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An acidified product with zinc, copper and tea tree oil compounds: a strategy to treat digital dermatitis in dairy cattle using HoofCare®

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ABSTRACT. - Mendonça APA, Moura RBR, Teodoro PHM, Ravetti R, Villettaz-Robichaud M, Rodrigues CA. **An acidified product with zinc, copper and tea tree oil compounds: a strategy to treat digital dermatitis in dairy cattle using HoofCare®**. *Pesquisa Veterinária Brasileira 46:00, 2026*. Departamento de Cirurgia Veterinária e Reprodução Animal, Faculdade de Medicina e Zootecnia, Universidade Estadual Paulista "Júlio de Mesquita Filho", Rua Prof. Doutor Walter Mauricio Corrêa s/n, Cx. Postal 560, Botucatu, SP 18618-681, Brazil. E-mail: celso.a.rodrigues@unesp.br

This study aimed to evaluate the effectiveness of an antibiotic-free footbath protocol with the product HoofCare® for the treatment, prevention, and control of digital dermatitis (DD) lesions in dairy cows, when used in combination with brushing the lesions with the same product. The study was conducted on a large commercial dairy farm in Brazil and lasted 15 weeks. The entire lactating herd, estimated at over 2,200 Holstein cows, was included. As this study was a continuation of the first investigation of the efficacy of this product in footbaths, the protocol was based on preliminary results on the optimal number of passages using pH changes and the effect of intensive footbathing with the product. The protocol consisted of a footbath with 10% HoofCare® applied once per day, three times a week, along with topical treatment of the DD lesions by brushing them three times a week on alternate days with the footbath. Data collection focused on the number of affected hind limbs and lesion classification (non-existent, inactive, and active) at eight inspection points. The results showed a significant reduction in the prevalence and incidence of DD lesions, from 49.85% to 17.17% and 20.91% to 1.80%, respectively. Furthermore, at the end, most hind limbs had no lesions (82.83%), followed by inactive lesions (13.22%), and active lesions (3.95%). Logistic regression analysis showed there was no significant impact of days in milk and lactation on lesion development ($p > 0.8$). Our findings demonstrated that this footbath protocol with HoofCare® combined with brushing lesions with the same product is effective in preventing, controlling, and treating DD lesions on a dairy farm.

INDEX TERMS: Digital dermatitis, footbath, treatment, dairy cattle, HoofCare®.

RESUMO.- [**Um produto acidificado com zinco, cobre e óleo de melaleuca: uma estratégia para tratar dermatite digital em vacas leiteiras usando HoofCare®.**] O objetivo do presente estudo foi determinar a eficácia do protocolo de pedilúvio sem antibióticos com o produto HoofCare® para o tratamento, prevenção e controle de lesões de dermatite digital (DD) em vacas leiteiras quando combinado com o pincelamento das lesões utilizando o mesmo produto. Este estudo foi realizado em uma grande fazenda leiteira comercial do Brasil durante 15 semanas. Todo o rebanho lactante estimado em mais de 2.200 vacas da raça Holandês foi incluído. Como este estudo foi uma continuação da primeira investigação da eficácia deste produto em pedilúvios, baseou-se o protocolo em resultados, o número ideal de passagens usando variações de pH, e o efeito do uso de pedilúvio intensivo com o produto. O protocolo consistiu em pedilúvio com HoofCare® 10% aplicado uma vez ao dia, três vezes por semana, associado ao tratamento tópico das lesões de DD pelo pincelamento três

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vezes por semana em dias alternados ao pedilúvio. A coleta de dados incluiu os membros pélvicos afetados e classificação das lesões (inexistente, inativa e ativa) em oito momentos de inspeção. Os resultados demonstraram uma redução significativa na prevalência e incidência de lesões de DD de 49,85% para 17,17% e de 20,91% para 1,80 respectivamente. Além disso, no final, a maioria dos membros pélvicos não apresentavam lesões (82,83%), seguido por lesões inativas (13,22%) e lesões ativas (3,95%). A análise de regressão logística mostrou que não houve impacto significativo dos dias em lactação no desenvolvimento da lesão ($p > 0,8$). Nossos resultados demonstraram que este protocolo de pedilúvio com HoofCare® combinado com pincelamento com o mesmo produto em ordenha rotativa é eficaz em prevenir, controlar e tratar lesões de DD na fazenda leiteira.

TERMOS DE INDEXAÇÃO: Dermatite digital, pedilúvio, tratamento, vacas leiteiras, HoofCare®.

INTRODUCTION

The management of digital dermatitis (DD) remains a challenge for dairy producers because it is a contagious, painful, widespread disease with multifactorial etiopathogenesis, and it is endemic in intensive housing systems (Wilson-Welder et al. 2015, Moreira et al. 2018, Caddey & Buck 2021). Thus, DD requires a combination of measures, such as clean facilities, hoof trimming, diet, genetics, and preventive treatments at the cow and herd levels (Palmer & O'Connell 2015). Although DD is one of the most investigated cattle hoof lesions worldwide (Orsel et al. 2018), there is a lack of efficient management strategies tailored to each different farm reality. In addition to the footbath protocol, a good individual treatment protocol for active lesions is necessary (Döpfer 2021, Chen et al. 2023), as several studies have shown that the cured stage was not achieved by footbaths alone (Smith et al. 2014, Marshal et al. 2024), including a previous investigation by this group (unpublished data).

