

Evaluation of the use of a copper and zinc chelate in the treatment of cows affected by digital dermatitis

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[Avaliação do uso quelato de cobre e zinco no tratamento de vacas acometidas por dermatite digital]

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ABSTRACT

The aim of this study was to evaluate the effectiveness of copper-zinc chelate spray in the treatment of digital dermatitis (DD). 39 limbs with DD injuries from 36 dairy cows were included, divided into three groups: group 1 treated with spray alone, group 2 with spray and bandage, and group 3 with spray and bandage only in the first 3 days. The cows were evaluated for locomotion score and qualitative analysis of the lesions on D0 (identification of the lesion and start of treatment), D3 (third day of treatment), D7 (seventh day of treatment) and D10 (tenth and last day of treatment). The results indicated that there were no significant differences between the groups in the analyzed variables. The three groups showed improvement in the locomotion score and regression from active to inactive lesion stages. However, at the end of treatment, active lesions were still present in both groups. It was concluded that the proposed 10 days of treatment were insufficient for complete healing of the lesions, suggesting the need to extend the days of application, given the significant regression of the lesions in the established period. More long-term trials are needed to evaluate the effectiveness of copper-zinc chelate spray.

Keywords: foot disease, dairy cattle, lameness

RESUMO

O objetivo deste estudo foi avaliar a eficácia do spray quelato de cobre-zinco no tratamento de dermatite digital (DD). Foram incluídos 39 membros com lesões de DD de 36 vacas leiteiras, divididos em três grupos: grupo 1 tratado apenas com spray, grupo 2 com spray e bandagem, e grupo 3 com spray e bandagem apenas nos primeiros três dias. As vacas foram avaliadas quanto ao escore de locomoção e à análise qualitativa das lesões no D0 (identificação da lesão e início do tratamento), no D3 (terceiro dia de tratamento), no D7 (sétimo dia de tratamento) e no D10 (décimo e último dia de tratamento). Os resultados indicaram que não houve diferenças significativas entre os grupos nas variáveis analisadas. Os três grupos apresentaram melhora no escore de locomoção e na regressão dos estágios de lesão ativa para inativa. Porém, ao final do tratamento, as lesões ativas ainda estavam presentes em ambos os grupos. Concluiu-se que os 10 dias de tratamento propostos foram insuficientes para a cicatrização completa das lesões, sugerindo necessidade de ampliação dos dias de aplicação, dada a regressão significativa das lesões no período estabelecido. Mais testes de longo prazo são necessários para avaliar a eficácia do spray quelato de cobre-zinco.

Palavras-chave: doença podal, gado leiteiro, claudicação

INTRODUCTION

Diseases related to the locomotor system are responsible for causing pain and lameness and are among the most important problems affecting

cattle, especially dairy breeds, surpassed only by reproductive problems and infections of the mammary gland; thus, these diseases cause significant economic losses to properties (Barker *et al.*, 2009; Berry, 2012; Gomez *et al.*, 2012).

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Hoof diseases are some of the problems that affect dairy production and are responsible for the increased incidence of lameness and pain, generating a significant impact on production and animal welfare (Pavlenko *et al.*, 2011). Digital dermatitis (DD) is considered an infectious disease and an important cause of lameness in dairy cattle. It is characterized by hyperkeratotic or ulcerative lesions, typically located on the plantar or palmar aspect of the foot, proximal to the interdigital cleft. Lesions are mostly present on the hindfoot and are associated with lameness, reduced milk production, diminished reproductive performance, and decreased animal welfare (Bruijn *et al.*, 2012; Higginson Cutler *et al.*, 2013; Dolecheck and Bewley, 2018).

DD is endemic in several countries around the world, with a prevalence ranging from 9.3% (Cramer *et al.*, 2008) to 83% (Holzhauer *et al.*, 2006). Other studies have reported a DD incidence above 90% in dairy herds (Jury *et al.*, 2021; Kofler *et al.*, 2022). In Brazil, prevalence rates range from 3.8% (Leão *et al.*, 2009) to 30.3% (Souza *et al.*, 2007). DD is a disease of multifactorial origin, and the risk factors involved include genetics, diet composition, nutritional and environmental management, concrete floors, humidity, a lack of hygiene in facilities, a lack of preventive trimming, and the introduction of infected animals into the herd (Silva *et al.*, 2001; Nishikawa and Taguchi 2008; Pavlenko *et al.*, 2011). It is considered a polymicrobial disease; the etiological agent often found in lesions is spirochete bacteria of the genus *Treponema* sp. Other bacteria have also been found, including *Fusobacterium necrophorum* and *Dichelobacter nodosus* (Mauchle *et al.*, 2008; Wilson-Welder *et al.*, 2015; Nielsen *et al.*, 2016; Moreira *et al.*, 2018).

Treatment is performed with systemic and/or topical antibiotics, of which the most widely used is long-acting systemic oxytetracycline at a dose of 20mg/kg or topical application of 2g to 25g, with or without the use of bandages, with satisfactory results (Nicoletti, 2004; Loureiro *et al.*, 2010; Cramer *et al.*, 2019).

