Efficacy of quaternary ammonium salt-based disinfectant or chelated copper-zinc mixture footbath solution in the control of digital dermatitis in dairy cows

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Abstract

Background: Bovine digital dermatitis (DD) represents a common cause of lameness in cattle both in
Taiwan and worldwide. DD can be controlled using footbaths but the traditional use of footbath with
copper sulfate is harmful to the environment. Therefore, the objective of this study was to use a
quaternary ammonium salt-based disinfectant or a chelated copper-zinc mixture for foot bathing as an
alternative to copper sulfate for DD in lactating cows. Moreover, a cleaning pool was established and
used prior to the DD foot bathing. Two groups of 27 lactating cows were checked and randomly assigned
to two treatment groups, which were 1% quaternary ammonium salt-based disinfectant (BCD) and 2.5%
chelated copper-zinc mixture (CZM). The experimental period lasted for 15 consecutive weeks and a
footbath was performed once a week.

Results: The cure rate proportion in the BCD group was significantly higher than the CZM, measuring at
67% and 38%, respectively (P = 0.03). The logistic regression analyses indicated that the odds (95% CI)
for the cure rate in the CZM group was 0.30 (0.095–0.948) times than in the BCD group (P = 0.04).
Following the 15 weeks of treatment, both the BCD and CZM groups exhibited a significant increase of
M0 in all cows (P < 0.05). Moreover, the proportion of M2, M3, and M4 in the BCD group was significantly
decreased (P < 0.05). Similarly, the M2 proportion was significantly decreased in the CZM group, while the
M1 proportion was significantly increased (P < 0.05). The proportion of cows with M0 in BCD was
significantly higher at the end of the experiment than those in the CZM group (P < 0.05). The proportion
of M1, M2, M3, and M4 demonstrated no difference between the BCD and CZM treatments at each stage
(P > 0.05).

Conclusion: The results illustrated that establishing a cleaning pool prior to foot bathing, in addition to, a
weekly footbath with a 1% quaternary ammonium salt-based disinfectant, provided additional control
over the prevalence of DD.

Background

Bovine digital dermatitis (DD) is an infectious skin disease that affects cattle worldwide [1]. DD has
become one of the most common causes of lameness in cattle in Europe and North America [2, 3, 4].
There is a similar situation in Taiwan. The painful skin lesions associated with DD lead to lameness,
declining body condition, decreased reproductive performance, and decreased milk production [5]. The
prevalence of DD in dairy cows ranges between 15 and 49% [6], depending on the dairy farm's hygiene
management and house type.

The exact cause of DD is still not fully understood, since it is a disease caused by multiple factors.
However, bacterial infections promote the majority of cases [7]. One hypothesis is that Treponema
bacteria in the environment and other bacteria (e.g., Dichelobacter nodosus) may cause or co-induce skin
macerations [8]. The skin between the hooves in cattle is a warm and humid place, which presents prime
conditions to promote the proliferation of these aforementioned pathogenic bacteria, thereby increasing
the chances of infection. Indeed, the wet and narrow barn floors, poor sanitary conditions, low-parity cattle with early lactation, and cattle with clinical or subclinical disease symptoms in the herd are all factors that contribute to the high incidence of DD [2, 4].

Infectious DD can be controlled by good hygiene management and footbaths with disinfectants or antibiotic solutions [9, 10, 11]. The traditional footbath, which uses copper sulfate contains heavy metal ions that are harmful to the environment. Hence, following a footbath, the solutions were usually discarded via the sewage treatment system, where the heavy metal concentration would remain for several months [12, 13]. The continuous accumulation of these metals in the environment could promote the co-selection of antibiotic-resistant microorganisms in the soil through co-resistance or cross-resistance and increase the concentration of copper and zinc in farmland soils [14, 15]. Copper and zinc form part of the List II Group of metals that have a deleterious effect on the aquatic environment. Moreover, the elements are the subjects of a current Directive 2006/11/EC reduction program on pollution caused by certain dangerous substances discharged into the aquatic environment [16].

