [11].

Incubation Processes

Eggs were incubated for 14 days in machines containing 37.5°C and 55% humidity after ozone application. The eggs were then transferred to machines containing 37.0°C and 75% humidity for hatching. The eggs were automatically turned every 2 h until the last three days of incubation.

Microbiological Analyses

After the application of ozone, 5 eggs were randomly taken from each group for microbiological analysis on the 1st, 7th and 14th day of incubation. Egg samples were transferred aseptically to sterile stomacher bags and 50 ml of sterile phosphate buffer solution was added (PBS; pH 7.2). The whole surface of the egg was washed in these solutions and total bacteria were detected [12]. After washing, sterile petri dishes were germinated. Ten fold serial dilutions of the samples were prepared using sterile PBS (Gentry and Quarles, 1972; Jones et al., 2002). Colonies were expressed as log cfu/egg. For determination of total mesophilic aerob bacteria (TMAB) appropriate dilutions were spread on plate-count agar (Merck, Whitehouse Station, NJ). Then petri dishes were incubated at 37°C for 48 h, according to American Public Health Association (1992).

Hatching

After the chick hatching was completed, the unhatched eggs were broken and macroscopic analysis was performed. Embryo dead stages were classified according to the Aygun et al., (2012) [4].

Chick Performance

At the end of the incubation, 75 chicks from each group were individually weighed at the beginning and end of the trial for performance data. Chicks were reared with 15 chick per 416 cm^2. Chicks were fed feed containing a diet (2,900 kcal ME/kg and 24% CP) during the 10-day growing period. During the treatment, the pens temperature was set to 33 °C. Continuous light application was made as lighting program during the rearing period. The chicks are individually weighed at the beginning of the reared period and at the end of the reared period in order to determine the relative growth (RG). During the reared period, chick deaths are monitored and recorded on a daily basis. Livability was calculated from these data.

Statistical analysis

Comparison of the means of the groups was made using analysis of variance. When the means were found to be different at 0.05 significance level, then Duncan multiple range test was applied.

RESULTS and DISCUSSION

Effects of ozone treatment on egg shell microbial load is presented in Table 1. The O7 ozone treatment significantly reduced total aerobic mesophilic bacteria by approximately 1.60 log units compared with the control group for the entire incubation period ($P<0.05$). However, there was no significant difference in the total aerobic mesophilic bacteria between the control and other ozone treatment groups. Whistler and Sheldon [13] that egg shell microbial load was approximately 2.8 log lower with ozone application compared to the control group. Rodriguez-Romo and Yousef [14] found a reduction microbial of 2.6 log on table eggs after an ozone application for 8 min (15 lb/in^2 gauge).

Egg weights and egg weight loss values and statistical results are given in Table 2. There was no statistically significant difference between the groups in terms of egg weights and egg weight loss. Egg weight loss ranged from 10% to 11% in all groups. The egg weight loss rates were determined between 10.57 and 11.30% among all groups. No statistically significant difference was found in egg weight loss during incubation from 0 to 14 day (C, 10.57%; O1, 10.63%; O3, 10.75%; O5, 10.72%; and O7, 11.30%). The egg weight loss until the 14th day of incubation generally ranges from 9.0 to 11.0% [4, 11, 15, 16]. Egg weight loss is an important parameter for optimum hatching results and high amount of water loss a disadvantage for the normal embryonic development [17-19]. Our results shown that ozone treatments does not have a detrimental effect on the eggshell cuticle. Peebles [17] stated that if the cuticle layer is damaged, more water is lost from the pores of the eggshell. On the other hand, Fuhrmann [20] reported that low ozone doses (10 ppm) destroyed the cuticle proteins completely. This difference may be due to the use of lower ozone doses in our study.

The differences between group means in terms of incubation characteristics and embryonic mortality were statistically insignificant (Table 3). There were no differences in the hatchability and embryonic mortality among groups. These results are consistent with studies of Aygun [4], Yildirim and Ozcan [21], Copur [22], and Fasenko [23] that various disinfectant applications (propolis, Oregano and Oregano, respectively) do not adversely affect hatching traits. On

the other hand, Williams [1] and Cadirci [24] stated that formaldehyde fumigation negatively affects incubation results. Ozon application (3.03% ozone, 2 h) significantly reduced hatchability compared to the C group [13].