However, the treatment of foot diseases in production animals should only be performed with drugs whose active ingredients are authorized for use in the respective category of

production animals, as this may imply the presence of residues (Laven and Logue, 2006). Previous studies have shown that systemic disease requires the discarding of milk for a prolonged period, with a variable cure rate (Leão *et al.*, 2005; Loureiro *et al.*, 2010).

Topical treatment protocols for DD with or without bandages and associated with surgical debridement have shown satisfactory results, with cure rates of 60% and 73%, respectively (Berry *et al.*, 2010; Toholj *et al.*, 2012).

However, the presence of bacterial strains resistant to some therapeutic protocols with antibiotics, increased restrictions regarding the presence of residues in milk, and the need to respect the milk withdrawal period have limited therapeutic options involving the use of antibiotics (Kamiloglu *et al.*, 2002; Cramer *et al.*, 2019).

Thus, antibiotic-free formulations have been studied to show their efficacy in the treatment and control of DD lesions (Hernandez *et al.*, 1999; Manske *et al.*, 2002; Silva *et al.*, 2005). Studies have shown the efficacy of these formulations in curing DD lesions using copper sulfate, salicylic acid and copper and zinc chelate (Teixeira *et al.*, 2010; Schultz and Capion, 2013; Dotinga, 2014).

Copper-zinc chelate spray is a commercial product (Repiderma[®] Dermatological Spray – Intracare/Elanco) composed of two minerals, which, despite being frequently used for the topical treatment of foot lesions, including DD, has not been scientifically and clinically analyzed in Brazil to confirm its effectiveness. Thus, the objective of this study was to evaluate a copper and zinc chelate in the form of a topical spray and some protocols for its use in the treatment of dairy cows affected by DD and kept in a *free-stall* regimen.

MATERIALS AND METHODS

The methodology was approved under protocol 0135/2019 by the Committee on Ethics in the Use of Animals (CEUA) of the São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science, Botucatu.

A total of 36 black and white Holstein cows (32 in the lactation period, two in the precalving period and two in the dry period) aged between two and a half to eight years were obtained from a single farm employing a *free-stall* system with a grooved concrete floor. The animals were divided into five lots according to their production categories and ages and maintained under the same management conditions. The cows' diets, based on their production level and nutritional requirements, consisted of corn silage, cottonseed, and mineral salt ad libitum in a separate trough, mash feed produced on the farm, and water ad libitum.

Milking was performed three times a day while the cows were housed in a waiting room with a rubber floor, where they were then bathed by a sprinkler. Simultaneously, the sheds that accommodated the animals were cleaned of manure by tractors with blades, followed by flushing with water. The cows' bedding consisted of sand, which was not periodically changed. The farm did not take preventive measures against foot diseases, such as footbaths or animal hoof trimming.

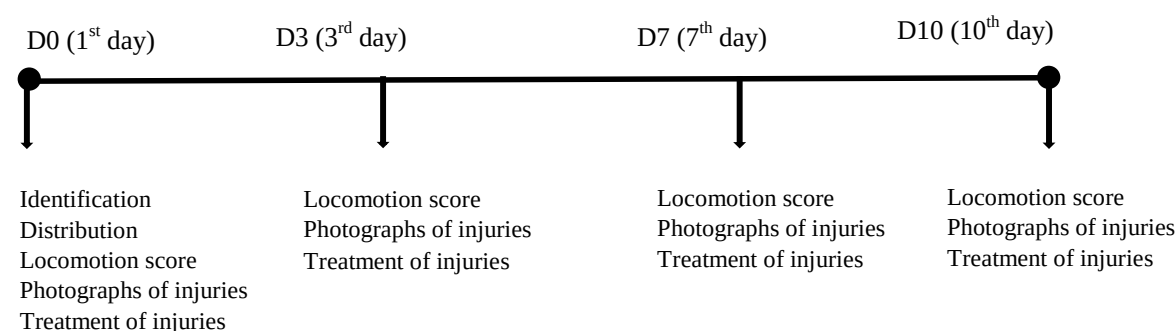
The examination was preceded by retention in a chute specifically designed for trimming the animal while it was upright. After the animals were restrained, information such as age, animal identification according to the tag number (ID), category, batch and experimental group was recorded. Cows were diagnosed with DD based on a direct inspection of the limbs and macroscopic visual physical examination of the

lesions on at least one of the limbs. In this study, animals with lesions from other claw diseases were not included.

The lesions were identified on D0, i.e., immediately before starting treatment, and then evaluated on D3 (three days after D0), D7 (seven days after D0), and finally D10 (ten days after D0) for all individuals. All animals were analyzed in terms of the locomotion score, and the lesions were photographed for later classification by an evaluator on D0, D3, D7 and D10.