There are a considerable number of compounds available for use in footbaths and as topical treatments in Brazil for dairy cattle (Silva et al. 2005, Teixeira et al. 2010, Moreira et al. 2018, Bomjardim et al. 2020, Moura et al. 2025). However, footbaths with copper sulfate and formalin remain the most used, even if they have limitations in effectiveness and varying degrees of toxicity to the environment, animals, and users (Jacobs et al. 2019).

A commercial product (HoofCare®) based on a combination of potent antimicrobials (copper, zinc and tea tree oil) has shown promising efficacy in the topical treatment of DD lesions (Mendonça et al. 2024b) and good empirical field results when used in footbaths. Due to its compositions and characteristics, it has the potential to be used in a practical and effective management, which is of utmost importance for Brazilian livestock farming. Therefore, the aim of this study was to determine the effectiveness of the antibiotic-free footbath protocol with 10% HoofCare®, combined with brushing the product as a topical treatment, as a prevention and control of DD lesions in dairy cows.

MATERIALS AND METHODS

Ethical approval. This research was approved by the Ethical Committee for the Use of Animals (CEUA, approval number: 0013/2022) of the "Faculdade de Medicina Veterinária e Zootecnia" (FMVZ) of the "Universidade Estadual Paulista 'Júlio de Mesquita Filho'" (Unesp) in Botucatu, São Paulo, Brazil.

Farm and management context. The study was conducted on a commercial dairy farm located in the city of Araras, São Paulo State, Brazil (22° 21' S latitude, and 47° 23' W longitude), with a lactating herd of approximately 2,200 Holstein cows. The cows were divided into eight lots based on category and productivity of the animals, housed in a free-stall barn with sand bedding, a slatted concrete floor, and a cross-ventilation system, which provided a temperature of around 21 °C. The farm's capacity was 290 cows per lot with milking three times a day in a rotary system and a cooling system with intermittent sprinklers in the milking waiting area. Simultaneously with milking, the barn aisles were flushed once per milking, three times per day, with scraping performed every 90 days. As there was no change in management for the experiment, the lactating herd turnover was dynamic, with daily introduction of new animals. During the experimental period, the overall turnover rate was 36.22%.

Standard hoof care on the farm consisted of preventive trimming before the dry-off period once a year and therapeutic trimming as needed. Specific management of DD prior to the study included inspections every two weeks in the rotary milking parlor with application of tetracycline spray to active lesions every 25 days. A footbath protocol with 5% copper sulfate twice weekly for lactating cows and once weekly for heifers and dry cows, and a footbath with 2% formalin once every two weeks for one specific lot of lactating cows.

The experiment was conducted from April to July 2022. The farm's average daily milk production exceeded 91,000 kg, with a mean bulk-tank somatic cell count of 224,187 cells/ml.

Footbath and brushing protocol for lactating cows. Two footbaths positioned at the milking exits in a barn were used for this experiment. Their dimensions were 190 cm (width) x 260 cm (length) for the right footbath and 192 cm x 270 cm for the left footbath. They were used simultaneously three times per week, once daily immediately after morning milking, and without pre-bathing for 15 consecutive weeks. As HoofCare® has a gel-like texture, all footbaths in this experiment were prepared as follows to achieve a 10% concentration: 20 kg of the product was added, followed by a small amount of cold water to aid in dilution. Water was added slowly at first to ensure complete dissolution of the gel, with the flow gradually increased until a minimum depth of 10 cm was achieved in the footbaths to ensure coverage of typical DD lesion sites.

In addition, all lesions brushing with HoofCare® were performed during morning milking in the rotary system, three times a week, on alternate days with the footbath. This brushing during milking was limited to the first month (four weeks) of the experiment to help control the outbreak of DD lesions on the farm observed in a previous study. In the subsequent 11 weeks of the experiment, individual brushing treatment was performed only when a DD lesion (active and inactive) was identified in the trimming chute, either during preventive (at dry-off) or corrective (any lameness cause, e.g., sole ulcer) hoof trimming.

This study was a continuation of the investigation into the effectiveness of the commercial product HoofCare® in footbaths. Based on a pilot study (Mendonça et al. 2024a) and considering the milking management, farm's infrastructure, and cows' preference for the left side due to the mats present on the flooring before the

footbath, the protocol was defined to include three footbath changes per day: one change for the right side footbath and two for the left side footbath. This approach allowed the solution to remain within the optimum pH threshold < 4.5 (Prastiwi et al. 2019).

It is noteworthy that, based on the findings of a previous study by our research team on intensive and exclusive use of footbaths with this product, footbaths needed to be associated with individual treatment of lesions for optimal effectiveness (Unpublished data).

Footbath protocol for dry cows and heifers. Based on the prevalence of active lesions in the fresh cows' group in a previous study (Unpublished data), the farm's footbath protocol for dry cows and heifers was insufficient. Therefore, in addition to the protocol proposed for lactating cows, a new protocol using 10% HoofCare® once a week was established for this category of animals at the beginning of the experiment. It is important to note that fresh cows were defined as those within the first 30 days in milk.

