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Research Article
Energized Oxygen Treatment in Drinking Water for Laying Hens: An Alternative Disinfectant

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Abstract

Background and Objectives: The objective of this study was to examine the effects of adding energized oxygen to drinking water for laying hens and evaluate quantities of microorganisms in fecal samples. **Materials and Methods:** Oxygen generated gas was hosed into the drinking water tank of the laying commercial farm. Cloacal swabs and conveyor samples taken for fecal samples were collected in three runs for microbiological analysis as follows: The first was before the application of energized O_2 gas as a control, the second was after one week and the last was two weeks later. **Results:** The total bacteria and *E. coli* count from control to successive samples in cloacal swabs and conveyor samples decreased. However, there were no effects on fungal count. **Conclusion:** Energized oxygen may be used as an effective disinfectant to reduce microbiological exposure and improve the health of laying hens.

Key words: Energized oxygen, laying hen, total bacteria/fungi count, total *Escherichia coli* count, drinking water disinfection

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Water is a physiologically necessity for all animals. Water consumption in poultry production is influenced by various factors, including animal species, activity, water quality, water temperature, environmental temperature, feed consumption and health[1-3]. Different water sources may be used in rural areas for animal consumption, such as springs, shallow, deep or artesian wells, lakes and creeks. However, the microbiological quality of water must be controlled to ensure safe use[4].

Disinfection is an essential part of an effective biosecurity program to prevent entry of disease agents and foodborne pathogens in birds[5,6]. Disinfectants used as drinking water sanitizers should inactivate microbes, control biofilms and neutralize undesired contaminants but should not leave behind residue. In poultry industry, sodium hypochlorite, chlorine gas, calcium hypochlorite, iodination, ultraviolet light and ozone applications (increased in recent years) have been used for drinking water sanitation as disinfectants/oxidizers[7,8].

"Energized oxygen (EO)" is a gas that is generated from the stratosphere through sunrays and takes all its strength and efficiency from oxygen through Profoks generators[9]. This technology derives all of the oxygen it produces from the air. EO, unlike other disinfectants, turns oxygen into pure oxygen in an hour and leaves no residue, free radicals, or oxidized toxic compounds in the environment[9].

The objective of this study was to evaluate the use of EO in drinking water to reduce *E. coli*, total aerobic bacteria and total fungi/yeast concentration in feces of laying hens.

MATERIALS AND METHODS

Ethics statement: This study was carried out in a commercial layer poultry farm of İşlerler Company in the Burdur Province of Turkey from January 2016 to April 2017.The owner of the farm provided their permission for the experiment.

Birds and housing: A total of 8500 29-week-old Lohmann laying hens were housed in 60×60 cm cages (8 birds in each cage) in a 25×10 m farm building. Water and feed were provided *ad libitum*. Average body weight of birds at 21 weeks (start to laying period) of age was 1650-1700 g and the average body weight at the beginning of the experiment was 1850-1900 g.

Energized oxygen device: The EO gas device was connected to the farm water supply system of the layer house. Generated gas was hosed and diffused into the 40 t drinking water tank at 1.6 g sec^{-1}.

Experimental design: In this experiment, cloacal swab and conveyor sample collection were conducted in three runs as follows: First was before setting up energized O_2 gas as a control, following one after one week and one after two weeks.

Conveyor samples: Samples were collected from the ends of the conveyor belts across the house. Belts were run before or during sampling to accumulate feces on the scrapers; 25×2 g feces were collected from each belt. Total fecal sample collection was 200-300 g.

Cloacal swabs: Cloacal swab samples were carefully obtained, taking care to avoid contamination from the outside of the cloaca of each bird and were placed in Amies transport medium. Five pens with five birds each were used for each treatment group, within a randomized block layout. Thus, a total of 25 birds were used for each treatment. All samples were cooled to 4-8°C in an icebox and immediately transported to laboratory for processing.

Microbiological analysis: twenty grams fecal samples were homogenized with 180 mL sterile saline solution from the conveyor belts. Five pooled swab samples from cloaca were put into a sterile falcon test tube (50 mL) with 45 mL sterile saline solution (0.85% NaCl; Sigma-Aldrich). Each sample mixed in this solution tube was brought to volume (10 mL) with 0.9% sterile saline solution. Samples (0.1 mL) were serially diluted via 10-fold dilutions (from 10^1 to 10^{10}). Violet Red Bile Lactose agar (VRBA, OXOID), Plate Count agar (PCA, OXOID) and Sabouraud Dextrose agar (SDA, OXOID) supplemented with chloramphenicol (0.05 mg mL^{-1}) were used for the enumeration of total aerobic bacterial count, *E. coli* and total fungal count, respectively. All PCA and VRBA plates were incubated at 37°C aerobically, for 24-48 h and all SDA plates were also incubated at 25°C, aerobically, for 5-10 days. Following incubation, colonies formed on double inoculated media were counted and the average number of colonies on double medium was taken. All microbial counts were converted to CFU×log 10 g^{-1} analysis to normalize data distribution[10].

RESULTS AND DISCUSSION

Water is essential to nutrient transportation, enzymatic and chemical reactions in the body, homeostasis and body temperature regulation in poultry[11,12]. In fact, high-quality drinking water can boost the immune system of poultry[13,14].

In this study, the effects of experimental treatments on microorganism counts were evaluated. EO-treated water significantly reduced quantities of total aerobic bacteria and *E. coli* in conveyor and cloacal samples (Table 1). Microbial results (total aerobic) from control to successive samples in cloacal swabs and conveyor samples were reduced from both in cloacal swabs and in conveyor samples (Fig. 1 and 2).