A total of 39 limbs with lesions were randomly divided into three groups for evaluation; some animals had their limbs categorized into different groups. The product was applied following the manufacturer's guidelines by spraying it along the entire length of the lesion until it was covered, leaving a green layer of spray over the entire surface area. The product bottle was kept 15–20cm from the injured skin during activation of the spray.

Subsequently, Group 1 (G1), consisting of 13 limbs with lesions treated with the zinc and copper chelate directly on D0, D3 and D7, was established. Group 2 (G2) comprised 13 limbs with lesions treated with the product on the same days in addition to bandaging with a crepe bandage shortly after the application of the spray. Group 3 (G3), which also consisted of 13 limbs, was bandaged after application of the product only on D0; only the spray was used on D3 and D7.



G1 - Group without bandages; G2 - Group with bandages; G3 - Group with bandages for the first 3 days.

Figure 1. Flowchart of the experimental design showing the days of the evaluation and the times and days of treatment of the lesions for the three groups.

The treatments were evaluated by determining the locomotion score (Sprecher *et al.*, 1997), as described in Table 1, at four time points (D0, D3, D7 and D10).

The animals were kept upright and contained in the chute for trimming, which allowed elevation of the animal's limb, a specific clinical examination of the digits and macroscopic

characterization of the lesion of the DD (Dirksen, 1993). During the examination, the digits were cleaned only with running water to remove dirt from the hoof and ensure better visualization of the wound. No antiseptic was used so as not to influence the treatment. After cleaning, the lesions were photographed, and then a spray of zinc and copper chelate was applied.

Table 1. Description of the locomotion score used to categorize the presence and degree of lameness in dairy cows. Adapted from Sprecher (1997)

Locomotion score	Clinical description	Description
1	Normal locomotion	Normal locomotion with a level back. Firm and long gait.
2	Irregular locomotion	Stands in station with a level back, but arches when walking. Slightly abnormal locomotion.
3	Moderate claudication	Arches back when in station and when walking. Walks with short steps.
4	Evident claudication	Arches back when in station and during locomotion. Favors one or more limbs, can support little weight on the others.
5	Severe claudication	The back is always arched. Reluctant to move, difficulty bearing weight on the affected limb.

The M scoring system (Kofler *et al.*, 2020) was used to evaluate the morphological and macroscopic characteristics of the DD lesions, example Fig. 2, which were classified by 3 experienced evaluators who were blinded at the time of evaluation. The evaluators classified the lesions into *M-stages* according to this M scoring

system. The lesions were categorized as shown in Table 2 at the four time points (D0, D3, D7 and D10). The digital files contained photographic images of the lesions, an evaluation form directly illustrating all stages of the M-scoring system for DD and a table with a legend describing the *M-stages*.

Table 2. System used to classify digital dermatitis lesions according to Kofler *et al.* (2020)

M-stages	Description
M0	Healthy skin with no visible signs of DD lesions.
M1	Initial stage of the disease; focal, reddish-gray, circumscribed and small lesion (less than 2cm in diameter) located in the epidermis of the interdigital cleft (plantar or dorsal) or around the crown of the hoof.
M2	Acute ulcerative lesion with a red or reddish-gray appearance, diameter greater than 2cm and commonly located on the skin of the plantar or dorsal interdigital region. The animals indicate pain when touched and pressure is applied to the wound. Presents with a strong odor.
M3	The lesion has a scarred appearance and is painless to the touch. A firm crust with black, gray, blue-green or brown coloration can be noted.
M4	Painless and chronic lesions of various sizes, presenting with characteristics of hyperkeratosis circumscripta or irregular proliferative growth (verrucous) with a grayish-brown coloration.
M4.1	Chronic lesion with M1-stage characteristics, developing into hyperkeratosis and/or a proliferative M4 lesion.



Figure 2. Representative photographs of the M-stages. (A) Representative image of M0, (B) representative image of M1, (C) representative image of M2, (D) representative image of M3, (E) representative image of M4, and (F) representative image of M4.1.

Agreement between the raters regarding the M-stages was assessed using the percentage agreement calculated with the following formula: $PA_0 = [(number\ of\ exact\ agreements / total\ number\ of\ observations) \times 100]$. Additionally, true agreement was assessed using the Kappa Fleiss (κ) test based on the different categories of degree of injury and the opinions of the six evaluators. Actual agreement was interpreted according to Landis and Koch (1977) as follows: ≤ 0 = poor; 0.01 to 0.20 = mild; 0.21 to 0.40 = regular; 0.41 to 0.60 = moderate; 0.61 to 0.80 = substantial; and 0.81 to 1.00 = almost perfect. The significance level was set at $P < 0.05$. The analyses were performed using the SAS version

9.4 (SAS Institute Inc., Cary, NC, USA) and GraphPad Prism version 8.4.3 statistical packages.

RESULTS

The locomotion scores of the animals in both groups were evaluated on the proposed evaluation days to measure the effectiveness of the three proposed therapeutic protocols. Moderate lameness (grade 3) was observed at the time of identification of the DD lesion and before treatment, evolving to normal locomotion in all three groups on D10, as shown in Table 3.

