Poultry Studies, 21(2), 61-64
http://doi.org/10.34233/jpr.1597124

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RESEARCH PAPER

POULTRY STUDIES

Alternative Application for Fumigation: Ozone Treatment During Incubation*

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*Presented In: International Poultry Congress 2024, Bursa, Turkey

Article History
Received: Oct 18, 2024
Accepted: Dec 02, 2024
First Online: Dec 05, 2024

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Keywords
Ozone
Incubation
Fumigation
Eggshell microbial load
Broiler

Abstract

In this study it was aimed to investigate effects of ozone treatment during incubation of broiler eggs as an alternative application for fumigation. A total of 240 eggs from 45-week-old Ross 308 broiler breeder flocks were utilized. The eggs were weighed and randomly assigned to two groups: one without ozone and one with ozone. A commercial ozone generator was placed in the incubator, with ozone gas generated for 1 minute every hour to provide ozone gas at a concentration of 0.050 ppm. Throughout the 18-day incubation phase, ozone gas was applied in three-day cycles. On the 18th day of incubation, six randomly selected eggs from each experimental group were placed in sterile bags to assess the microbial load of the eggshell. Furthermore, six eggs were randomly selected from each experimental group to assess embryo growth. The total aerobic bacteria and Coliform sp. count were found higher in control than ozone group ($P < 0.01$). The yeast mold count, egg weight, embryo weight and embryo yolk sac weight were found to be similar between the groups ($P > 0.05$). It can be determined that using ozone instead of fumigation during incubation in broiler chicken eggs reduces eggshell total aerobic bacteria and coliform counts, but not affected to embryo traits such as weight, yolk sac weight and length.

Introduction

In poultry, from the moment the egg is laid until it is incubated, it is exposed to many environmental conditions and interventions such as collection, transportation and storage, and the eggshell can be contaminated by microorganisms. As a matter of fact, it has been emphasized in various studies that many different microorganisms such as *Salmonella*, *Streptococcus, Escherichia coli, Staphylococcus* and *Yersinia* can be found on eggshells (Jones *et al*., 2004; Musgrove *et al*., 2008; Koç, 2015). Microorganisms that multiply under incubation conditions quickly enter the egg through the pores on the eggshell, damaging the developing embryo and reducing success in incubation (Berrang *et al.*, 1999). Therefore, sanitation is an extremely critical and necessary issue in hatching eggs. Many different methods are used for sanitation of hatching eggs. Among these include fumigation with formaldehyde gas (Ledoux, 2002), immersion in disinfectant solutions, and spraying with disinfectant solutions (Moats, 1981). Among these methods, especially the use of disinfectant solutions causes wetting of the eggshell and therefore an increase in bacterial permeability, and the fumigation method with formaldehyde gas has decreased in use due to its toxic and carcinogenic effects. Therefore, in recent years, emphasis has been placed on alternative methods

for sanitation of hatching eggs. Among these; use of UV light (Al-Shammari *et al*., 2015), application of colloidal silver to eggshells (Batkowska *et al.*, 2017) and use of various natural/herbal extracts (Uluçay and Yıldırım, 2010; Çopur *et al.*, 2011; Batkowska *et al*., 2018). In recent studies, the use of ozone gas for sanitation has been mentioned (Wlazlo *et al*., 2020; Gogaev *et al.*, 2021). One well-known very reactive antibacterial agent is ozone (O_3). Ozone is in gaseous form at room temperature and is colorless and has a distinctive odor. FDA accepted ozone application as an antimicrobial agent for foods in 2001. Ozone has other benefits in addition to its bactericidal action, such as low toxicity and ease of handling (Braun *et al.*, 2011). The embryo development is an important factor. Therefore, we applied the ozone treatment as continuously during the 18 day of incubation period in 3 days-cycle to bring to light effects on embryo development, as stimulating effect or inhibiting effect. This study was conducted to investigate effects of ozone treatment during incubation of broiler eggs as an alternative application for fumigation.

