RESEARCH NOTE

EVALUATION OF CUPROHCA® AS AN ALTERNATIVE TO THE COPPER SULPHATE FOOTBATH

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ABSTRACT

The aims of this study were to determine the effective ratio of a new commercial footbath, namely CuproHCa®, against bacterial load and to reveal the number of cows that could pass from the ideal concentrations of CuproHCa® footbath solution without disrupting the effectiveness relative to copper sulphate ($CuSO_4$). A total of 80 Holstein Friesian cows under each footbath regimen walked in the footbath twice a day after four consecutive milkings. After every 10 passes from the footbaths, 33 samples for each solution totalling 66 samples were subjected to pH, oxidation reduction potential (ORP), microbiological, and volumetric analysis. The pH value was 4.08 after 230 cows passed through in $CuSO_4$, whereas pH value in CuproHCa® footbath showed minimal change over time (1.90±0.08). The overall ORP value of CuproHCa® (343.21±12.31 mv) was higher than $CuSO_4$ (294.97±61.40 mv, $p<0.001$). The overall bacterial load of CuproHCa® (0.44±0.18 ng/ml) was higher than $CuSO_4$ (0.37±0.21 ng/ml, $p=0.007$). The volumetric copper analysis in CuproHCa® (10.02±0.81) was higher than $CuSO_4$ (9.22±0.84), ($p<0.001$). In conclusion, based on pH and ORP analysis, 10% CuproHCa® has less effect compared to 4% $CuSO_4$. After 280 cows passed through the footbath, CuproHCa® has a better antibacterial effect than $CuSO_4$.

Key words: *bacterial load, copper, cow, pH, volumetric.*

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INTRODUCTION

Footbaths are commonly used to control the infectious hoof disease in dairy facilities (Speijers *et al*., 2012, Izci and Cuhadar 2021). Antibiotic solutions should be less preferred among veterinarians and farmers due to antimicrobial resistance and economic losses (Hyde *et al*., 2017). Bacteriostatic agent copper sulphate ($CuSO_4$) appears as the most efficacious agent to include in a footbath program but negative environmental consequences, due to soil accumulation, limits the frequency of its use (Solano *et al*., 2016). The European Union Biocidal Products Direction already prohibits the use of $CuSO_4$ as a footbath due to environmental concerns. Inactivity of $CuSO_4$ after 200 to 300 cows passed through may also restrict its use among farmers due to economic reasons (Cook *et al*., 2012).

Acidifying agents for $CuSO_4$ have been used to achieve less copper ratio in footbath solutions to protect environmental harm (Reichenbach *et al*., 2017). The lower pH value results with maximum solubility of copper ions to get better antibacterial activity (Williams *et al*., 2019). Reducing the amount of copper in the $CuSO_4$ concurrently reduces the cost of the solution (Cook, 2017). A new commercial product sold in the market, namely CuproHCa®, composed of 10% copper and 1% zinc that contains less copper than $CuSO_4$, may encourage farmers to apply footbaths.

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The bacterial load and pH are some of the indicators to evaluate the effectiveness of footbath (Cook, 2017). The pH of disinfectants in footbaths higher than five does not have antiseptic properties (Izci and Cuhadar 2021). In order for copper to be effective, it must be in ionic form (pH must be lower than 3.8). When copper sulphate liquid is contaminated with organic substances such as urine and feces, it quickly neutralizes and loses its antiseptic feature (Van Amstel *et al.*, 2006).

This was a two-part study. The first part, a preliminary laboratory trial, was aimed to determine the effective ratio of CuproHCa® and $CuSO_4$ against bacterial load in the laboratory trial. The second part, a field study, was aimed to reveal the number of cows that could pass from the ideal concentrations of CuproHCa® footbath solution without disrupting the effectiveness relative to $CuSO_4$. The hypothesis of the present study was that CuproHCa® would have a less or more effect on pH and bacterial load compared with the most commonly used footbath agent $CuSO_4$.

MATERIALS AND METHODS

The Atatürk University ethical committee approved the study protocol of this research (decision no 77/2021). The trial was performed in two stages namely, the preliminary laboratory trial and the field study.

Preliminary Laboratory Trial

In order to determine at what concentrations of CuproHCa® (Metal-Kim,İstanbul,Turkey) was effective against *Escherichia coli*, *Enterococcus faecalis, Staphylococcus aureus*, *Candidatus amoebophilus asiaticus*, and *Pseudomonas aeruginosa,* concentrations of 2.5%, 5%, 7.5%, and 10% were prepared. One-hundred microliters of each concentration were added to sterile antibiotic disc papers which were kept in sterile conditions for one hour then, antibiograms were performed in accordance with the disc diffusion method. The antibacterial activity of each concentration was scored from one (no bacterial inhibition) to five (inhibited whole bacteria) according to the avarage of three replication experiments.