Table 3. Median (1st and 3rd quartiles) locomotion scores of the treated groups at all evaluation times

Groups	D0	D3	D7	D10
G1	3 (2–3) ^A	1 (1–2) ^A	1 (1–2) ^A	1 (1–2) ^A
G2	2 (2–3) ^A	2 (1.5–3) ^B	2 (1–2) ^A	1 (1–2) ^A
G3	2 (2–3) ^A	2 (2–2.5) ^B	2 (1–2) ^A	1 (1–2) ^A

(A–B) Different letters between lines indicate significant differences ($P < 0.05$).

The Friedman test, which compares locomotion scores across different days within the groups, showed that the treatments were effective at improving locomotion. A significant difference was observed between D0 and the other days of treatment, as shown in Figure 3.

All DD stages of all groups and time points were classified according to the M scoring system by the evaluators. Subsequently, they were further categorized into stages, which aimed to classify the lesion as 0 = no lesion, 1 = inactive lesion and 2 = active lesion, as shown in Table 4.

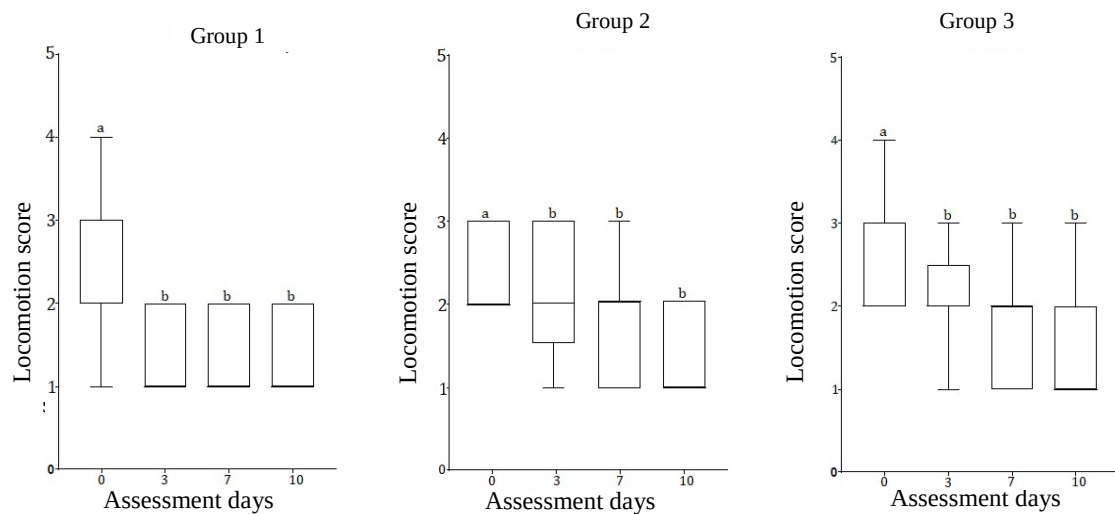


Figure 3. Results of the evaluation of the locomotion score of the three groups treated with a topical application of a copper and zinc chelate in spray form. The box plots show the 25th and 75th percentiles and the median (x) values. (a–d) Different letters between the box plots indicate a significant difference ($P < 0.05$).

Table 4. Median (1st and 3rd quartile) stage classifications for the treated groups at all evaluation times

Groups	D0	D3	D7	D10
G1	2 (1–2) ^{Aa}	1 (1–1) ^{Aa}	1 (1–1) ^{Aa}	1 (1–1) ^{Aa}
G2	2 (1–2) ^{Aa}	1 (1–2) ^{Aa}	1 (1–2) ^{Aa}	1 (1–2) ^{Aa}
G3	2 (1–2) ^{Aa}	1 (1–2) ^{Aa}	1 (1–1.5) ^{Aa}	1 (1–1) ^{Aa}

(A–B) Different letters between columns indicate significant differences ($P < 0.05$).

(a–b) Different letters between the lines indicate significant differences ($P < 0.05$).

The Mann–Whitney U test, which compared the lesion stages among the groups, did not show a significant difference. The Friedman test did not show a significant difference when comparing time points within the same group. However, the stages of the lesions on D0 and D10 differed from each other, i.e., the lesions that were in the active stage on D0 were in the inactive stage on

D10, suggesting that the treatment used for all three groups led to a transition between the stages of the lesions. Only one lesion each in Groups 1 and 2 reached the M0 stage. Interobserver agreement was absent or very low at most time points for the different groups, as evidenced by the results of the Kappa test in Table 5.