Evaluation method. Only hind limbs were included in the study. The diagnosis of DD lesions was made only by visual inspection after washing the feet with running water. Hind limbs were evaluated for the presence or absence of DD lesions. If a lesion was detected, it was classified according to the M-stage scoring (Kofler et al. 2020) as active (M1, M2 and M4.1) or inactive (M3 and M4), as done in previous studies (Solano et al. 2016, Mendonça et al. 2024b). If a cow presented with a lesion on either (or both) hind limbs, it was classified as "with DD". The assessments were performed at eight timepoints (D0 = four days before the start of the protocol, week 2, w.3, w.4, w.5, w.7, w.9, and w.15). They were always conducted during morning milking in the rotary system, before the footbath, and on the same day of the week by the same person, to reduce any interference of subjectivity. Furthermore, the adhesion of the product to the hooves was also observed through visual inspection immediately after the footbath or hours later in the pen and during milking, as a way of monitoring the application of the footbath.

Three outcomes were established with the aim of determining the effectiveness of the protocol in the prevention, control and treatment of DD lesions. Preventability was defined by incidence, the control by prevalence, and the ability to treat DD lesions using three possible end statuses for the lesion (non-existent, inactive, and active). These were based on the M-scoring system: 0 = non-existent for M0, 1 = inactive for M3 and M4, and 2 = active for M1, M2, and M4.1 (Kofler et al. 2020, Mendonça et al. 2024b) according to the methodology used in our previous study.

Statistical analysis. Two types of analysis were performed: the first considered the cow as the statistical unit without considering herd turnover, while the second considered the hind limb in a statistical population as a unit. Data were recorded in Excel (Microsoft Inc.) and analyzed using SAS software (SAS Institute Inc., Cary/NC).

Prevalence of DD lesions (categories 0 to 2) was assessed at eight timepoints (D0, w.2, w.3, w.4, w.5, w.7, w.9 and w.15). It was calculated considering the proportion of cows or hind limbs affected with DD lesions within the herd milked on the day. To calculate the incidence of new lesions, the proportion of hind limbs that developed inactive or active lesions between evaluation points (i.e., during each interval) within the susceptible population (those with non-existent DD) was determined for both types of statistical unit of interest, cow and limb level. Additionally, lesion progression was analyzed based on changes in status over time: lesions initially classified as non-existent, inactive, or active were monitored for permanence or change in status until week 15 (D104). Frequencies and proportions for these categories were obtained using PROC FREQ and PROC MEANS in SAS, allowing for identification of lesion transitions and control of prevalence and incidence over time.

For the limb-based analysis, only cows that remained in the lactating herd throughout the 104-day follow-up period and had at least one DD lesion on the hind feet were included. Thus, all hind limbs that were not present, regardless of reason, during the 15-week study were excluded from the analysis to allow statistical comparisons over time on the same limbs. McNemar's test and chi-square analyses were used to assess lesion changes from the beginning to the end, following previously described methods (Smith et al. 2014, Solano et al. 2017b). To evaluate the impact of lactation number and days in milk (DIM) on DD lesion development and healing, logistic regression was performed, with lesion development as a binary outcome (0 = no lesion, 1 = lesion developed). The leg (left or right) was included as a covariate. Additionally, to assess the influence of lactation and DIM on lesion healing time, a Cox regression model was applied. The distribution of lesions between left and right limbs was compared using chi-square tests. Results were considered statistically significant at $p < 0.05$.

Limitations. The main limitation was the absence of a control group. This approach was deemed necessary due to observations of severe active DD cases emerging in other pens during our previous study on the same farm, also considering the estimated losses ranging from US$126 to US$133 per clinical case (Cha et al. 2010), as well as their negative implications for future lactations (Akin & Akin 2018) and animal welfare. In addition, a positive group was unfeasible due to the decision to include the entire lactating herd. Therefore, it was decided to evaluate only the protocol in question, using the moment before the start of the experiment (D0) as a reference for the other moments evaluated.

RESULTS

Adhesion of the product to the hooves

HoofCare®, a blue-colored product, was observed adhering to the pastern and hoof region immediately after the footbath, presenting an intense blue-green appearance that persisted for up to 48 hours, followed by a softer blue coloration.

Overall prevalence and incidence of cows with DD lesions

Four weeks (30 days) after initiating the protocol (footbath + brushing), corresponding to the fifth week of evaluation, a regression in the number of cows with DD lesions in the lactating herd was observed from 355 (Day 0) to 157 (w.4). In relation to active lesions, the prevalence decreased from 73.24% (260 of 355) to 21.66% (34 of 157) of the affected cows.

The lactating herd size increased from 2,232 cows on D0 to 2,454 on D104, with an occupancy rate of 109.9% and 120.9%, respectively. The median number of cows and occupancy rate of the study were 2,283 and 112.5%. To assess the overall effect of the footbath protocol under real-world conditions on a commercial dairy farm, the prevalence and incidence of cows with DD lesions at each time point were determined, regardless of the affected limbs. The overall prevalence and incidence are illustrated in Figure 1, where it is evident that both the prevalence and incidence of cows with lesions decreased from 15.91% (355 out of 2,232) and 2.83% (54 out of 1,908) to 5.42% (133 out of 2,454) and 0.26% (6 out of 2,311) over the 104 days (15 weeks) of the protocol. Among the 133 DD lesions observed in week 15, 90 were classified as inactive and 43 as active lesions.

It is important to note that incidence increased in week 9, followed by a reduction in the subsequent assessment (w.15). The prevalence of lactating cows within the herd with active and inactive lesions throughout the study is demonstrated in Figure 2, which shows a reduction of ~10% of cows with active lesions compared to the prevalence before the protocol.