Field Study

After the optimal ratio of footbath solutions were obtained, the field study was employed. This was designed to be randomized and controlled but not blinded. The study was conducted at the Food and Livestock Application and Research Center, Atatürk University, Erzurum from August to October 2021. A total of 80 Holstein Friesian cows were utilized in the study. All animals were milked twice daily and were housed in cubicles with solid concrete floors and with an automatically scraping system. Exclusion criteria included if the cow had any illness, infectious foot disease, or lameness.

Cows of the farm, under each footbath regimen, walked in the footbath twice a day after four consecutive milkings. The footbath used was a split walk-through footbath, consisting of two baths separated by concrete. The first bath measured 880 cm long × 90 cm wide × 15 cm high, and it was used as a wash bath filled with only water. The second bath measured 220 cm long × 90 cm wide × 15 cm high and was used as a treatment bath. To prepare both footbath solutions, water was added to a depth of 11.25 cm and then the solution was added up to 12.5 cm (total bath volume is 247.5 cm^3).

pH and ORP

The bath volume was calculated, after every 10 cows passed, by immersing the ruler to the bath. Two 10 ml tubes (one for pH, ORP, and volumetric analysis and another for microbiological analysis) were taken into the centre of the treatment bath prior to cow passes and subsequently every 10 cow passes. Thus, 33 samples for each solution, a total of 66 samples, were subjected to the pH, ORP, and microbiological analysis. The pH and ORP values of samples were immediately measured with a pH meter (Adwa, Romania).

The 5-7% prepared Brain Heart Infusion Agar and Sabouraud Dextrose Agar were used for bacteriological and mycological analysis, respectively. After centrifugation, a sample was taken from the bottom sediment with a sterile loop and planted by purification method. For bacteriological analysis, the petri dishes were incubated for 24-48 hours in aerobic conditions and 72 hours in anaerobic conditions at 37°C. For mycological analysis, samples were incubated at 22°C for 5-10 days. Identification was made from bacterial colonies that grew after incubation. Biochemical tests such as Gram stain, Catalase, oxidase, coagulase, gelatin, H2S, carbohydrate fermentation tests (O/F, TSI, MR-VP), and UV lamp appearance were performed for identification purposes. Sabouraud Dextrose Agars incubated for mycological analysis were examined macroscopically and microscopically. Colonies with spore and hyphae structures were typed (Quinn, 2011).

Volumetric analysis

The volumetric analysis of copper for each footbath solution was performed by using titration method (Kolthoff and Stenger, 1947).

Statistical analysis

Data were analysed using commercial software (Version 13.2.2; MedCalc, Ostend, Belgium). Significance was set at $p<0.05$. A paired sample t-test was employed to determine the differences between pH, ORP, bacterial load, and volumetric analysis. Odd ratios were employed for negative and positive fungi samples. An average reduced amount of footbath solutions were calculated using chi-square analysis.

RESULTS

Preliminary Laboratory Trial

The 10% CuproHCa® inhibited whole bacterial activity, whereas no inhibition zone was observed at the concentration of 2.5%. Both the 5% and 7.5% concentrations (scored as four) inhibited four of five bacteria.

Field Study

The average reduced amount of footbath solutions after whole cows passed for CuproHCa® and $CuSO_4$ were 122.76 cm3, and 128.76 cm3, respectively. The overall pH value of $CuSO_4$ was 3.51±0.60 ($p<0.001$). The pH value was 4.08 after 230 cow passes in $CuSO_4$, whereas pH value in CuproHCa® footbath showed minimal change over time (1.90±0.08) (Fig. 1A). The overall ORP value of CuproHCa® (343.21±12.31 mv) was higher than $CuSO_4$ (294.97±61.40 mv, $p<0.001$) (Table 1). As the number of cow passes increased, the ORP level decreased in both footbath solutions (Fig. 1B).