Table 5. Interobserver agreement in the evaluations from three evaluators at all times for all groups

Groups	D0	Kappa agreement values (95% CI)		
		D3	D7	D10
G1	- 0.08 (-0.55–0.39)	-0.08 (-0.49–0.33)	0.28 (-0.23–0.79)	0.62 (-0.07–1.00)
G2	0.076 (-0.31–0.46)	-0.17 (-0.14–0.49)	0.13 (-0.38–0.64)	0.22 (-0.08–0.53)
G3	- 0.04 (-0.66–0.57)	0 (-0.37–0.36)	-0.06 (-0.39–0.27)	0.19 (-0.19–0.5)

Agreement measured using κ was interpreted as ≤ 0 = absent; 0.01 to 0.20 = very low; 0.21 to 0.40 = considerable; 0.41 to 0.60 = moderate; 0.61 to 0.80 = substantial; and 0.81 to 1.00 = perfect (Landis & Koch, 1977).

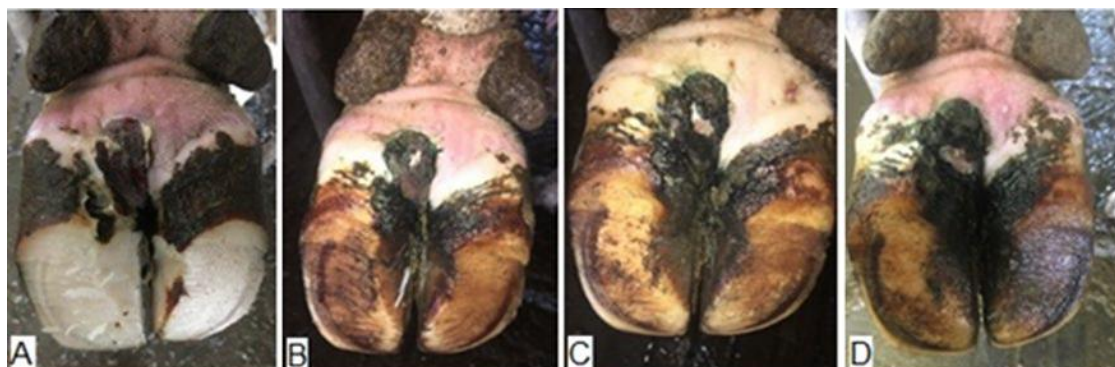


Figure 4. Animals with treated limbs in Group 1 (without bandages). (A) D0, initial day of treatment; (B) D3, 3rd day after treatment initiation; (C) D7, 7th day of treatment; (D) D10, 10th day of treatment.



Figure 5. Animals with treated limbs in Group 2 (with bandages). (A) D0, initial day of treatment; (B) D3, 3rd day after treatment initiation; (C) D7, 7th day of treatment; (D) D10, 10th day of treatment.



Figure 6. Animals with treated limbs in Group 3 (bandage until the 3rd day). (A) D0, initial day of treatment, (B) D3, 3rd day after treatment initiation; (C) D7, 7th day of treatment; (D) D10, 10th day of treatment.

The graph in Fig. 7 illustrates the evolution of the lesions in Group 1, showing that at the beginning of treatment, 13 active lesions, i.e., stage 2, were present in high quantities. However, they decreased in activity during treatment, and on the last day of evaluation, 10 inactive lesions were at stage 1, only two active

lesions were present, and one limb showed complete healing of the lesion, with a classification of M0 stage. As shown in the graph in Fig. 8, at the beginning of treatment, all lesions (13) were active (stage 2), and this grade prevailed throughout treatment.

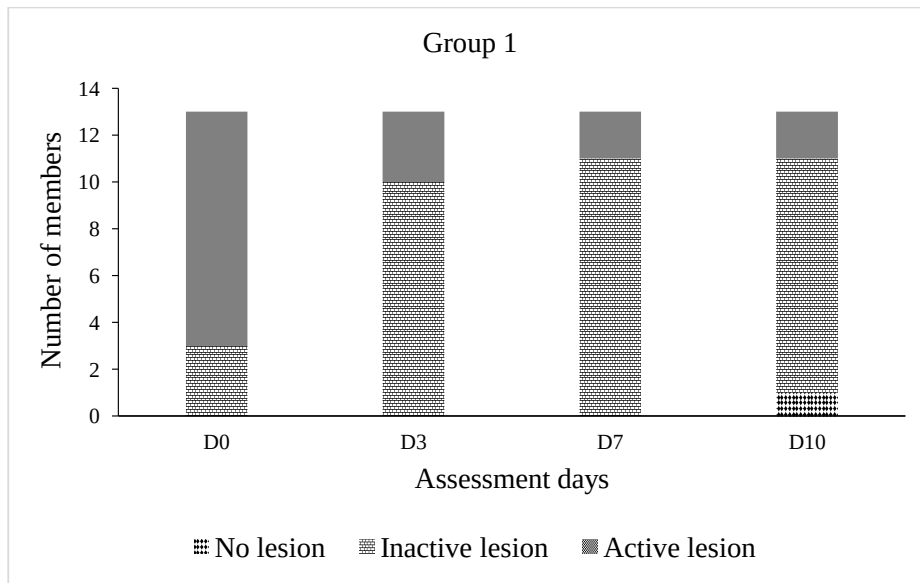


Figure 7. Clinical evolution of the lesions during the 10 days of treatment in G1. Lesion stage transitions (active, inactive and nonexistent) can be observed.

