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APPLICATION OF SLIGHTLY ACIDIC ELECTROLYZED WATER FOR DISINFECTION IN A BROILER FARM

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ABSTRACT With the rapid development of intensive animal farms, epidemic diseases in animal farms are more and more sever, it is necessary to develop effective disinfectants for reducing pathogens in animal farms to improve the health of animals. Slightly acidic electrolyzed water (SAEW, pH 5.0-6.5) generated by electrolysis of a dilute NaCl solution or HCl in an electrolytic cell shows promise as an environmentally friendly broad spectrum microbial decontamination agent. The efficiency of SAEW for inactivating aerobic plate counts (APC) in broilers' houses was examined and compared with chemicals which are commonly used. The effect of SAEW on the production performance of broilers was also determined. Results indicate that SAEW with available chlorine of 80 mg/l can effectively reduce the population of APC in broilers' houses. The percent of reduction of APC in the indoor air by SAEW (80.2%) was higher than that of povidonolodine solution (75.2%), glutaraldehyde-containing disinfectant (68.7%) and iodine-containing disinfectant (54.0%). The APC in the disinfection pool treated with SAEW was 0 cfu/ml in the first 6 h, after 12 h the number of bacteria sharply increased. Moreover, the percent reduction in APC on feed carts treated with SAEW and sodium dichloroisocyanurate for 1 min was 92% and 85%, respectively. According to statistical analysis of broiler production performance, the survival rate and feed/gain was 94.5% and 1.85, respectively for using SAEW. Therefore, SAEW is an available option for disinfection in poultry farms with harmless to human and animals and no environmental pollution.

Keywords: Slightly acidic electrolyzed water, Poultry farms, Disinfection, Pathogens.

INTRODUCTION With the level of large-scale and intensive broiler feeding enhanced, in the high-density rearing conditions, the broiler houses are filled with a variety of harmful gases and pathogenic micro-organisms, while broiler chickens grow fast with poor physique easily impacted by pathogenic bacteria and viruses surrounding in the environment, so disinfection is particularly important. At present, however, the chemical disinfectant is the most commonly used which brings drug residues and environmental problems. Therefore, it is very necessary to found a SAEW type disinfectant and SAEW disinfection process which is efficiently and environmentally friendly.

Disease outbreaks increase farm costs, decrease income and have a negative impact on consumer confidence. Cleansing and disinfection play an important role in the prevention and control of infectious diseases of man and animals. Their adequate application in animal husbandry during production, transportation of animals, etc. will lead to a significant reduction in number of pathogenic microorganisms. Generally, chlorinate-based, formaldehyde, glutaraldehyde disinfectants are generally used as in poultry farms of China. But these chemicals are highly caustic and potential harmful to human and animals. Therefore, developing new disinfectants with high efficiency, harmless and no pollution is necessary to animal farms.

Acidic electrolyzed water (AEW) or electrolyzed oxidizing water has been regarded as a novel antimicrobial agent with environmentally friendly broad spectrum microbial decontamination in recent years. Many studies have proved that AEW has powerful bactericidal activity for reducing pathogens (Kim et al., 2003; Park et al., 2004; Huang et al., 2008). However, the utilization of AEW has limited potential for long-term applications because of its strong acidity ($\text{pH} < 2.7$) (Ayebe et al., 2005; Guentzel et al., 2008). At this low pH, dissolved Cl_2 gas can be rapidly lost due to volatilization, decreasing the bactericidal activity of the solution with time (Len et al., 2002, Cui et al., 2009) and adversely affecting human health and the environment. Moreover, the strong acidity of AEW may cause corrosion of equipment and consequently limit its practical application.

Slightly acidic electrolyzed water (SAEW) with a pH value of 5.0-6.5 and containing higher hypochlorous acid (HOCl) is generated by electrolysis of a dilute hydrochloric acid and/or NaCl solution in a chamber without a membrane. SAEW has been reported as an advantage to antimicrobial activity agent with low available chlorine and high stability (Abadias et al., 2008; Cao et al., 2009; Quan et al., 2009; Koide et al., 2009), and there has been a growing interest in SAEW as an alternative method of disinfection recently. The application of SAEW may improve the bactericidal activity with maximizing the use of hypochlorous acid, reduce corrosion of surfaces, and minimize human health and safety issues from Cl_2 off-gassing (Guentzel et al., 2008).