Bacillus spp. and *Dichelobacter* spp. were positive in all footbath solution specimens. The presence of fungi in CuproHCa® (n=27) and $CuSO_4$ (n=16) solutions did not significantly differ ($p=0.051$). The overall bacterial load of CuproHCa® (0.44±0.18 ng/ml) was higher than $CuSO_4$ (0.37±0.21 ng/ml), ($p=0.007$). The level of bacterial load was gradually increased in both

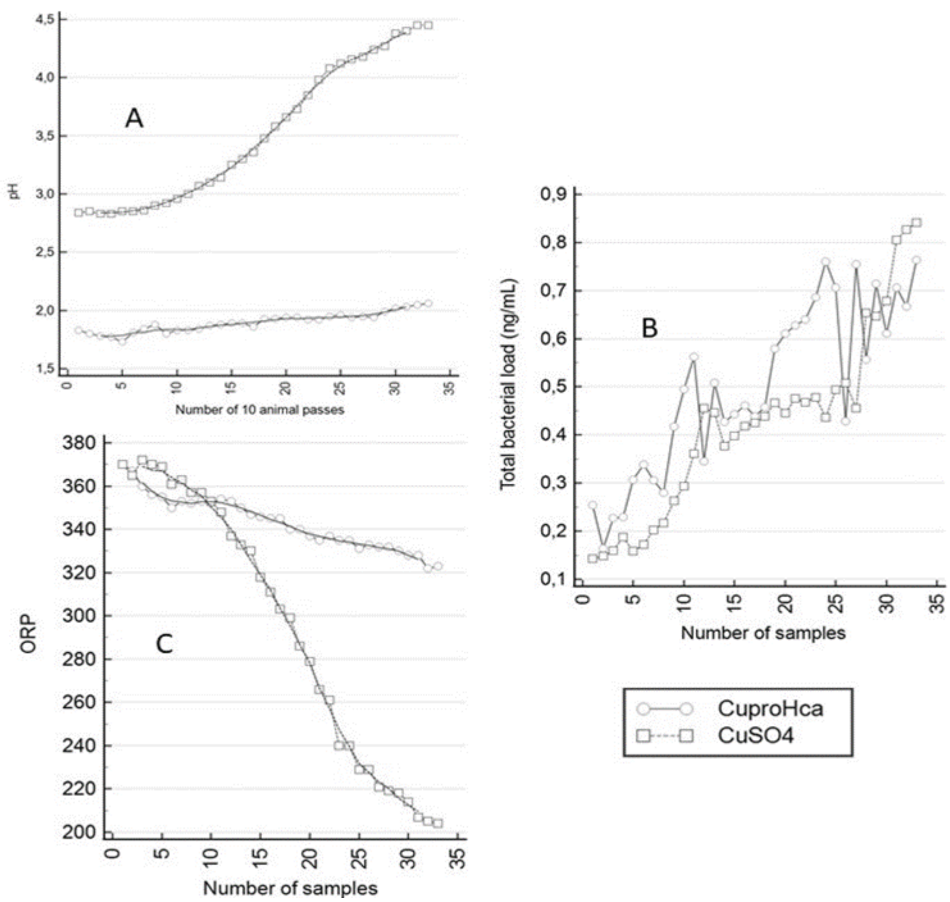

Figure 1. (A) The pH levels of CuproHCa® and $CuSO_4$ solutions after every 10 animals pass the footbath. (B) The oxidation reduction potential (ORP) levels of CuproHCa® and $CuSO_4$ solutions after every 10 animals pass the footbath. (C) The total bacterial load of CuproHCa® and $CuSO_4$ solutions after every 10 animals pass the footbath.

Table 1 The pH, oxidation reduction potential (ORP), total bacterial load (TBL), and remaining copper ratio (RCR) differences between 10% CuproHCa® and 4% $CuSO_4$.

Parameters	10% CupraHCa® (n=32, Mean ± SD)	4% $CuSO_4$ (n=32, Mean ± SD)	*p* value
pH	1.90±0.08	3.51±0.60	<0.001
ORP (mv)	343.21±12.31	294.97±61.40 mv	<0.001
TBL (ng/ml)	0.44±0.18	0.37±0.21 ng/ml	0.007
RCR (ppm)	10.02±0.81	9.22±0.84 ppm	<0.001

mv: millivolt, ng/ml: nanograms per millilitre, ppm: parts per million.

groups and the bacterial contamination of $CuSO_4$ exceeded the CuproHCa® after 280 cows passed the footbath (Fig. 1C). The copper ratio in the remaining solution was lower in $CuSO_4$ (9.22±0.84 ppm) than CuproHCa® (10.02±0.81 ppm), ($p<0.001$).

