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DISINFECTION EFFICIENCY AND PREVENTION OF MASTITIS BY SLIGHTLY ACIDIC ELECTROLYZED WATER IN A DAIRY FARM

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ABSTRACT Microbial contamination is an important issue in dairy farms. Slightly acidic electrolyzed water (SAEW) with a strong bactericidal activity, low cost and no chemical pollution due to its rapid decomposition is a novel disinfectant. SAEW (pH 5.0-6.5) containing higher hypochlorous acid (HClO) is produced by electrolyzing a mixture of dilute sodium chloride and hydrochloric acid solution in a non-membrane electrolytic cell. The disinfection effect of SAEW on cow's teats, milking cups, towels and hands of the workers was investigated. The prevention of mastitis in dairy cows by SAEW was also examined. Results show the percentage reduction of aerobic plate count (APC) on the cow's teats, towels and the milking cups treated by SAEW with ACC of 40 mg/l achieved 91.2%, 97.1% and 93.3%, respectively. Using ACC of 20 mg/l SAEW to flush the cow's teats, the APC was reduced by 93.6%. The percentage reduction of APC on the towels and hands of workers was 97.1% and 93.9% by SAEW, 91.3% and 89.3% by NaClO at ACC of 20 mg/l, respectively. Moreover, after 15 days disinfection test, cow teats dipped with SAEW at ACC of 20 mg/l before milking and post-milking resulted in the percentage reduction of mastitis positive of 27.8%. But the control (dipped with povidone-iodine solution, PVP-I) had a high number of positive cows with a mastitis positive increase of 21.4%. The finding of this study indicates that SAEW may be a promising disinfectant to microbial contamination control in dairy farms and to prevent mastitis in cows.

Keywords: Slightly acidic electrolyzed water, Dairy farms, Disinfection, Mastitis, Feet bath.

INTRODUCTION Milk production is an important industry in China. According to regulations, the dairy farm must be cleaned and sanitized regularly. Good cleaning practices in dairy farms help reduce the incidence of disease like mastitis and ensure the production of high quality milk. Mastitis is commonly a severe problem of dairy farmers worldwide, which is a result of the interaction between the cow, pathogen, and the environment (Huton et al., 1990). The best way to prevent mastitis occurrence is to keep the animals clean (WHO, 1984). Cow teats must be disinfected with iodine or other

chemical disinfectants before or post-milking. Chlorinated alkaline cleaners and iodine compounds have been commonly used as antiseptics and disinfectants in dairy farms of China. These chemicals are highly caustic and can cause serious burns of the skin and eyes (Walker et al., 2003). Therefore, developing an effective disinfectant with high efficiency, harmless and no pollution is crucial to dairy farms.

SAEW with a pH value of 5.0-6.5 and higher hypochlorous acid (HClO, approximately 95%) generated by electrolysis of a mixture of dilute sodium chloride and hydrochloric acid solution in a non-membrane electrolytic cell has a strong bactericidal activity and no chemical pollution due to its rapid decomposition (Kim et al., 2003; Park et al., 2004; Ayeben et al., 2005; Huang et al., 2008; Ayebah and Hung, 1995; Cui et al., 2009; Ding et al., 2010). The bactericidal activity of hypochlorous acid (HOCl) is 80 times than that of hypochlorite ion (ClO^-) for inactivating *E. coli* at a same chlorine concentration and treatment time (Anonymous, 1997). Therefore, 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). Cao et al. (2009) reported that a 100% inactivation (reduction of 8.2 log10 CFU/ml) of *S. enteritidis* was resulted in using SAEW at an ACC more than 4 mg/l for 3 min. SAEWwith pH of 6.1 and ACC of 20 mg/l reduced by 1.5 $\log_{10}$ CFU/g for total aerobic bacteria and 1.3 log10 CFU/g for moulds and yeasts on fresh cut cabbage (Koide et al., 2009). The disinfectant efficacy of SAEW was equivalent to or higher than that of NaOCl solution (pH 9.6, about 150 mg/l available chlorine). However, little information is available on the application SAEW in decontamination of dairy farms.

The objective of this study was to evaluate the decontamination efficiency SAEW on cow's teats and feet, milking cups, towels and hands of the workers, and examine the prevention of mastitis in dairy cows by SAEW.