The objectives of this paper were to investigate the disinfection effect of slightly acidic electrolyzed water in broiler houses, to determine the influence of SAEW on the production performance of broilers and to provide some technical parameters for practical use.

MATERIALS AND METHODS

Experimental broiler farm The experiments were carried out in one of the broiler farms in a large-scale commercial broiler company in Penglai, Shandong, China, from July to September in 2008. The farm has 21 broiler houses, with a dimension of $102 \times 8 \times 3$ m (length \times width \times height) of each house. All houses have a multi-span structure, with three stacking layers of cages from bottom to top, and four rows of cages in width. The distance among the rows is 50 cm. Three aisles are designed in the middle and both sides of houses. Every house accommodates 14586 chickens. Manual spraying disinfection with chemicals is used and a sodium hydroxide pool in the entrance of house is designed.

Preparation of slightly acidic electrolyzed water Slightly acidic electrolyzed water (SAEW) was generated using a SAEW generator (Shenyang Dongyu Xinbor Technology Co. Ltd.), basically consisting of an electrolytic cell with anode and cathode electrodes and no separating membrane. Aqueous HCl (5%, v/v) and NaCl (10%, w/v) solutions were pumped into a chamber to mix with tap water by two adjustable valves, respectively. The mixture was electrolyzed continuously to produce SAEW water with pH of 6.52, oxidation reduction potential ORP of 821.5 mV and ACC of 150 mg/l. The pH and ORP values were measured using a dual scale pH/ORP meter (HM-30R, DKK-TOA Corporation, Tokyo, Japan) with a pH electrode (GST-5741C) or an ORP electrode (PST-5721C). The available chlorine concentration (ACC) was determined by a colorimetric method using a digital chlorine test kit (RC-2Z, Kasahara Chemical Instruments Corp., Saitama, Japan).

Using SAEW in disinfectant pool at the entrance The disinfectant pools at entrances of four broiler houses were selected, with two for treatments and two for control. The pool size is 140 × 70 × 10 cm. Forty liters of SAEW with ACC concentrations of 60-150 were poured into the treatment pool to disinfect the shoes of workers when entering and leaving broiler houses. The change of available chlorine concentration of SAEW in treatment pools was monitored at 0, 1, 2, 4, 6, 8, 10, 12 and 24 h. In the meanwhile, 1 ml of SAEW in the pool was collected and mixed with 9 ml of neutralizing buffer solution in a sterile EP tube, and then the aerobic plate count (APC) was determined by placing 1 ml of the mixture serially diluted (1:10) in the sterile saline solution, and appropriately diluted solutions (0.1 ml) were surface plated in triplicate on plate count agar (PCA, Merck, Germany) and incubated at 37°C for 48 h before counting. The sodium hydroxide solution with a pH of 14 in another two pools was used as control. Six repeats were conducted on SAEW with three different ACC concentrations (81-150 mg/l) and three repeats were done on the control.

Spraying disinfectant with SAEW in broiler houses One broiler house was selected to test the disinfecting efficiency of spraying SAEW. Another broiler house was selected as control, and the disinfectant was povidoneiodine solution (diluted to 1:1000), glutaraldehyde-containing disinfectant (diluted to 1:500) and iodine-containing disinfectant (diluted to 1:1000), respectively. A 200 l of SAEW at 60-100 mg/l of ACC (treatment) or chemicals (control) was sprayed to the house at 8:30 and 14:30 every day. During spraying, the windows and door in the houses were closed and the fans were stopped for 20 min. The air samples were collected for 5 min before and after 15 min of spraying at the twenty different positions in the house (5 points per row, totally 4 rows), with sampling plates (9 cm in diameter) containing nutrient agar. In the meanwhile, the samples were also collected from the surface of floor, wall, water line and feed line, respectively using a sterile cotton swab. The swab was rinsed completely in 2 ml of neutralizing buffer solution, and then 100 µl was pipetted and spread on plates. The plates were incubated at 37°C for 24 h for determining the aerobic plate count (APC). The percent reduction of APC was calculated by (Percent reduction of APC % = (APC before disinfection - APC after disinfection) / APC before disinfection × 100).