Despite these findings on bacteria counts, this effect was not observed for total fungi/yeast counts. In general, yeast and molds, especially fungal spores, are more resistant than bacteria to disinfectants[15]. Newell *et al.*[16] proposed that fungal cells may have a barrier to one or more biocides, or to inactivate a biocide due to the presence of existing enzymes. There is little published information available on the effect of drinking water disinfection on laying hens, as most research has been conducted on broiler farms[4,17-19]. Disinfecting water, equipment and controlling microbiological issues are crucial to minimize water-borne diseases for poultry. According to previous studies diseases that can be transmitted to the bird flock though drinking water may originate from water contamination by feces and secretions of sick birds, such as in the case of Salmonella, Campylobacter, Pseudomonas and *E. coli*, respectively[4,20-24].

Contamination of table eggs by these microorganisms significantly affects both poultry production and public health. High eggshell contamination is positively correlated with total airborne bacteria count in the housing system and initial eggshell contamination[25]. Water supply must therefore be well control led within laying hen production enterprises and bio security control measurements must be developed to ensure safe egg production[26].

Several studies have reported that drinking water acidification and disinfection is essential to improve performance and reduce microbial contamination[27,28]. Commonly used disinfectant methods include chlorination, iodination, ultraviolet light and ozone applications[8]. Chlorination is a highly accepted method in water disinfection for human and animal drinking purpose and has been used for many years[17,29]. Chlorine levels between 1 and 2 ppm in drinking water are recommended and are well tolerated by birds[30,31].

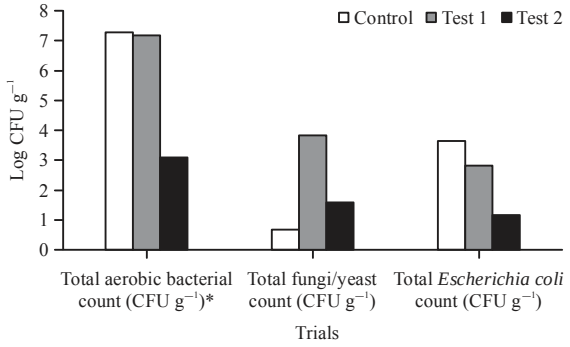

Fig. 1: Differences between microorganism counts of conveyor samples across sampling time

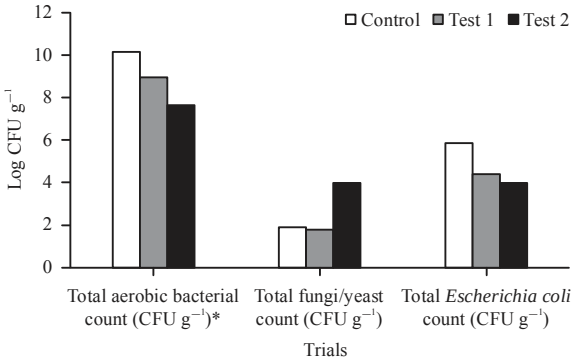

Fig. 2: Differences between microorganism counts of cloacal swab samples

Table 1: Total microorganisms count (control and one and two weeks after energized O_2 gas hosed)

Samples	Aerobic bacteria (CFU g^{-1})*	Fungi/yeast (CFU g^{-1})	*Escherichia coli* (CFU g^{-1})
Conveyor samples			
Control	10.21	1.89	5.91
First sample	8.99	1.80	4.43
Second sample	7.69	3.99	3.97
Cloacal swabs			
Control	7.33	0.69	3.65
First sample	7.21	3.84	2.81
Second sample	3.11	1.60	1.16

*CFU g^{-1}: Colony-forming unit in per gram of samples (log_{10})

Adding organic acid to drinking water helps reduce pathogens in the water and crop/proventriculus to regulate gut microflora, increase the digestion of feed and improve growth performance[28]. Acikgoz *et al.*[17] used formic acid as a disinfectant in drinking water but did not find significant reductions in total organism and *E. coli* counts in intestinal microflora in broilers. Chaveerach *et al.*[32] found that total aerobic bacteria was significantly higher in the cecal content of broilers compared with the untreated group via acidified drinking water.

The physicochemical properties of ozone: Its relatively high solubility in water and high redox potential, which destroys the structure of microorganisms, have enabled its commercial application in the 1880 s for deodorization of industrial waste and disinfection of drinking water[33]. Furthermore, since ozone is unstable in water, in which some ozone-resistant compounds occur, including pesticides and chlorinated solvents, only partial oxidation may take place, while EO is stable in water and compatible with animal and human health[9,34]. Use of EO prevents unfavorable color, smell, cloudiness and moss growth regardless of disinfection properties; it also does not cause abrasion or corrosion in water pipes and it can also be used safely in food products[9].

CONCLUSION

Energized Oxygen can be sued as a disinfectant to reduce total aerobic bacteria and *E. coli*, in addition, treatment can also prevent moss build up. Further investigations are necessary to confirm and increase knowledge for more statistically supported data to study the effects of EO treatment on bird health and production.

SIGNIFICANCE STATEMENT

This is the first study to evaluate EO usage in drinking water for laying hens and measure microbial levels. Using EO to reduce microbiological exposure may provide an effective disinfectant method to improve bird health. This method could be useful for farmers to resolve problems related to disinfection, water hygiene and water quality.

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