Materials and Methods

This study was carried out in Bursa Uludağ University Faculty of Agriculture Research and Application Unit. Practices regarding the care and use of animals for research purposes were in accordance with the laws and regulations of Turkey and approved by the Animal Use and Ethical Committee of Uludağ University (Approval Number 2023-12/02). A total of 240 eggs obtained from 45-week-old Ross 308 broiler breeder flocks were used in the study.

The non-fumigated eggs were weighed and randomly divided into two groups: the group without ozone (control group) and the group with ozone (n: 3 trays, 40 eggs/tray). The eggs were placed into two incubators with the same features, which were calibrated before the experiment. A commercial ozone generator placed in the incubator and ozone gas activated for 1 minute per hour and provide ozone gas at the level of 0.050 ppm. Ozone gas was applied in 3-day cycles during the 18-day incubation period. During this period, a temperature of 37.2-37.5ºC and 55% of relative humidity were applied in incubators.

On the 18th day of incubation, randomly selected six eggs from each experimental group were sampled to determine the microbial load of the eggshell. Egg samples were placed in sterile containers containing 50 mL of phosphate buffered saline solution. Serial dilutions of samples in phosphate buffered saline were placed on sterile substrates to obtain total aerobic bacteria, coliforms, yeast-molds (Gentry and Quarles, 1972; Jones *et al*., 2002). After the incubation period, colonies were counted, and the result was expressed as colony forming unit (CFU)/1 mL of egg liquid. The plate count agar, violet, red bile agar, potato malt agar was used for total aerobic bacteria, coliforms, yeast-molds count respectively. On the 18th day of incubation, besides, six eggs were randomly sampled from each experimental group for determination of egg weight at transfer, embryo weight, embryo yolk sac weight and embryo length. The embryo length was measured from the tip of the beak to the tip of the longest toe by placing the embryo face down on a flat surface and straightening the left leg. The embryo parameters were determined by the formula given below;

$$\text{Embryo ratio (\%)} = \frac{\text{Embryo weight without yolk sac}}{\text{Egg weight}} \times 100$$

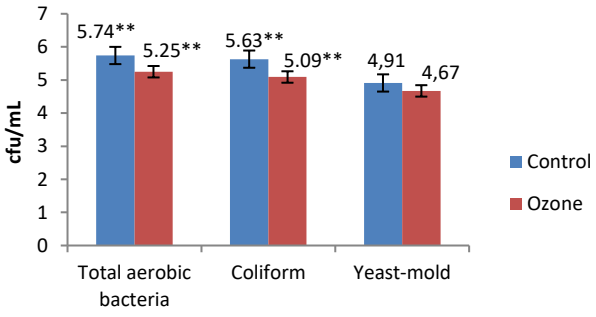
$$\text{Embryo yolk ratio (\%)} = \frac{\text{Embryo yolk sac weight}}{\text{Egg weight}} \times 100$$

Statistical analysis

The study was conducted on a completely randomized design and data was analyzed by analysis of variance using General Linear Models (Minitab, 2013). Analysis of percentage data were conducted after arcsine square root transformation of the data. Differences in investigated traits were analyzed by two sample T-test (Minitab, 2013). Data were presented as mean ± standard error in all the tables. Differences were considered significant at $P \leq 0.05$ and the statistical difference at $P < 0.10$ was described as a tendency.

Results

The effect of ozone application on eggshell microbial load at 18 days of incubation are given in figure 1. The total aerobic bacteria count was found higher in control group than ozone group (5.74 *versus* 5.25 cfu/mL respectively; P<0.01). The number of *Coliform sp*. was found higher in control group than ozone group (5.63 *versus* 5.09 cfu/mL respectively; P<0.01). The yeast mold count was found similar between the groups (P>0.05).



Bacterial countof eggshell at 18 day of incubation

Figure 1. The effect of ozone application on eggshell microbial load

The effect of ozone application on embryo parameters at 18 days of incubation are given in Table 1. The egg weight, egg weight at transfer, embryo weight, embryo yolk sac weight, embryo length, embryo ratio and embryo yolk ratio were found to be similar between the groups (P > 0.05). But egg weight at transfer was numerically lower in ozone group than control group (P=0.069). The embryo length was numerically higher in ozone group than control group (P=0.082).