Hand-washing with SAEW Three workers were selected, and their hands were washed with SAEW at 60 mg/l of ACC) for 0.5, 1 and 3 min, respectively. Before and after washing, an area of 2 × 2 cm of both hands was sampled with a sterile cotton swab.

Effect of SAEW on the production performance of broilers Four houses with chickens at a same age were divided into 2 treatment groups and 2 control groups. For treatment groups, SAEW with available chlorine concentration of 60-100 mg/l were used for spraying disinfection, in disinfectant pool at the entrance to the broiler house and hand-washing disinfection of workers. The procedure of disinfection with SAEW was the first 3 days after the chickens coming into the houses without disinfection, once a day from the 4th to the 11th days, twice a day from the 12th to the 39th days, no disinfection in the last 3 days before market and during immunization periods. The disinfection in the control group was used povidonolodine solution, glutaraldehyde-containing disinfectant and iodine-containing disinfectant in turn. The disinfection method of the control group was same as the treatment group. No sampling in the 4 houses during the entire experiment in order to avoid the stress caused by frequent access, which would reduce the production performance of the broilers. The whole experiment lasted 42 days, then the statistics of all production indexes such as survival rate, weight and feed conversion ratio.

RESULTS AND DISCUSSION

Disinfection efficiency of SAEW in the disinfecting pool Figure 1 illustrates the available chlorine concentration change in SAEW used in the disinfecting pool at the entrance to the broiler house. The available chlorine concentration of SAEW decreased with time, and the lower initial available chlorine concentrations, the faster it reduced. At an initial ACC of 81 mg/l, the ACC of SAEW dropped to 0 mg/l after 24 h and no bactericidal activity.

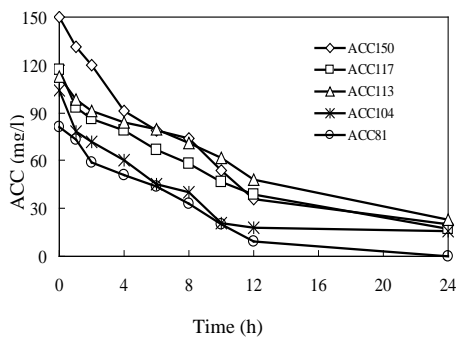


Figure 1. Available chlorine concentration change in SAEW used in the pool at the entrance to the broiler house

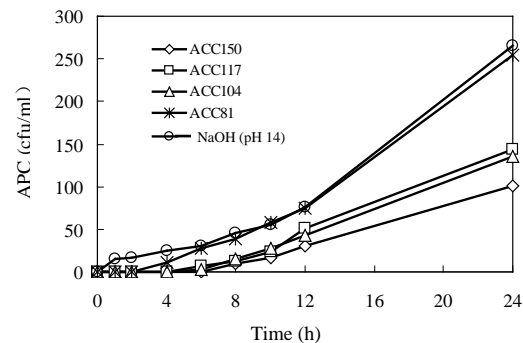


Figure 2. Comparison of APC in the pool using SAEW and NaOH solution

Figure 2 shows the population of APC in the disinfection pool at the entrance to the broiler house with SAEW and sodium hydroxide solution. As can be seen the data in Fig. 2, the population of APC in the pool with both disinfectants increased. The bactericidal activity of SAEW increased with increasing ACC. At a same time, the population of APC in the pool with SAEW (ACC > 80 mg/l) was significantly lower than that with sodium hydroxide solution. The population of APC in the pool increased slowly in the first 12 h using SAEW, and thereafter dramatically enhanced, when the SAEW should be replaced. Moreover, the bactericidal activity of SAEW at ACC of 80 mg/l is equivalent with the sodium hydroxide solution at a pH value of 14. Therefore, SAEW with an ACC of 80-120 mg/l is suitable for use in the pool at the entrance to the broiler houses and to be replaced every 12 h to keep high disinfection effect.