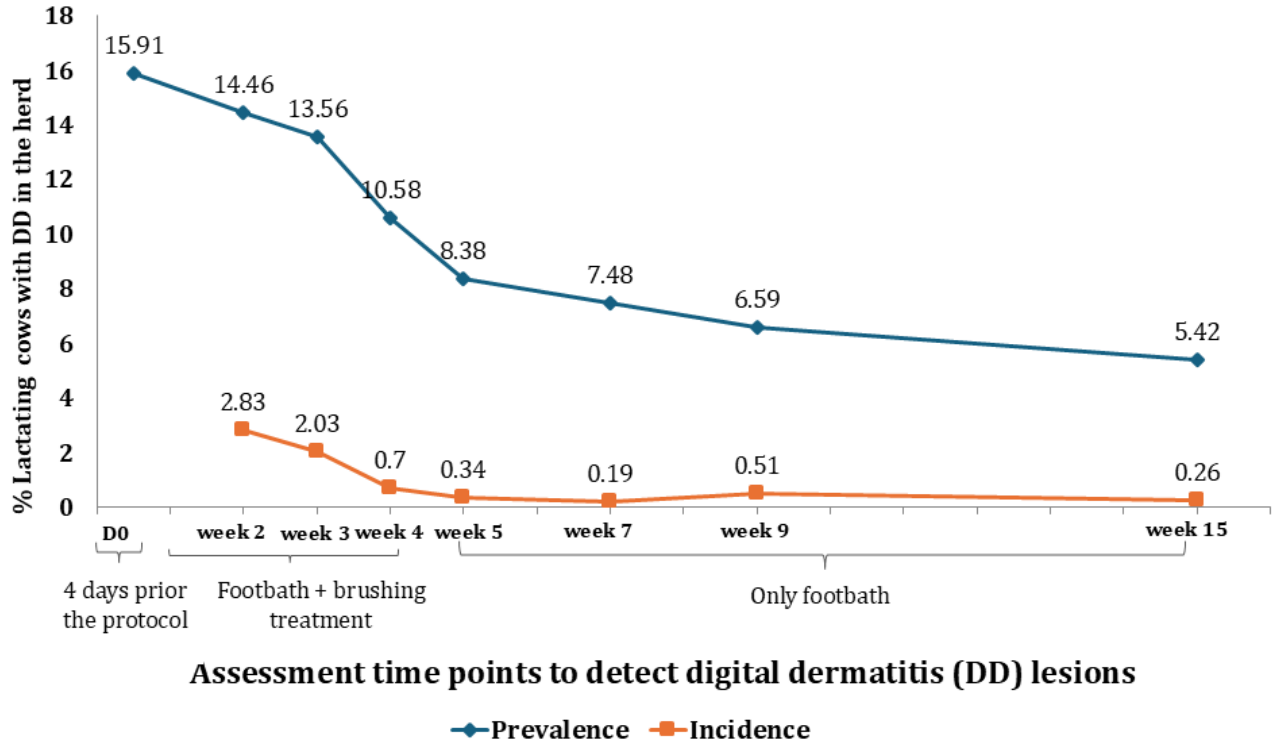

Fig. 1. Prevalence and incidence of lactating Holstein cows with digital dermatitis during a 15-week footbath protocol using 10% HoofCare® and a four-week brushing treatment at the beginning using the same product.