The effects of ozone application on BW d 1, BW d 10, RG, and livability are given in Table 4. Differences between groups in terms of BW, RG and livability were found to be statistically insignificant. It has been determined that ozone application does not have a positive or negative effect on growth characteristics and livability. Pre-incubation disinfectant applications did not have a negative effect on the performance of chicks [4, 22, 23].

CONCLUSION

Ozone egg has not affected hatching traits and chick performance positively or negatively. Ozone has significantly reduced microbial load on the surface of eggs. According to the current study, ozone can be used as an alternative to chemical disinfectants in the disinfection of eggs.

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CONFLICT of INTEREST

The authors declares that they are no conflict of interest.

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Table 1. Effects of ozone on total aerobic mesophilic bacteria during incubation period (log cfu/egg)

Treatment	1 d	7 d	14 d
C	2.89[a]	3.04[a]	3.07[a]
O1	2.91[a]	2.94[a]	3.11[a]
O3	2.83[a]	2.90[a]	2.90[a]
O5	2.33[a]	2.42[a]	2.50[b]
O7	1.21[b]	1.28[b]	1.48[c]
SEM	0.022	0.022	0.025
P value	<0.05	<0.05	<0.05

[a-c] Different letters in the same column are statistically important ($P<0.05$).
C: Control (benzalkonium chloride), O1: 1 ppm Ozone, O2: 3 ppm Ozone, O5: 5 ppm Ozone, O7: 7 ppm Ozone.
SEM: Standard error mean

Table 2. Effect of ozone on egg weight loss during incubation period

Treatment	Set egg weight (g)	Transfer egg weight (g)	Egg weight loss (%)
C	12.11	10.83	10.57
O1	12.18	10.88	10.63
O3	12.20	10.88	10.75
O5	12.19	10.89	10.72
O7	12.12	10.75	11.30
SEM	0.07	0.07	0.22
P value	>0.05	>0.05	>0.05

C: Control (benzalkonium chloride), O1: 1 ppm Ozone, O2: 3 ppm Ozone, O5: 5 ppm Ozone, O7: 7 ppm Ozone.
SEM: Standard error mean

Table 3. Effect of ozone on fertility, hatchability, and embryonic mortality.

Treatment	Fertility (%)	Hatchability of set eggs (%)	Hatchability of fertile eggs (%)	Embryonic mortality (% of fertile eggs)		
				1-9 d	10-16 d	17-18 d
C	90.00	84.67	94.07	3.65	0.77	1.51
O1	90.67	80.00	88.07	8.24	0.23	1.46
O3	90.67	83.33	91.95	6.62	0.71	0.71
O5	90.66	84.67	93.36	4.42	2.22	0.00
O7	88.00	79.33	90.18	8.25	0.77	0.80
SEM	1.522	2.376	1.934	1.868	0.814	0.668
P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

C: Control (benzalkonium chloride), O1: 1 ppm Ozone, O2: 3 ppm Ozone, O5: 5 ppm Ozone, O7: 7 ppm Ozone.
SEM: Standard error mean

Table 4. Effect of ozone on BW, relative growth and livability

Treatment	BW d 1 (g)	BW d 10 (g)	Relative growth	Livability (%)
C	8.67	45.62	427.77	98.67
O1	8.88	44.76	408.85	100.00
O3	8.82	46.57	426.80	100.00
O5	8.83	44.84	412.58	100.00
O7	8.86	45.06	411.97	97.33
SEM	0.074	0.632	7.306	0.592
P value	>0.05	>0.05	>0.05	>0.05

C: Control (benzalkonium chloride), O1: 1 ppm Ozone, O2: 3 ppm Ozone, O5: 5 ppm Ozone, O7: 7 ppm Ozone.
SEM: Standard error mean