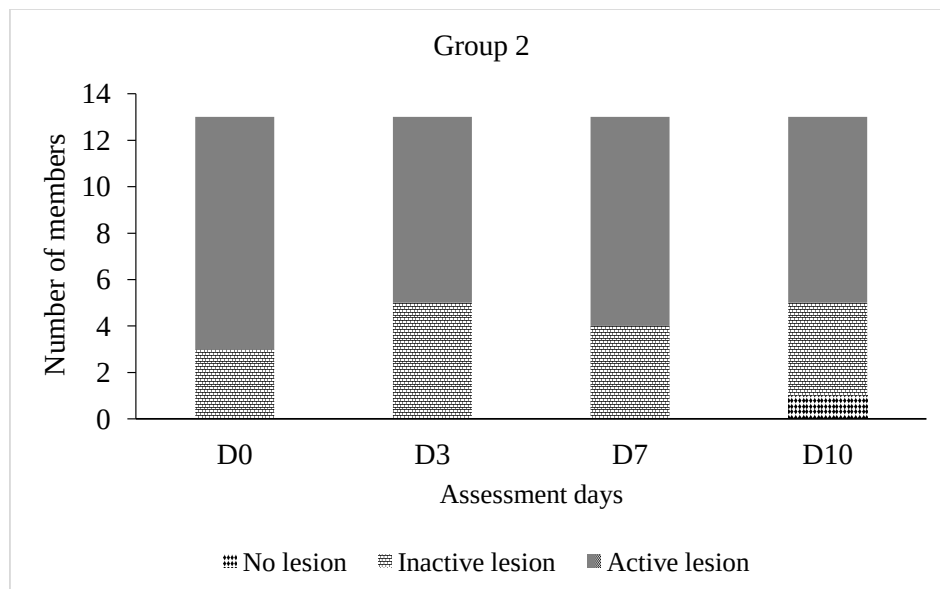


Figure 8. Clinical evolution of the lesions during the 10 days of treatment in G2. Lesion stage transitions (active, inactive and nonexistent) can be observed.

On the last evaluation day, inactive lesions (stage 1) were present in small numbers (4), while more active lesions were present (8). One limb showed complete healing of the lesion (stage 0).

The graph in Fig. 9 shows that at the beginning of treatment of Group 3, more lesions (11) were active (stage 2), but this quantity decreased during treatment. On the last day of evaluation, most lesions (9) were inactive (stage 1), while only a small number (4) were active.

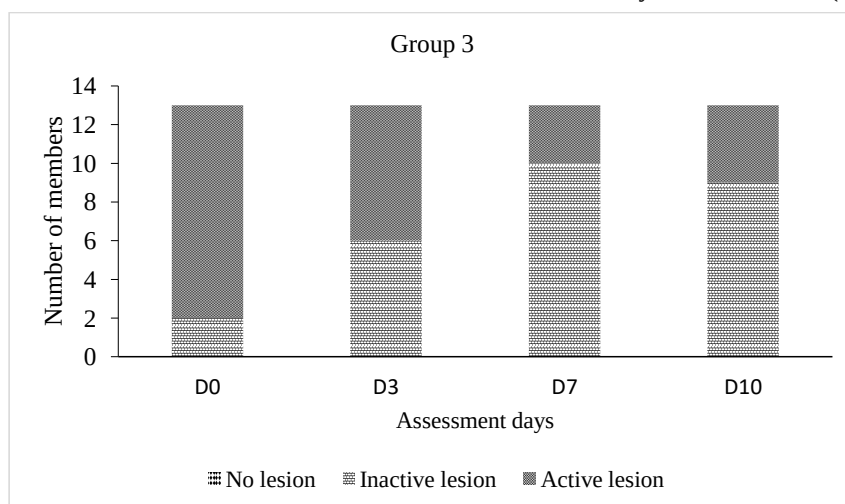


Figure 9. Clinical evolution of the lesions during the 10 days of treatment in G3. Lesion stage transitions (active, inactive and nonexistent) can be observed.

DISCUSSION

The cows from the same farm were maintained under the same environmental conditions and subjected to unchanged routine farming activities; milking and diet provided homogeneity to the experimental groups. This standardization contributed to the reduction in stress and discomfort caused by the changes inherent to the proposed experimental protocol (Nicoletti, 2004, Loureiro *et al.*, 2010). The use of a containment chute suitable for trimming, without the need for chemical agents for restraining the animals, was effective because no accidents or difficulties occurred during data collection (Desrochers *et al.*, 2001, Loureiro *et al.*, 2010).

When evaluating the lameness of selected animals at different stages of DD, no significant differences were observed among the three groups evaluated in terms of the locomotion score. Throughout the treatment, the three groups showed a lower degree of locomotion; namely, the animals presented a normal gait at the end of the three proposed treatment protocols, as shown in Table 4. Thus, regardless of the use of the

dressings, the treatment influenced improving the locomotion score.