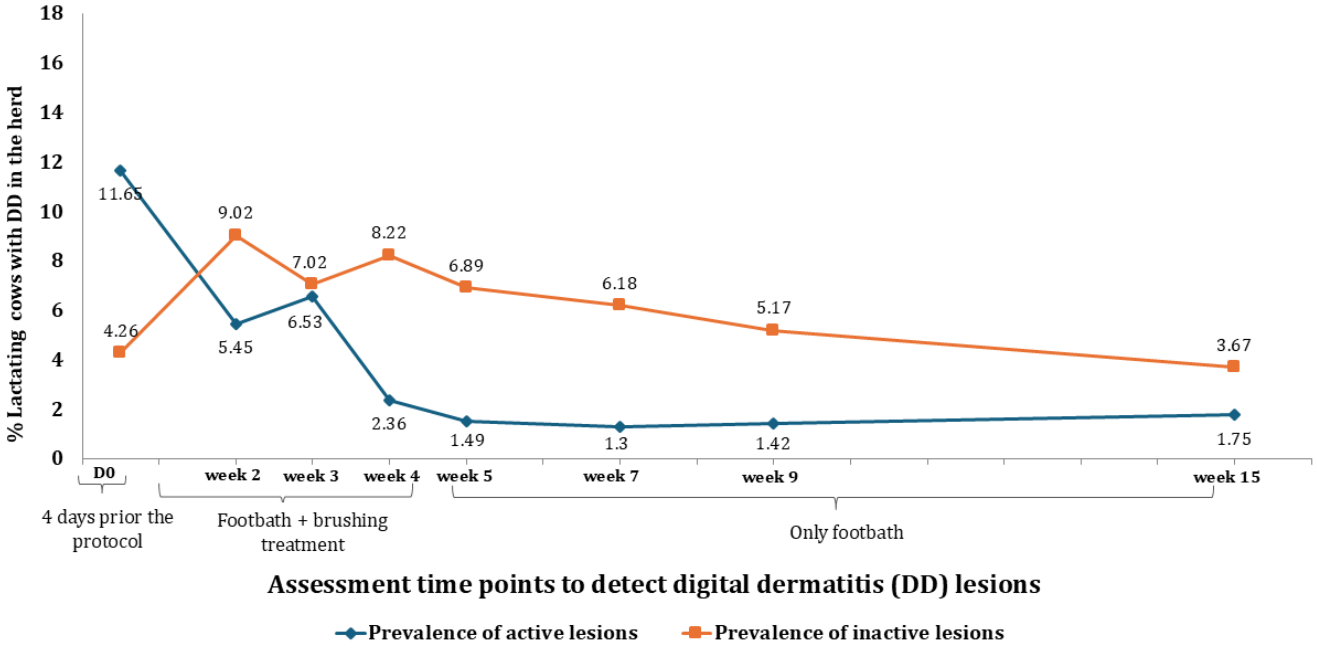

Fig. 2. Prevalence of lactating Holstein cows with digital dermatitis lesions active and inactive during a 15-week footbath protocol using 10% HoofCare® and a four-week brushing treatment at the beginning using the same product.

Prevalence and incidence of DD lesions on hind limbs in a static population

To evaluate the ability to prevent, control, and treat DD lesions in the dairy herd, all cows that were not present in the lactating herd during the entire study period were excluded from this specific analysis. Therefore, of the 562 cows (1,124 hind limbs) assessed with DD lesion in at least one hind limb throughout the experiment, 233 cows were excluded due to: drying off during the study (105), calving after the start of the protocol (73), being sold (47), missing information (6), and death (2). Among the reasons for sale, the following stand out: reproduction (7), hoof problem (7), high SCC (6), old age (4), and others (15).

A total of 329 cows in the lactating herd throughout the study were included in the analysis, regardless of whether they had lesions at baseline, resulting in 658 hind limbs observed for the presence or absence of lesions over the 15 weeks of the protocol. The cows' median number of lactations was 3.0, with an IQR from 1.0 to 4.0; the median days in milk (DIM) was 162 days, with an IQR of 73 to 242.

The prevalence of hind limbs with DD lesions gradually decreased over 15 weeks from 49.85% (328/658) on D0 to 17.17% (113/658) on D104, proportionally to the increase in the prevalence of limbs without DD lesions. Similarly, the incidence of new lesions reduced throughout the study, as shown in Figure 3, from 20.91% (69/330) on week 2 to 1.80% (9/501) on D104. Among these, six lesions were inactive, and three were active, based on recurrent cases. It is important to highlight that in the first four weeks of the study, the footbath was associated with brushing the product on lesions. The regression of prevalence and incidence continued, even after individual topical treatment ended (w.4), with only the footbath.

Outcomes of DD lesions in a static population

The evolution of the lesions from the beginning to the end of the study, along with their frequencies according to three possible lesion stages (non-existent, inactive, and active), is shown in Figure 4. The cure rate was 70.12% (230/328) for inactive and active lesions combined, and the prevention rate was 95.46%, as 315 limbs having non-existent lesions at D0 remained lesion-free until week 15.

It was observed that before the establishment of the protocol, 50.15% (330/658) of the hind limbs did not present DD lesions, and that 95.45% (315/330) of these remained without lesions, with only 4.55% (15/330) developing lesions. Of the 49.85% (328/658) of the limbs that presented lesions before the application of the protocol, 70.12% (230/328) evolved to the non-existent stage, and 29.88% (98/328) remained with lesions, either active or inactive. However, it is worth noting that only 22.45% (22/98) of these lesions were active, with the majority (77.55%, 76/98) being inactive. Thus, at the end of the study on D104, 82.83% (545/658) of limbs were without lesions and only 17.17% (113/658) with DD lesions were identified.

The McNemar statistical test suggested that there was a significant change in the stage of the lesions between D0 and D104 ($p < 0.0001$). Furthermore, the chi-square test indicates that the proportions of lesions between the stages from baseline to the end of the study are not equal and non-random ($p < 0.0001$), reinforcing that there was a significant change in the state of the DD lesions over time, as shown in Table 1.

Logistic regression analysis was performed for DIM and the number of lactations. There was no significant impact of either DIM ($p = 0.577$) or lactation number ($p = 0.623$) on lesion development, as well as the absence of significant lesion prediction ($p > 0.8$).