MATERIALS AND METHODS

Preparation of slightly acidic electrolyzed water SAEW was produced by using a SAEW generator designed by the Key Laboratory of Agricultural Bio-Environmental Engineering of the Ministry of Agriculture and manufactured by Dongyu Xinber Co., Ltd., Shenyang, China, which basically consisting of a non-membrane electrolytic cell with anode and cathode electrodes. A mixture HCl (5%, v/v) and NaCl (10%, w/v) solution was pumped into a chamber to mix with tap water by two adjustable valves, respectively. The mixture was electrolyzed continuously to produce SAEW with pH of 6.52, oxidation reduction potential ORP of 821.5 mV and ACC of 150 mg/l. The SAEW made of above was diluted in sterile deionized water to obtain different available chlorine concentrations required. 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). The detection limit is 0-300 mg/l.

Experimental dairy farm and subclinical mastitis test The experiments were conducted in a dairy farm, HuiHuang Industrial Co., Ltd. at the city of Xinzhou, Shanxi province, China. There were totally 406 dairy herds, and 139 milk cows in the lactation

period which were divided into four groups and kept in four different cowsheds. The dairy cows were sent to milking in four batches, twice a day for each cow and milking time was 8: 30-10: 30 and 20: 30-22: 30. Before the experiment, the detection of subclinical mastitis were conducted on 139 lactation cows using California Mastitis Test Kit (CMT), to determine the number of infected heads in each cowshed and record the ear tag.

Disinfection of SAEW for cow teats, towels, milking cups and hands of workers Sixteen lactation cows were selected for disinfection experiments with SAEW. The cow teats were cleaned and disinfected before- and post-milking with SAEW at available chlorine of 20, 40, 60 and 80 mg/l for 1 and 15 s. Four cows were used for each ACC level. The chemical disinfectant, povidone-iodine solution (1:4, v/v) commonly applied in the dairy farm to disinfect the teats was used as the control. The disinfection procedure for the control was same as the treatment.

The towels usually used to swab the teats, milking cups and workers' hands were cleaned with SAEW at ACC of 20, 40 and 80 mg/l for 0.5 and 1 min, respectively. The NaClO solution at 20 mg/l of ACC was used as the control. Before and after disinfection, an area of 4 $cm^2$of the teats and their surrounding skin or workers' hands was completely swabbed with a sterile cotton swab. The swab was rinsed fully in 2 ml of neutralizing buffer solution in EP tubes, and then 100 μl was pipetted and spread on plates. The plates were incubated at 37ºC for 24 h for determining the aerobic plate count (APC). The percentage reduction of APC was calculated by (Percentage reduction of APC % = (APC before disinfection - APC after disinfection) / APC before disinfection × 100).

Prevention of subclinical mastitis of cows with SAEW Depending on the cowshed and different milking batches, the dairy cows were divided into four groups (two treatment and two control). The experimental procedures in trials are as follows:

Treatment 1 (No. 2 cowshed, 39 cows): before milking, washing the breast of cows with SAEW at ACC of 20 mg/l for not less than 15 s to each cow, thereafter wiped the breast with sterile towels; after milking, dipping each teat with SAEW at ACC of 60 mg/l for 15 s. Fresh SAEW was used for each cow.

Treatment 2 (No. 3 cowshed, 36 cows): the disinfection approach was same as treatment 1 before milking and after milking, but the SAEW was used continuously.

Control 1 (No. 1 cowshed, 38 cows): rinsing the breast with warm water before milking; then wiped with sterile towels; after milking dipping each teat with 0.5% povidone-iodine solution (PVP-I) for 15 s. Fresh PVP-I solution was used for each cow.

Control 2 (No. 4 cowshed, 26 cows): the disinfection approach was same as control 1 before milking and after milking, but the PVP-I solution was used continuously.

The trial period was 15 d. The subclinical mastitis detection was conducted before and after experiments.

RESULTS AND DISCUSSION

Disinfection efficiency of SAEW for cow teats Table 1 shows percentage reduction of APC on the teats by povidone-iodine solution (PVP-I) and SAEW at available chlorine concentration of 20, 40, 60 and 80 mg/l for 1 and 15 s. With increasing the available chlorine concentration and treatment time, the bactericidal activity of SAEW increased. When ACC of 40 mg/l, the bactericidal activity of SAEW (percentage reduction of APC of 71.7%) was stronger than that of povidone-iodine solution (percentage reduction of APC of 69.6%). The percentage reduction of APC reached 97.4% by SAEW at ACC of 80 mg/l. There were no negative effect on the skin of cows treated with SAEW.