DISCUSSION

This study compared the efficacy of a new commercial product (CuproHCa®) against $CuSO_4$. The 10% of CuproHCa® was selected for field study because of inhibition of all bacterial activity at this ratio. The reason for choosing 4% $CuSO_4$ was because it contains the same amount of copper as 10% CuproHCa®. As the pH values of CuproHCa® showed minimal change throughout the experiment, it could be preferred for footbath solution over the 4% $CuSO_4$. Acidifiers have been utilized to increase the pH for improving the solvency and activity of solution. They also reduce the concentration of copper, thereby decreasing environmental risk (Burgi *et al.*, 2015, Holzhauer *et al.,* 2012). Previous studies have reported that pH is one of the main factors for assessing footbath activity. The pH of footbaths becomes higher after the 250 cow passes (Cook *et al.*, 2012), a similar finding was obtained in 4% $CuSO_4$ footbath (230 cows). Concentrations of CuSO4 between 2% and 10% have been used (Speijers et al., 2010). Based on the findings obtained from the current study, 4% $CuSO_4$ could be used as a footbath in dairy herds due to economic reasons and environmental hazard but future studies are warranted to evaluate the effectiveness of this rate over the digital dermatitis lesions. In this study, although pH of $CuSO_4$ gradually increased over time, it was below five even after 320 animals passed. This finding was consistent with a previous study reporting this number as 260 cows (Prastiwi *et al.*, 2019).

The ORP is the oxidation and reduction power of a liquid substance expressed in millivolt and this value is used to evaluate the quality of the water. If the ORP is positive, it indicates that the water can be oxidized. ORP and pH are inversely proportional, as pH increases, ORP decreases (Suslow, 2004). In the present study, the increase in pH level caused the ORP level to decrease. It can be concluded that in addition to pH, ORP values can also be utilized to evaluate the effectiveness of the footbath. The ORP level in both solutions decreased over time as the number of cows passed the footbath which is congruent with a previous study that states ORP levels are strongly related to water disinfection potential (Suslow, 2004).

Volume losses in footbaths occur due to inappropriate size and design (Izci and Cuhadar, 2021). In this study, the height of the footbath walls was 15 cm, whereas the liquid depth was 12.5 cm which is tall enough to cover the feet (Cook *et al.*, 2012). A 50% decrease in initial concentration of footbath after 320 cow passes were observed in the present study. This finding is similar to the findings of Holzhauer *et al.* (2004), who reports that 300 to 320 cow passages reduces half of the initial concentration of footbath (Holzhauer *et al.*, 2004). Previous study that used a small length of footbath and a lower wall height caused higher footbath reductions approximately a 50% decrease after 200 cow passes (Ariza *et al.*, 2019). The lesser number of animals that pass the footbath is likely due to using a shorter length of bath. A similar statement has also been submitted previously (Ariza *et al.*, 2019).

Measuring the optical density is a method to quantify bacterial growth (Koch, 1970). It is mainly used to monitor growth of bacteria in liquid solutions (Haase *et al.*, 2017). The bacterial load of footbaths elevated with the number of cows passes on the solution. A similar finding is obtained with the previous work of Ariza *et al.* (2019). Although the overall bacterial load of CuproHCa® was higher than $CuSO_4$, the bacterial load of $CuSO_4$ exceeded the CuproHCa® after 280 cows passed the footbath. This is mainly related to the defecation amount in footbaths.

Increased ratio of feces in footbaths can cause higher pH levels and lower disinfectant properties.

The high amounts of copper in slurry wastewater disposed to land can lead to high rates of copper in soil (Jondreville *et al*., 2003). Copper soil levels on farms should be taken into consideration to protect soil productivity and poisoning. A previous study has reported that the farms using copper as a footbath have an average of 10.3±12.02 ppm copper concentration from manure (Downing *et al*.,2010), a similar finding was obtained from the remaining footbath solutions in this study regardless of used footbath.

Researchers have examined the efficacy of footbath solutions for treatment (Cook *et al*., 2012, Speijers *et al*., 2012) or prevention of digital dermatitis (Teixeira *et al*., 2010). The effects of CuproHCa® solution on cows with infectious foot diseases should be investigated to estimate their effectiveness on herd-level. Moreover, the effects of this new footbath solution on cow lameness score is also needed to be determined.

In conclusion, 10% CuproHCa® has less effect on pH and ORP than 4% $CuSO_4$. After 280 cows pass the footbath, CuproHCa® has a better antibacterial effect than $CuSO_4$. Effectiveness of this new footbath solution on infectious foot diseases should be evaluated before routine usage in dairy farms.

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STATEMENT ON COMPETING INTEREST

The authors have no competing interests to declare.

AUTHOR'S CONTRIBUTION

LEY designed and supervised the study. LEY, OTO, and YK collected the samples. CO performed laboratory analysis. LEY analyzed the data. The first draft of the manuscript was written by LEY and OTO. All authors contributed to the critical revision of the manuscript and have read and approved the final version.

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