Table 1. The effect of ozone application on embryo parameters

Embryo parameters	Control	Ozone	P - Value
Egg weight, g	68.84 ± 0.39	68.77 ± 0.29	0.802
Egg weight at transfer, g	63.43 ± 0.48	62.32 ± 0.24	0.069
Embryo weight, g	28.26 ± 1.81	30.28 ± 0.37	0.202
Embryo yolk sac weight, g	17.46 ± 1.37	15.74 ± 1.49	0.237
Embryo length, cm	14.00 ± 0.10	14.98 ± 0.50	0.082
Embryo ratio, %	44.55 ± 2.53	48.57 ± 0.52	0.115
Embryo yolk ratio,%	27.54 ± 2.36	25.26 ± 2.40	0.324

Discussion

Braun *et al*., (2011) suggested that ozone gas is suitable for treatments in hatcheries. Thus in the study, total aerobic bacteria and *Coliform sp*. count were higher in control group than ozone group. Similar to our findings Koç and Aygün (2021) reported that the 7 ppm ozone application reduced total aerobic mesophilic bacteria on egg shell. But, they also reported that 1, 3, 5 ppm ozone application did not have any effect on total aerobic mesophilic bacteria on egg shell. However, Rodriguez-Romo and Yousef (2005) reported that after ozone application reduction in egg shell microbial load. And, Whistler and Sheldon (1989) reported that the application of ozone gas to eggs reduced the number of *E. coli,Pseudomonas, Fluorescens, S. typhimurium, Proteus* species and *Aspergillus fumigatus* species on the egg shell. However, Bailey *et al.* (1996) found that after treatment with 0.2-0.4ppm ozone, 90.9% of egg shells remained contaminated with *Salmonella* until hatching. In the study there was no any differences for yeast mold count og egg shells between the groups.

In the study, embryo length was tend to be higher in ozone group than control group. Thus, Gogaev *et al*. (2021) discovered that the weight and length of quail embryos in incubated eggs treated with ozone for 20 minutes at a dosage of 10 mg/m3 were considerably greater than the control, 10- and 30-minute treated groups. However, in the study there were no any differences for egg weight, embryo weight, embryo yolk sac weight, embryo ratio and embryo yolk ratio between the groups. Thus, according to Fuhrmann *et al*. (2010) lower than 50 mL L^{-1} ozone dosages resulted in oxidative reactions at the egg surface, which are likely innocuous to the developing embryo. Ozone exhibits dosage dependence. The survival and development of chick embryos exposed to ozone in ovo are significantly influenced by their impact and reaction time (Hoffman *et al*., 2005). Oxygen is typically depleted from the embryo during egg incubation. As ozone disintegrates into its constituent atoms, ideal conditions for embryonic development are created (Gogaev *et al*., 2021). Low ozone levels (10 ppm) totally damaged the egg cuticle proteins, according to Fuhrmann *et al*. (2010). More water escapes the eggshell through its pores if the cuticle layer is compromised (Peebles, 1998). Thus, in the study, egg weight at transfer was tend to be lower in ozone group than control group. It may also be due to the use of very low ozone doses in our study. However, Koç and Aygün (2021) reported that there was no any differences for egg weight and transfer egg weight between the ozone groups and control, but the transfer egg weight was numerically lower in the 7 ppm ozone application group than control and 1, 3, 5 ppm ozone groups.

Conclusion

Ozone has considerably decreased the amount of microorganisms on egg surfaces. It can be concluded that ozone application (0.050 ppm) as an alternative to fumigation during incubation in broiler chicken eggs is effective in reducing of eggshell microbial load without harmfull effect on embryo development. However, it should be recommended to perform extended studies to investigate the various application ways of ozone in egg fumigation with considering effecting mechanism related to embryo development and hatchability.

Ethical Statement

This study was approved by the Bursa Uludağ University Animal Experiments Local Ethics Committee (Approval no: 2023-12/02).

Funding Information

The authors received no specific funding for this work.

Presented In: International Poultry Congress 2024, (8-11 May 2024) Bursa, Turkey

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