A 4% copper sulfate footbath solution with dimensions of 220 cm long x 90 cm wide x 15 cm high could allow 300 cow passes, while the 2% copper sulfate footbath solution can be used for about 168 passes [17]. Currently, there are limited studies that have analyzed the use of quaternary ammonium salt-based disinfectants for foot bathing. The quaternary ammonium salt-based disinfectant is often used in livestock farms to inhibit a variety of viruses and bacteria. The quaternary ammonium compounds, specifically didecyldimethylammonium chloride and alkyl (C12–C16) dimethylbenzylammonium chloride, act as broad spectrum bactericidal and fungicidal disinfectants in the prevention of DD [18]. Manure and slurry contamination are known to inactivate most hoof-care products [19, 9]. However, it is currently unclear as to whether pre-cleaning the feet prior to the footbath would increase the effect of footbath agents. Therefore, the objective of this study was to evaluate DD controlling efficacy for copper sulfate foot bathing alternatives on lactating cows. Firstly, a cleaning pool setup would be instilled, while the foot bathing would be performed using either a quaternary ammonium salt-based disinfectant or a chelated copper-zinc mixture.

Results

The proportion of cows presenting signs of lameness (lame: locomotion score > 2; non-lame: locomotion score ≤ 2) at the start (1st week) and the end (15th week) of the experiment is illustrated in Table 1. There was no difference in the proportion of lameness between the BCD and CZM groups (P > 0.05). The logistic regression analyses indicated that the odds (95% CI) of lameness for cows in CZM were 1.167 (0.320–4.259) times the odds of lameness cows in BCD (P = 0.82).

The cure rate for the DD lesions is defined as the proportion of the transition from the active phase to the non-active stage (M1/M2 to M0/M4) between the trial’s start and endpoint. The cure rate proportion for the DD lesions is shown in Table 2. Overall, the cure rate proportion in the BCD group was significantly higher than the CZM, measuring at 67% and 38%, respectively (P = 0.03). The logistic regression analyses
indicated that the odds (95% CI) for the cure rate in the CZM group was 0.30 (0.095–0.948) times than in the BCD group ($P=0.04$).

The proportion and numbers of DD on the left front, right front, left hind, and right hind foot of the cow at the various different stages of the experiment (start: 1st week; end: 15th week) are shown in Table 3. Only the cows in the BCD groups demonstrated a reduction in DD prevalence on both the hind feet at the conclusion of the experiment. The cows which underwent CZM treatment only showed exhibited an improvement to the right hindfoot.

Figure 1 shows the distribution of DD lesion M-stage scoring categories in the BCD and CZM groups for the individual foot throughout the experiment (start: 1st week; end: 15th week). Following the 15 weeks of treatment, both the BCD and CZM groups exhibited a significant increase of M0 in all hind feet of the cows ($P<0.05$). Moreover, the proportion of M2, M3, and M4 in the BCD group was significantly decreased ($P<0.05$; Fig. 1A). Similarly, the M2 proportion was significantly decreased in the CZM group, while the M1 proportion was significantly increased ($P<0.05$; Fig. 1B).

The distribution of the DD lesion M-stage scoring categories for the cows in the BCD and CZM groups throughout the experimental duration (start: 1st week; end: 15th week) is shown in Fig. 2. Indeed, the proportion of cows with M0 (normal and healthy) in BCD was significantly higher at the end of the experiment than those in the CZM group ($P<0.05$) (Fig. 2A and 2B). The proportion of M1, M2, M3, and M4 demonstrated no difference between the BCD and CZM treatments at each stage ($P>0.05$).