Table 1. Comparison of disinfection effect of SAEW and povidone-iodine solution for inactivating aerobic plate count (APC) on cow teats

Disinfectants	**Available chlorine (mg/l)**	**Percent reduction of APC (%)**	
		5 s	15 s
SAEW	20		85.0
	40	71.1	91.2
	60		95.1
	80		97.4
Povidone-iodine		69.6	92.0

Disinfection efficiency of SAEW for towels, milking cups and hands of workers Figures 1 and 2 show the disinfection effect of SAEW for towels, hands of workers and milking cups, respectively. SAEW is effectively to reduce the APC on the towels, workers' hands and milking cups. The percentage reduction of APC on the hands and towels reached 93.4% and 97.1% by SAEW at an ACC of 20 mg/l, respectively. The bactericidal activity of SAEW was more powerful than that of NaClO solution at a same ACC. The disinfection effect of SAEW was more significant to reduce the APC on the milking cups with washing with water followed by SAEW rinsing. This may be related to the organic matter attached on the milking cups, which decreased the bactericidal activity of SAEW (Toshihiro, 1990).

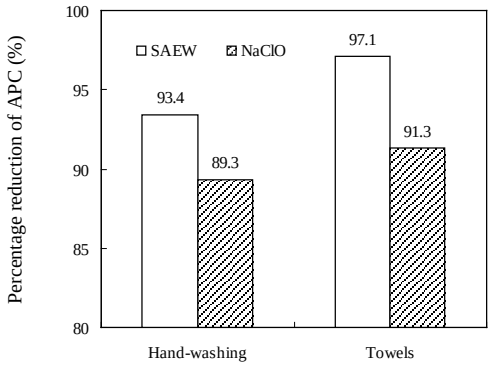

Figure 1. Comparison of disinfection efficiency between SAEW and NaClO solution at ACC of 20 mg/l for hand-washing and towels.

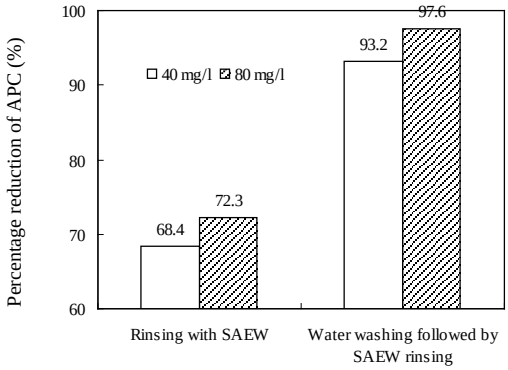

Figure 2. Disinfection effect of SAEW at ACC of 40 and 80 mg/l for milking cups.

Effect of SAEW on prevention of subclinical mastitis of cows Table 2 shows the prevention and treatment of subclinical mastitis of dairy cows with SAEW (treatment) and 0.5% PVP-I (control) commonly used in the dairy farms. After 15-day trial, SAEW has a significant effect on the prevention and treatment of subclinical mastitis for dairy cows. Results indicate that the timely replacement of disinfectants had the more pronounced effect than the continuous use of the same kind of disinfectant in dipping teats, because the bactericidal activity of disinfectants were greatly influenced by the environment, pH and organic matter. The ACC of SAEW declined rapidly by the presence of organic matter like milk on the teats. It suggests that the disinfectant used for teat dipping should be timely replaced.

From the data of control 2, the positive rate increased by 11.5%. It indicates that the cow teats must be treated with disinfectants for appropriate time to allow bactericidal action fully obtained. Probably, when removing the dipping cup quickly, the disinfectant remained on the teats is very little and affected by organic matter, and its active ingredients disappear soon, can not completely kill the micro-organisms. By comparing the results of the treatment and the control, it can be found that the disinfection procedure plays an important role in the efficiency of disinfectants.

In the current operation mode of this farm, the breast of dairy cows are not sterilized before milking, but rinsed with warm water only; micro-organisms on teats can not be reduced markedly, and will enter the milk with the milking machine pipeline. In the study, the breast of dairy cow washed with warm water followed by rinsing SAEW at a lower of ACC is effective to prevent the occurrence of subclinical mastitis.

Table 2. Effect of SAEW on the preventing mastitis occurrence of dairy cows

Groups	Number of cows	Positive number before treatment	Positive number after treatment	Positive rate before treatment	Positive rate after treatment	Change of positive rate*
treatment 1	39	18	13	46.2%	33.3%	12.9%
treatment 2	36	18	16	50%	44.4%	5.6%
control 1	38	20	18	52.6%	47.4%	5.2%
control 2	26	12	15	46.2%	57.7%	-11.5%

* The negative indicates the increase of mastitis positive.

CONCLUSION SAEW with a mild pH 5.6-6.5 is not only effective to reduce the population of APC on the cow teats, towels, hands of workers and milking cups, also could prevent the occurrence of subclinical mastitis. The findings from the current study imply that SAEW with environment friendly, low cost, non-corrosive, high stability and bactericidal activity, and a less potential health hazard to the worker and environment could be an attractive candidate for application in dairy farms.

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