Spraying disinfection effect in broiler houses Figure 3 presents the spraying disinfection efficacy of SAEW, povidonolodine solution, glutaraldehyde-containing disinfectant and iodine-containing in broiler houses. Results show that the percent reduction of APC in the indoor air reached to 80.2% by SAEW at available chlorine of 80 mg/l, which is higher than that of povidonolodine solution (75.2%), glutaraldehyde-containing disinfectant (68.7%) and iodine-containing disinfectant (54.0%).

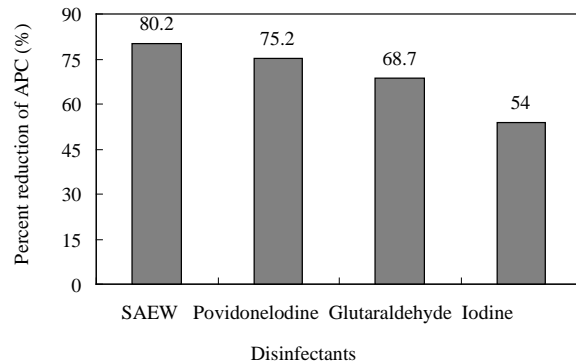


Figure 3 Comparison of disinfection efficiency of SAEW and other three disinfectants for reducing APC of air in broiler houses

In the process of spraying disinfection in the broiler house, droplets with disinfectant fell down and then inactivated the APC on the walls, water line, feed line and ground. The disinfection effect of SAEW and other three chemical disinfectants was compared and the results are shown in Table 1. As can be seen the data in Table 1, SAEW and the three chemical disinfectants can effectively reduce the APC on the walls, water line, feed line and ground. And the bactericidal effect of SAEW is stronger than that of three different disinfectants.

Table 1. Efficiency of different disinfectants for inactivating APC on the surface of wall, water and feed lines, and ground in the broiler house

Disinfectants	Percent reduction of APC (%)			
	Wall	water line	feed line	ground
SAEW	77.9	73.5	70.0	76.6
Povidonolodine solution	73.8	67.4	60.5	68.7
Glutaraldehyde-containing	68.7	62.0	50.4	65.6
Iodine-containing	61.3	50.2	60.0	69.7

Disinfection effect of SAEW for hand The percent reduction of APC on the workers' hands is shown in Fig. 4. The percent reduction of APC of 94% was resulted by SAEW at ACC of 60 mg/l for 0.5 min and 100% reduction was obtained by SAEW treated for 3 min. Furthermore, due to non-corrosive and non-irritating SAEW is an ideal disinfectant for hand-washing. Hand-washing disinfection can not only reduce the transmission of the pathogenic micro-organisms, but also conducive to the workers' health.

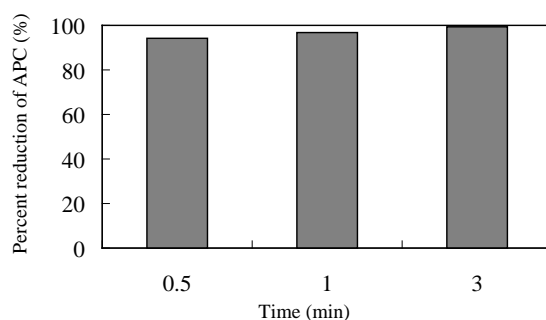


Figure 4. Percent reduction of APC on the hands by SAEW for different time

Influence of SAEW on the production performance of broilers Table 2 gives the effect of SAEW on the production performance of broilers. The survival rate and weight of broilers for the treatment groups was slightly higher than that of the control, whereas the feed conversion ratio of the treatment group (1.86) was lower than that of the control (1.87). Results indicate that SAEW is no negative effect on the production performance of broiler.

Table 2. The influence towards the production performance

Groups	Survival rate (%)	Weight (kg)	Feed conversion ratio
Treated	94.5	2.43	1.86
Control	93.3	2.42	1.87

CONCLUSION Slightly acidic electrolysed water with a pH of 5.0-6.5 is effective to reduce the population of APC in broiler houses. Comparing with the chemical disinfectants, SAEW had stronger bactericidal activity to kill the bacteria in broiler houses. The production performance of broilers was not affected by SAEW which used to disinfection. The findings of this work indicate that SAEW has a great potential in the poultry farms with environment friendly, low cost, non-corrosive and non-irritating.

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