Initially, and in addition to DD, some experimental cows were found to suffer from double soles, sole ulcers, sole hemorrhage circumscribed, or white line abscesses. The proportion of double soles, sole ulcers, sole hemorrhage circumscribes, and white line abscesses in the BCD group were 0.0%, 55.6%, 11.1%, and 11.1%, respectively. Likewise, there were 12.5% double soles, 25.0% sole ulcers, 0.0% sole hemorrhage circumscribes, and 25.0% white line abscesses in the CZM group. Following the experimental period, the cure rate of sole ulcer, sole hemorrhage circumscribed, and white line abscess in the BCD group was 40.0%, 100.0%, and 0.0%, respectively. Comparatively, the cure rates of a double sole, sole ulcer, and white line abscess were 100.0%, 100.0%, and 0.0%, respectively, in the CZM group. Only in the CZM group was found a 33.3% new incidence rate of sole ulcer.

**Discussion**

The locomotion score is used to evaluate the movement status of a cow, and thus can be implemented to classify the condition of a lame cow [20, 21]. The data produced by this study present no significant difference in lameness (locomotion score >2) between the BCD and CZM groups at the end of the experiment (Table 1). In this study, some experimental cows suffered from a double sole, sole ulcer, soles hemorrhage circumscribed, or white line abscesses concurrently. These hoof diseases could affect the locomotion scores. Preliminary observations showed that a sole ulcer was the most detrimental to the locomotion score. No statistical analysis was performed for these non-DD hoof diseases due to the limited cow numbers. Moreover, the locomotion score would be similarly affected if DD failed to be
completely healed in any foot. Therefore, neither footbath treatment applied in this study can completely and effectively eradicate all hoof diseases within our experimental time period.

Approximately 90% of all hoof lesions affect a hindfoot, and the majority (70–90%) affect the lateral claw [22]. Similarly, DD is common in the hind feet [23]. These hoof lesions are related to potential metabolic and physical factors and are also closely related to the environment of the house. The floor type will affect the degree to which the hoof is soiled with organic matter and slurry, and potentially aggravate the presence of infectious diseases in the deep tissues of the claws [15]. In this study, DD mainly occurred in the hind hooves. The proportions of DD following BCD and CZM treatments were 7.1% and 0% on the forefeet, and 92.9% and 100% on the hind feet, respectively (Table 3). Although the number of cows with DD decreased in both groups after 15 weeks, the proportions of DD in BCD and CZM still reached 71.4% and 100% in the hind feet, respectively (Table 3). Cattle commonly have lesions on both hind feet simultaneously [23]. Indeed, Laven [24] revealed that 51% of cattle in a herd had a disease on both feet, 22% were identified with it only on the left foot, and 27% contained it only on the right foot. Although both hind feet are exposed to the same risk factors concomitantly, DD occurs only on one hindfoot in some cattle.

Although the feet in both treatment groups did not completely become M0, there was a significant increase in M0 categorizations on all hind feet for each treatment group (Fig. 1). Moreover, the increase of M0 in the hind feet of the BCD group was mainly observed through a significant decrease in the M2, M3, and M4 categories. Conversely, the increase of M0 in the hind feet of the CZM group was mainly due to a significant decrease in M2. After 15 weeks of treatment, the DD cure rate proportion following BCD treatment was significantly higher than from the CZM ($P = 0.03$), while the odds (95% CI) of the cure rate for cows in CZM were $0.30 (0.095−0.948)$ times that in BCD ($P = 0.04$) (Table 2). Overall, the DD cure rate was improved following BCD treatment than CZM.

To understand the effect of treatment on DD in experimental cows, the proportion of different types of DD lesions M-stages scoring were categorized for each treatment group at different experimental stages (start: 1st week; end: 15th week) (Fig. 2). Indeed, BCD provided a significantly higher proportion of cattle with M0 at the end of the experiment ($P < 0.05$) (Fig. 2), indicating better efficacy on controlling DD by BCD.