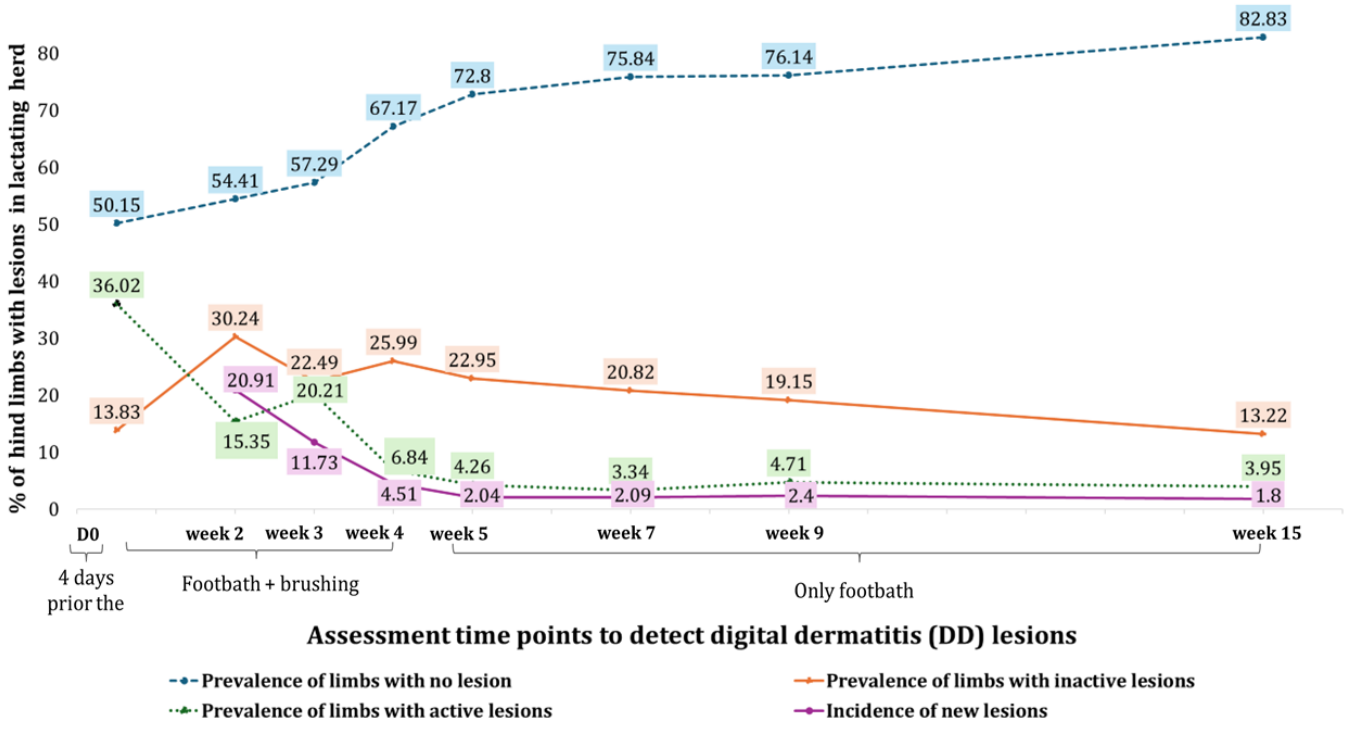

Fig. 3. Evolution of prevalence and incidence of digital dermatitis lesions in 658 hind limbs of lactating 329 cows over 15 weeks with a 10% HoofCare® footbath and with a four-week brushing treatment at the beginning.

Frequency of cows with lesions by hind limb in a static population

Of the 329 cows monitored throughout the study, the most had lesions in both hind limbs with 28.88% (95/329), followed by 22.19% (73/329) with lesions only in the right limb, and 19.76% (65/329) only in the left limb. By the end of the study, at D104, there was a reduction in cows with lesions, with 7.29% (24/329) presenting lesions on both limbs, 8.51% (28/329) only on the left, and 11.24% (37/329) only on the right limb.

Overall prevalence and incidence of fresh cows with DD lesions

To determine the effectiveness of the new footbath protocol for dry cows and heifers, implemented alongside the protocol for lactating cows, we tracked the occurrence of cases of DD lesions in cows entering the milking herd and recorded the number of calvings during the corresponding period. As shown in Table 2, the proportion of fresh cows with DD decreased throughout the study, while the total number of calvings increased. It is important to highlight that the prevalence on D0 of fresh cows in the lactating herd with lesions was 6.20% (22/355).

DISCUSSION

By focusing only on cows, without considering herd turnover, it was possible to calculate overall prevalence and incidence over the period, providing an understanding of the potential impacts of such hoof care protocols on a commercial farm. A comparison of the prevalence and incidence of cows with lesions showed a constant reduction up to week 15. However, in week 9, the incidence increased by 0.32%, which coincided with the increase in the concentration of calvings that occurred during that period and a higher occupancy rate (114.4%; 2,322 cows), resulting in greater pressure for the development of lesions. Another point that needs to be considered is the interruption of topical treatment in week 4, which may contribute to increasing the challenge. The subsequent steady reduction in