The locomotion scores of the animals in G1, especially from D3 onwards, decreased more rapidly than those of the other groups. This observation is corroborated by other studies, which showed that the use of topical substances that have in their formula copper-zinc sulfate, acidified ionized copper solution and acidified sodium chlorite solution contribute to improving the locomotion score of the treated animals (Britt *et al.*, 1996; Stevančević *et al.*, 2009; Klawitter *et al.*, 2019).

The results obtained here were seen in other studies that compared the effect of topical application of two solutions for the treatment of DD, one containing iodine and the other containing copper, which also resulted in a lower percentage of animals with lameness at all follow-up times (Paudyal *et al.*, 2020).

However, at the end of the treatment days, most lesions present in both groups were inactive, also classified as chronic, and presented lower pain sensitivity compared to active lesions. This factor may have masked the perception of

improvement in the lameness score, and these chronic lesions serve as a reservoir, with the possibility of evolving into active lesions (Alsaad *et al.*, 2022).

Therefore, the active infection could be under the crust visualized at stages M3 and M4 (Berry *et al.*, 2012; Krull *et al.*, 2016; Plummer and Krull, 2017). This is consistent with the claim that *Treponema* spp., the main pathogen of DD (Moreira *et al.*, 2018), can migrate, penetrate the deep dermis and form a biofilm, favoring its proliferation and persistence of the infection (Beninger *et al.*, 2018; Lux *et al.*, 2001).

In all groups evaluated, the lesions presented macroscopic changes and changes in M-stage after topical application of copper and zinc chelate due to mechanisms inherent to antiseptics that prevent the development of bacterial resistance. Zinc sulfate has bactericidal activities, as it penetrates the deepest layers of the tissue. Copper is an oxidizing agent with important deleterious effects on microorganisms, acting as an astringent and bactericidal and fungicidal agent (Stevančević *et al.*, 2009).

Interobserver agreement (Table 5) for the DD score was absent in most evaluations. The number of animals used for the study together with the short period of follow-up for the proposed treatment may have influenced the classification of the lesions by the evaluators, in contrast to other studies proposing the same method of evaluation of agreement (Solano *et al.*, 2017, Jacobs *et al.*, 2018).

The classification of the M-stage is a subjective evaluation; in previous studies that used the Kappa test, the evaluators underwent training with direct visualization and photographs of lesions to classify the lesions (Relun *et al.*, 2012, Solano *et al.*, 2017). In the present study, the evaluators did not undergo training, and lesion evaluations were performed only with photographs. The clarity of the image together with the focal length may have influenced the classification of the lesions by the evaluators and the subsequent statistical analyses.

Table 4 suggests that no significant differences were present between the groups throughout the treatment period. Nevertheless, the topical use of a zinc and copper chelate in spray form,

regardless of the use of bandages, contributed to the lesions transitioning from the active stages (stage 2—M1, M2 and M4.1) to the inactive stages (stage 1—M3 and M4) of digital dermatitis. This result was also observed by Jacobs *et al.* (2018), who compared the efficacy of a topical treatment with a tetracycline solution with that of a commercial product containing copper and zinc and showed that the product was superior in the clinical cure of active lesions within one week of treatment.

Holzhauser *et al.* (2011) also described the effect of topical treatment with a copper zinc chelate gel on the healing of M2 lesions (transitioning to M0) compared to that of a chlortetracycline spray at the end of 28 days of treatment. The number of active lesions decreased, but at the end of 10 days of treatment, both groups presented with active DD when analyzing the evolution of lesions throughout the treatment period for all groups in graphs 1, 2 and 3. Complete healing of the lesion implies evolution to the M0 stage, which rarely occurs in treatments lasting less than one week (Krull *et al.*, 2016).

Notably, in this study, the transition from the active (M1, M2 and M4.1) to inactive (M3 and M4) stages was also analyzed. However, studies have shown that lesions in the M4 stage can regress to the active stage, and the possibility of transitioning to M0 is relatively low when treatment lasts less than 4 weeks (Berry *et al.*, 2012, Weber *et al.*, 2019).

In addition, stage M4 is considered a chronic DD lesion characterized by hyperkeratosis, which hinders the penetration of substances in topical treatments and makes therapy difficult. Other studies indicate that lesions in stage M4 persist longer than those in other stages and are also sources of bacterial populations, contributing to the occurrence of new lesions or the recurrence of existing lesions (Relun *et al.*, 2012, Schultz and Capion, 2013; Biemans *et al.*, 2018; Klawitter *et al.*, 2019). This finding indicates its importance in the treatment of lesions considered inactive.

Alsaad *et al.* (2022) showed that weekly treatment of 26 stage M2 lesions with a salicylic acid paste and bandages resulted in regression to stage M0 in four weeks. The elimination of

Treponema spp. from deep epidermal layers was observed via PCR.