Researchers believe that the quaternary ammonium salt-based footbath is not as effective as copper sulfate in controlling DD [18]. It was evaluated in vitro by the minimum bactericidal concentration (MBC) and the minimum inhibitory concentration (MIC) of various disinfectants and copper sulfate, in the absence or presence of manure, for a *Treponema phagedenis*-like microorganism using either a 30 second or a 10 minute exposure time. The result indicated that in the absence of manure, copper sulfate was the most effective against *Treponema* microorganisms in vitro (lowest MIC and MBC). However, the presence of manure in the solution severely decreased the effectiveness of the copper sulfate. When organic matter such as manure was present, it required a higher concentration of disinfectants to inhibit or kill the *Treponema* microorganisms [25]. The manure and other organic matter cause the majority of
footbath products to lose their disinfectant and antibacterial abilities. Additionally, organic matter causes many footbath products to become neutralized over time [19; 9]. Here, the addition of a cleaning pool forced the cows to clean their feet before the foot bathing treatment with BCD or CZM. Ultimately, this promoted a reduction in the proportion of organic matter attached to the hooves. It is hypothesized that establishing a cleaning pool set up prior to foot bathing, or using a different concentration of the copper-zinc mixture employed in this study (2.5% vs 5%) could be the reasons our data are different from previous publications.

**Conclusions**

Overall, this study demonstrated that the use of a cleaning pool before foot bathing, in addition to, using a 1% quaternary ammonium salt-based disinfectant for foot bathing once a week has a controlling efficacy on the prevalence of DD.

**Methods**

**Cattle and herd management**

The cows were all housed together in a free stall with rubber beds and solid concrete floors, which were scraped by a tractor 6 times a day. The cows did not have access to pasture during the study period. The cattle were milked and fed a total mixed ration twice daily and had free access to fresh water and salt blocks.

**Experimental Design**

This study was conducted from August 2019 to March 2020 in a dairy barn of the Hsinchu Branch, Livestock Research Institute in Miaoli County, Taiwan. Two groups of 27 lactating cows were randomly assigned to two treatment groups, which were 1% quaternary ammonium salt-based disinfectant and 2.5% chelated copper-zinc mixture. Initially, all experimental cows had at least one foot with DD. There were 3 cows were dried during the experimental period, so only 24 experimental cows left in chelated copper-zinc mixture group. The experimental period lasted for 15 consecutive weeks with foot bath once a week. The lactation days cows ranged from 40 to 200 days.

A water-based cleaning pool was located in front of the footbath to clean the hooves and reduce the manure and organic dirt attached to the hooves from entering the footbath. The cleaning pool dimensions were 300 cm long × 77 cm wide × 20 cm high, while, the footbath dimensions were 400 cm long × 154 cm wide × 18 cm high. The cleaning pool and footbath volumes were 346 and 924 liters, respectively. The footbath solution was initially measured out into a container, before being added to the footbath, and water was added to provide a final depth of 15 cm. The cleaning pool and footbath were both made of cement and built on the milking parlor entrance path. This ensured that all the cows passed through the cleaning pool before first, then footbath and milking.
The chemical composition and concentration for each group of footbath agents are 2.5% chelated copper-zinc mixture (CZM, active ingredients Cu\(^{2+}\) and Zn\(^{2+}\), Intra-Bath, Intracare BV, Veghel, Netherlands) and 1% benzalkonium chloride disinfectant (BCD, active ingredient NR\(_4^+\) (Quaternary ammonium compound), Anti B-25, China Chemical & Pharmaceutical Co., Ltd., Taiwan). The footbath solutions were refreshed daily along with water change of cleaning pool, and therefore no more than 60 cow passes occurred in each footbath.