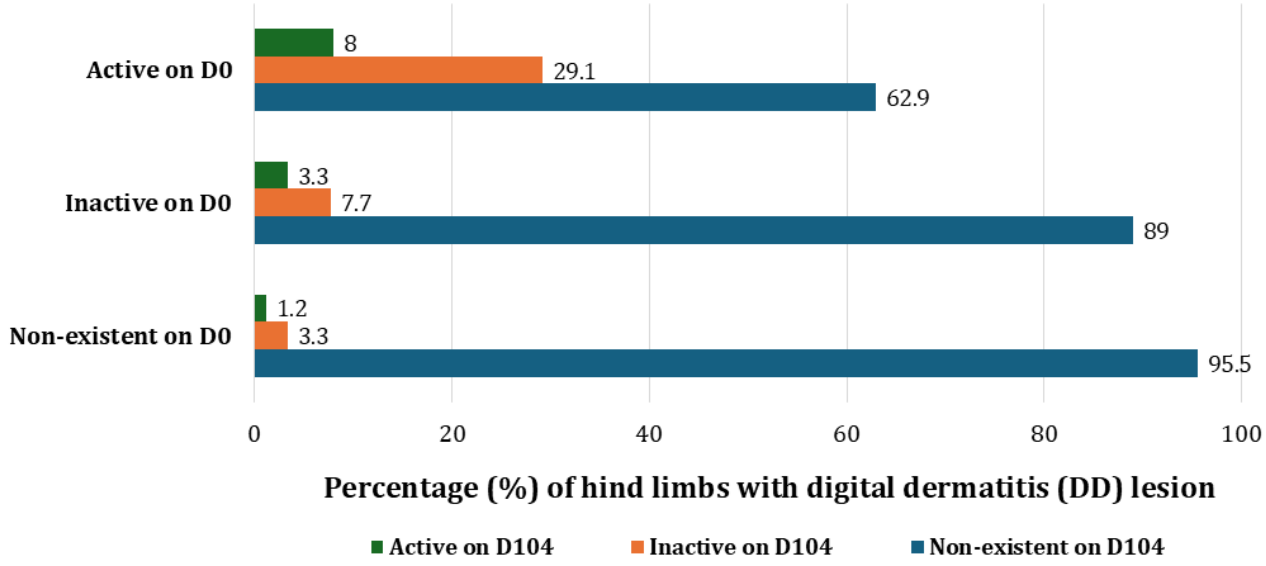

Fig. 4. Progression of 658 hind limbs of 329 Holstein cows with different stages of digital dermatitis lesions subject to a footbath protocol with 10% HoofCare® three times a week for 104 days and with a four-week brushing treatment at the beginning.

Table 1. Distribution of the evolution of digital lesions in lactating cows over 15 weeks under a 10% HoofCare® footbath protocol, per hind limb

Evolution from the initial stage to the final stage	Number of hind limbs	Percent of the population
No lesions throughout the study	315	47.87
New lesions developed	15	2.28
Lesions recovered (healed)	230	34.95
Lesions became inactive	76	11.55
Lesions became or remained active	22	3.34
TOTAL	658	100

Table 2. Proportion of digital dermatitis lesions and incidence in fresh cows over 15 weeks using a 10% HoofCare® footbath protocol

Assessment time point (week)	2	3	4	5	7	9	15
No. of fresh cows with DD lesions in the herd	10	4	0	0	0	0	0
No. of calvings during the period	114	65	69	64	155	123	393
Percentage of fresh cows entering the herd with lesions (%)	8.77	6.15	0.00	0.00	0.00	0.00	0.00

DD = digital dermatitis.

incidence and prevalence indicates that the footbath protocol was effective in controlling and preventing DD lesions, even after topical treatment was discontinued. Reinforcing this fact, the prevalence of cows with active lesions decreased by 10%, with some fluctuation, which is expected due to the natural response of lesions to topical treatment and environmental challenges (Holzhauer et al. 2011).

The 86.92% reduction observed in the number of lactating cows with active lesions, decreasing from 260 to 34 over a four-week protocol, suggests that topical treatment was effective in promoting healing of the lesions, corroborating the finding that most M2 lesions evolve to healing stages in the second week after topical treatment (Holzhauer et al. 2011). It also corroborates the findings of a brief study on the efficacy of brushing with the HoofCare® product in the treatment of DD lesions without pre-cleaning (Mendonça et al. 2024b) and the fact that it can take about 42 days for complete healing, i.e., reaching a non-existent stage (Nielsen et al. 2009). In addition, the combination of footbaths and topical treatment was deemed necessary, even in an intensive footbath protocol (Smith et al. 2014, Marshal et al. 2024), as demonstrated in a previous study (unpublished data). This combination of footbath and individual topical treatment is beneficial, with greater therapeutic power than either alone (Relun et al. 2012).

A commercial farm, especially a high-performance farm like the one in this study, has a flexible, dynamic herd, making it difficult to assess the impact of the protocol at different levels. Therefore, the creation of the static population was necessary, and was performed as recommended (Smith et al. 2014, Solano et al. 2017b). This approach allowed us to observe that the prevalence of limbs without lesions increased by 32.68% throughout the study, accompanied by a 32.07% reduction in limbs with active lesions and a 19.11% reduction in the incidence of new lesions. However, it is important to note that this static population was inserted into a dynamic environment, subject to several conditions of the farm, such as overcrowding.

The free-stall farm had an efficient cleaning system that, combined with the cooling system, promoted a low proportion of members (< 50%) with moderate and very dirty scores (Cook 2010). However, living conditions may not have remained constant over time and may have deteriorated — an aspect not measured in this study. The farm's occupancy rate ranged from 110% to 121% throughout the experimental period. This, associated with the cumulative effect of locomotion and longer time spent standing on concrete floors with greater accumulation of manure (Olmos et al. 2009), is an important risk factor for the development of DD lesions (Palmer & O'Connell 2015) because excess moisture can lead to maceration of the digital skin (Krull et al. 2016). Even so, a regression in the incidence and prevalence of the disease reinforces the effectiveness of the protocol evaluated in controlling DD under challenging conditions.

The percentage of limbs with inactive lesions increased by 16.41% in week 2, while that with active lesions decreased by 20.67%, which is consistent with what is expected after two weeks of an effective protocol (Holzhauer et al. 2011). This oscillation between active and inactive lesions indicates that the protocol, combining footbath and topical treatment, promoted not only prevention, with 47.87% of limbs never developing lesions, but also a good treatment rate for DD lesions (70.12%), with the majority being inactive (23.7%). Furthermore, some "falling of flaps" were observed at timepoint assessments, which is the first sign of efficient prevention using footbaths according to Döpfer (2021). Unlike some studies that evaluated the efficacy of footbath protocols that considered therapeutic power as the transition from active lesions to non-existent and inactive (Relun et al. 2012, Chen et al. 2023, Marshal et al. 2024), the present study chose to analyze them separately, given the impact of inactive lesions in the herd on the spread of the disease, as they are considered an important reservoir (Berry et al. 2012, Solano et al. 2017a, Biemans et al. 2018). Thus, the lesion was considered healed (cured) only when the non-existent stage (M0).