This and other studies comment on the importance of treating lesions considered active (M2), painful phases and the spread of the disease among healthy individuals (Somers *et al.*, 2005, Alsaad *et al.*, 2022).

In the present study, the 10 days proposed for treatment and observation for the transitions of the M-stages of the lesions of both groups did not allow the observation of the probability of lesion recurrence. This information is worth investigating, as some studies have shown that the complete cure of active lesions with copper and zinc chelate based products occurs over a mean time of 28 days of treatment (Relun *et al.*, 2012).

Jacobs *et al.* (2018) reported that the probability of recurrence for lesions treated with a product containing copper, zinc and aloe vera was lower after 15 days of observation. In the present study, only one limb with active DD treated in Groups 1 and 2 progressed to M0; however, the size of the lesions and the use of bandages contributed to faster healing.

Thus, larger lesions may be associated with a greater presence of microorganisms and a more harmful etiology, causing a lower cure rate (Elliott *et al.*, 2007, Shahabaddin *et al.*, 2007, Nishikawa and Taguchi, 2008).

Another observation to be considered was the grouping of active lesions (M1, M2 and M4.1), which may have contributed to diluting the effect of the treatment of a more susceptible stage with respect to a more resistant stage. Thus, future studies should focus on investigating single stages (Jacobs *et al.*, 2018).

We observed that the use of bandages did not cause significant differences between the groups. However, bandages are commonly used as part of therapeutic protocols for foot diseases because of their ability to prevent trauma and environmental influences, thus producing an environment that favors healing (Kamiloglu *et al.*, 2002, Nicoletti, 2004).

In this study, animals from Groups 1 and 3 received treatment without bandaging for a

certain period. Thus, the results suggested that the lesions of these groups took longer to transition to the inactive stage because they were unprotected and therefore influenced by external factors (Capion *et al.*, 2018). When DD is prevalent in the herd and the facility tends to have poor hygiene, the cows may become reinfected (Capion *et al.*, 2012).

Figure 6 shows that most lesions tended to be active throughout treatment, suggesting that even with the use of bandages, the presence of moisture may have contributed to increasing the transition time between the stages of the lesions.

A study conducted by Klawitter *et al.* (2019) revealed that the use of bandages to treat stage M2 injuries achieved a cure rate of more than 70% and reduced progression to chronic stage M4. For the treatment of the M2 DD stage, dressings reinforced with cotton, bandages and tar were used for waterproofing, ensuring protection against environmental factors for longer periods of time and resulting in better wound healing.

In this study, G2 received two crepe bandages on the injured limbs to protect against external factors that could harm the healing process. However, on the days when the injuries were assessed and the dressings were changed, they were damp; namely, the dressings did not protect the injuries from environmental humidity.

Humidity is one of the factors that contributes to the development of digital dermatitis, and studies have shown that environmental factors, such as humidity and poor hygiene in facilities, hinder the wound healing process (Silva *et al.*, 2001; Dotinga, 2014).

Importantly, the effectiveness of dressing use is influenced by the treatment time, stage and location of the injury, and the cleanliness and humidity of the facilities. This finding has also been observed in previous studies by Klawitter *et al.* (2019).

The type of dressing also influences the healing process. Studies have shown that applying a dressing reinforced with cotton, gauze, and compresses increases the likelihood of DD healing, as these dressings offer better protection

of the wound against external factors (Toholj *et al.*, 2012; Klawitter *et al.*, 2019).

Notably, before performing bandage treatment, the type of dressing to be used, environmental factors, material costs, time and skill in handling the animal and removing the dressing, when necessary, must be known to prevent the development of other injuries (Anderson and White 2000; Toholj *et al.*, 2012; Klawitter *et al.*, 2019).

The extremities of the limbs were washed only with water to remove any adhered dirt (feces and mud), helping to improve the contact between the lesion and the product without interfering with the aseptic treatment of the injured surface. The same methodology was used by Jacobs *et al.* (2018), who observed that washing the toes with water alone improved foot hygiene and the contact surface for the product being applied.

Therefore, it is known that the microminerals present in the copper and zinc chelate formula are responsible for participating in and regulating certain aspects of the healing and tissue regeneration processes (Schwartz *et al.*, 2005; Polefka *et al.*, 2012; Lin *et al.*, 2017), and we believe that the addition of the chelate to the formula helped to improve the bioavailability of the microminerals and, therefore, their actions on the lesion.

However, studies are needed to determine the long-term effects of this spray (Holzhauer *et al.*, 2011; Relun *et al.*, 2012).

CONCLUSIONS

The topical application of a copper and zinc chelate in the form of a spray on active lesions of digital dermatitis in cows promotes the transition to other less infectious stages and contributes to improving the locomotion score. However, the 10-day period was not long enough for complete regression of the lesions (M0), making it necessary to extend the treatment period. No difference in treatment was observed among the three groups in terms of whether a bandage was used.

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