## Recording Of Locomotion And Assessment Of Dd

Prior to, and at the conclusion of the study, the farm crew graded all the cattle with locomotion scores. Furthermore, a veterinarian performed the DD scoring and the hoof trimming alongside maintaining records of additional hoof diseases throughout the study, i.e., from the start (1st week) to the end (15th week). For ethical and welfare reasons, any detected active DD lesion was treated by the veterinarian with an individual topical dressing of copper sulfate. Further hoof diseases required the veterinarian to perform either hoof trimming, 1 to 3 topical dressings, or hoof shoe sticking, according to symptoms presented. Topical dressings were changed every 3 days, and if the hoof shoes were not worn out, they were removed after approximately 14 days.

Locomotion scores ranged from 1 to 5, whereby 1 indicated normal (i.e. a cow that stands and walks with a flat back and an ordinary gait); 2 indicated mildly lame (i.e. a cow that stands with a flat back but arches the back when walking with a normal gait); 3 indicated moderately lame (i.e. a cow that has an arched back while standing and walked with a gait described as short strides in ≥ 1 limb); 4 indicated (i.e. a cow that has an arched back while standing and walking and favors ≥ 1 limb). Finally, 5 indicated severely lame (i.e., the cow has an arched back while standing and walking and refuses to place or possesses great difficulty placing weight on ≥ 1 limb) [26, 22].

All the cow’s feet were washed with water before the examination. The feet were scored according to the 4-point nominal scale M-stage system [27, 28] that reflects the different clinical stages of DD during the course of the disease. DD lesions were categorized according to the M-scoring system: M0, normal digit skin without signs of DD; M1, early, small, circumscribed red to grey lesions less than 2 cm in diameter; M2, red or red grey, active, ulcerative lesions > 2 cm in diameter; M3, healing stage where a firm scab-like material has covered the DD lesion; M4, late chronic lesions that may have either a thickened epithelium, a proliferative filamentous, or a scab-like mass.

The DD was defined as clinically cured by the progression of the wound lesion from the active stage (M1 or M2) to the non-active stage (M0 or M4) [29, 30].

## Statistical analysis

The data were entered in Excel (Microsoft Inc.) and analyzed using SAS Version 9.4 (SAS Institute Inc.) [31]. The locomotion score variable was combined into binary outcomes for analysis of either lame
(locomotion score > 2) or non-lame (locomotion score ≤ 2) cows. Proc FREQ was used to compare each variable (lameness, cure, M score, and DD stage) outcome between the two treatment groups at the start and end of the experiment. Proc GENMOD was used to analyze the categorical responses of lameness and the cure using logistic regression at the start and end of the experiment. The odds ratio and 95% CI risk between the two treatment groups at the end of the experiment were calculated.

**Abbreviations**

DD
digital dermatitis.

**Declarations**

**Ethics approval and consent to participate**

The experiments were performed in accordance with the Republic of China (Taiwan) regulations and approved by the Institutional Animal Care and Use Committee (IACUC) of Livestock Research Institute, (Approval number: 109-8).

**Consent for publication**

All authors give consent for publication.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article. All raw data can be provided under request.

**Competing interests**

The authors declare no conflict of interest.

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**Authors’ contributions**

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References


Tables

Table 1

The proportion of cows with signs of lameness (locomotion score > 2) at the start (1st week) and the end (15th week) of the experiment. The odds ratio was used to demonstrate lameness in the CZM group in comparison to the BCD group at the end of the experiment.

<table>
<thead>
<tr>
<th>Items</th>
<th>Treatments¹</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCD (%)</td>
<td>CZM (%)</td>
</tr>
<tr>
<td>Proportion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start (1st week)</td>
<td>13%</td>
<td>40%</td>
</tr>
<tr>
<td>End (15th week)</td>
<td>20%</td>
<td>26%</td>
</tr>
<tr>
<td>Odds ratio at End (95% CI² risk)</td>
<td>Baseline</td>
<td>1.167 (0.320 - 4.259)</td>
</tr>
</tbody>
</table>

¹Treatment: BCD (footbath with 1% quaternary ammonium salt-based disinfectant, n = 27), CZM (footbath with 2.5% chelated copper-zinc mixture, n = 24).