Logistic regression analysis did not show significant associations between the number of lactations and DIM in the static population of 329 cows, despite these two characteristics being considered risk factors for the development of DD lesions, consistent with the fact that the disease is multifactorial (Palmer & O'Connell 2015). Regarding the prevalence of hind limbs affected, there was a significant reduction in cows with DD lesions in both hind limbs, reinforcing that the protocol was effective.

Another important consideration was the change in the footbath protocol for dry cows and heifers, together with the protocol investigated for the lactating herd. When evaluating the incidence of new lesions, the percentage corresponding to fresh cows reduced by more than 8% after three weeks of protocol, reaching 0%, which remained until the end of the study, suggesting that the protocol was adequate for this category and may have had a positive impact on lactating cows. This finding supported the need for adequate preventive management for dry cows, which contrasts with the research conducted by Holzhauer et al. (2024), who reported a higher prevalence of lesions in herds that utilized footbaths solely for lactating cows compared to those without footbaths. This discrepancy may be attributed to an inadequately defined and executed protocol, highlighting the importance of considering the frequency of footbath application, the number of passages appropriate for the substances used, and their concentrations.

Regarding the characteristics of HoofCare®, it was observed that the product exhibits good adhesion to the pastern and foot, promoting effective coverage and greater contact of the compounds with the dermis and hoof. This fact is in accordance with a study that investigated liquid bandages as antibacterial protection, finding that formulations containing zinc and copper were more effective, with the capacity to contact-kill bacteria and less metal ion leaching (Bastos et al. 2020). Visual observation of product adhesion in this study was possible due to the use of the brilliant blue dye, another point that can be useful for monitoring the application of footbaths on a farm, such as dilution concentration and which cows were footbathed. It is also important to highlight that although the length of the footbaths was shorter (~40 cm) than the recommended (Cook 2017), the good adherence with the reduction of prevalence and incidence suggested that HoofCare® requires a short contact to act, a fact that may contribute to the effectiveness of the footbaths protocol as evidenced by Ferraro et al (2024). However, further investigations, such as quantification of adherence, are needed to validate these findings.

Regarding the solution changes to maintain the antiseptic effect, the replacement of the footbath solution in this study was based on the realities of a commercial farm and on a previous study on pH in lactating and dry herds, both of which respected the recommended range below 5.0 (Prastiwi et al. 2019). Due to the HoofCare® product composition, fewer changes were required than in previous protocols adopted by the farm, in accordance with other studies that used copper in combination with an acidifier (Manske et al. 2002, Holzhauer et al. 2012, Pirkkalainen et al. 2024). Furthermore, the product contains another powerful antimicrobial and anti-inflammatory compound, tea tree oil. This oil (Cox et al. 2000, Bakkali et al. 2008, Ramadan et al. 2020) may have further contributed to good cure and prevention rates by possibly reducing inflammation, given the inflammatory process during DD lesion development (Pirkkalainen et al. 2024). Nevertheless, further research is needed to improve knowledge about the composition of the product and its action when applied in the footbath.

Based on the findings of the present study, a practical protocol is suggested for the management of DD lesions on Brazilian free-stall farms: the association of weekly footbaths (three days per week) using HoofCare® with brushing the same product on DD lesions without prior cleaning during the milking routine. Regarding the frequency of brushing, it is recommended to start with three days a week to control outbreaks, as performed in this experiment, and subsequently adjust according to the specific conditions on the farm, management practices and status of the disease in the herd. In this context, other protocols could be investigated with different frequencies of product brushing, always combined with frequent footbaths.

It is important to highlight that the results of this study reinforce the need to treat active lesions individually, with footbaths being sufficient when prevalence and incidence rates are controlled, as well as the need for effective preventive management during the dry period, and not just throughout lactation.

CONCLUSION

The investigated antibiotic-free protocol with HoofCare® demonstrates promising effectiveness in controlling, preventing, and treating digital dermatitis (DD) lesions in a commercial dairy herd. Long-term benefits can be expected, such as increased productivity and animal longevity. However, further investigation is needed to confirm these impacts, including correlation with milk productivity and herd health metrics before and after protocol implementation.

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Conflict of interest statement.- The authors declare that, although this study received funding and technical support from the company that manufactured the product evaluated (Salmix), this company did not exert any influence on the analysis of the data or the interpretation of the results.

Credit author statement.- Ana P.A. Mendonça, Piero H.M. Teodoro, Celso A. Rodrigues: Study conception and methodology. Ana P.A. Mendonça, Raymis B.R. Moura: Material preparation and data collection. Renato Ravetti: Laboratory analyses of pH and provided chemical support for the study. Ana P.A. Mendonça, Marianne Villettaz-Robichaud: performed the analyses. Ana P.A. Mendonça, Marianne Villettaz-Robichaud, Celso A. Rodrigues: Writing and reviewing the manuscript. All authors contributed to the final manuscript and have read and approved the submitted version.

Data availability statement.- The data used in this study are available and can be accessed upon request from the corresponding author.

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