²CI: confidence interval.

The odds ratio for demonstration of lameness at the end stage of experiment was calculated using logistic regression.

Table 2

The proportion of cows providing evidence of a cure from the DD lesions at the start (1st week) and the end (15th week) of the experiment. The odds ratio for a cure of the lesions in the CZM group in comparison to the BCD at the end of the experiment.
<table>
<thead>
<tr>
<th>Items</th>
<th>Treatments$^1$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCD (%)</td>
<td>CZM (%)</td>
</tr>
<tr>
<td>Proportion</td>
<td>Start (1$^{\text{st}}$ week)</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>End (15$^{\text{th}}$ week)</td>
<td>67%</td>
</tr>
<tr>
<td>Odds ratio at end (95% CI$^2$ risk)</td>
<td>Baseline</td>
<td>0.300 (0.095 - 0.948)</td>
</tr>
</tbody>
</table>

$^1$Treatment: BCD (footbath with 1% quaternary ammonium salt-based disinfectant, n = 27), CZM (footbath with 2.5% chelated copper-zinc mixture, n = 24).

$^2$CI: confidence interval.

The odds ratio for evidencing cure of the lesions at the end stage of experiment was calculated using logistic regression.

Table 3

The proportion of DD (M1, M2, M3, and M4) on the left front, right front, left hind, and right hind foot of the cow at different stages of the experiment (start: 1$^{\text{st}}$ week; end: 15$^{\text{th}}$ week).

<table>
<thead>
<tr>
<th>Treatments$^1$</th>
<th>Items</th>
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<th>Right front</th>
<th>Left hind</th>
<th>Right hind</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCD (%)</td>
<td>Start (1$^{\text{st}}$ week)</td>
<td>0</td>
<td>7.1</td>
<td>42.9</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>End (15$^{\text{th}}$ week)</td>
<td>14.3</td>
<td>14.3</td>
<td>28.6</td>
<td>42.8</td>
</tr>
<tr>
<td>CZM (%)</td>
<td>Start (1$^{\text{st}}$ week)</td>
<td>0</td>
<td>0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>End (15$^{\text{th}}$ week)</td>
<td>0</td>
<td>0</td>
<td>57.1</td>
<td>42.9</td>
</tr>
</tbody>
</table>

$^1$Treatment: BCD (footbath with 1% quaternary ammonium salt-based disinfectant, n = 27), CZM (footbath with 2.5% chelated copper-zinc mixture, n = 24).

Figures
Figure 1

The distribution of digital dermatitis (DD) lesion score categories of left front, right front, left hind, and right hind feet at the different stages of the experiment (start: 1st week; end: 15th week) following treatment with A) benzalkonium chloride disinfectant (BCD), B) chelated copper-zinc mixture (CZM). The DD lesions were categorized according to M-stage scoring: M0, normal digit skin without signs of DD; M1, early, small, circumscribed red to grey lesions < 2 cm in diameter; M2, red or red grey, active, ulcerative
lesions > 2 cm in diameter; M3, healing stage, where a firm scab-like material has covered the DD lesions; M4, late chronic lesions that may have thickened epithelium, proliferative filamentous, or a scab-like mass, * $P < 0.05$.

Figure 2

The distribution of digital dermatitis (DD) lesion score categories for cows at various stages of the experiment (start: 1st week; end: 15th week) following treatment with A) benzalkonium chloride disinfectant (BCD), B) chelated copper-zinc mixture (CZM). The DD lesions were categorized according to the M-stage scoring: M0, normal digit skin without signs of DD; M1, early, small, circumscribed red to grey lesions < 2 cm in diameter; M2, red or red grey, active, ulcerative lesions > 2 cm in diameter; M3, healing stage, where a firm scab-like material has covered the DD lesions; M4, late chronic lesions that may have a thickened epithelium, proliferative filamentous, or a scab-like mass, * $P < 0.05$. 