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Original Article

Efficacy of non-antibiotic treatment options for digital dermatitis on an organic dairy farm

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Highlights

- Digital dermatitis is a painful condition that can result in lameness and decreased performance.
- Two non-antibiotic formulations resulted in earlier transition to M0 lesions than no treatment
- A copper sulphate and iodine combination was most effective in reducing lesion size, pain, and lameness
- A honey and iodine combination reduced lesion size but effects were transient.

Abstract

The objective of this study was to evaluate the efficacy of two non-antibiotic treatment options for digital dermatitis (DD) in an organic certified dairy farm. A randomized clinical trial

was conducted using 70 multiparous Holstein cows with an early DD lesion at a USDA certified organic dairy farm in Northern Colorado, USA. Cows were enrolled in the study based on the presence of early DD lesions (scores M1 and M2) and randomly assigned to one of three treatments: (1) topical application of copper sulfate and iodine (CUI); (2) topical application of honey and iodine (HOI); and (3) control subject to no treatment (CON). Cows were evaluated at enrolment and on days 3, 12, 28, and 120 post treatment for pain and lesion size and received a locomotion and a lesion score. Cure was defined as the transition from active to non-active stages (M1/M2 to M0 or M4).

The formulations had variable effects on the treatment of DD. The cure rate was numerically higher for CUI on all follow up days. The proportion of cows experiencing pain on d3 after treatment was greater in CON, followed by HOI and CUI. However, this proportion increased in HOI during the follow up period. The CUI group had a greater reduction in lesion size and larger lesions persisted in HOI. Non-antibiotic treatment formulations were partially effective in the treatment of DD in organic dairy cows: The two non-antibiotic formulations resulted in an earlier transition to mature lesions compared with the control group. The CUI combination was the most effective treatment in reducing lesion size, pain, and lameness in affected cows. However, this combination had short-term efficacy, which did not persist throughout the duration of the study. The HOI combination produced only transient reduction in lesion size.

Keywords: Dairy cows; Digital dermatitis; Non-antibiotic treatment; Organic

Introduction

Digital dermatitis (DD) is a contagious, painful, wart-like disorder of the digits of dairy cows, characterized as a superficial inflammation of the skin (Berry et al., 2012). Although the precise etiology of this disease is unknown, DD is considered a multifactorial polybacterial condition, with *Treponema* spp. being the most commonly isolated pathogens (Döpfer et al., 1997; Krull et al., 2016). The prevalence of this disorder ranges from 15 to 49% in conventional dairy farms and has been estimated to be about 10% in organic dairy operations (Pinedo et al., 2017; Solano et al., 2017). In a recent U.S. Department of Agriculture (USDA) survey, 31% of the farms with bred heifers and 75% of the operations with cows reported at least one case of DD, with higher percentages in medium and large dairies [1]. As this is a painful condition, DD results in lameness and represents a serious concern due to decreased milk production and reduced welfare of dairy cows (Shearer et al., 2015; Biagiotti, 2016; Pinedo et al., 2017). Consequently, early detection and treatment are crucial for restoring digital function and cow comfort.

Various antimicrobial formulations are available for the treatment of DD (Shearer et al., 1998; Pol and Ruegg, 2007; Shearer et al., 2015). However, due to prohibitions in the use of antibiotics, organic dairy farms in the US face serious limitations in the administration of therapeutic resources and, consequently, non-antibiotic treatment options have been explored as alternatives for the treatment of DD lesions (Jacobs et al., 2018). The application of topical therapies under a bandage is the most common procedure for DD treatment in organic dairies

[1] See: NAHMS, 2018. National Animal Health Monitoring System. Dairy 2014: Health and Management Practices on U.S. Dairy Operations, 2014. Fort Collins, Co: USDA APHIS Veterinary Services Centers for Epidemiology and Animal Health. https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy14/Dairy14_dr_PartIII.pdf (accessed 22 November 2019).

and poultices or emulsions are used to create ointments applied on the DD lesions (Biagiotti, 2016; Pinedo et al., 2017). However, the efficacy of these preparations has not been fully investigated, and there is a need for scientific evidence regarding cure rates of DD lesions. Previous studies that tested products containing mixtures of soluble copper, a peroxide compound, and a cationic agent have demonstrated moderate efficacy in the treatment of DD lesions (Laven and Logue., 2006) and studies using copper sulfate, iodine, and honey suggest their potential for use in the treatment of DD (Durani and Leaper., 2008; Holzhauer et al., 2012; Oelschlaefel et al., 2012).

In this study, two options for the treatment of DD lesions, using formulations prepared by combining copper sulfate, honey, and iodine were investigated. The hypothesis was that these formulations would range in effectiveness compared to no treatment. Therefore, the short- and long-term effects of two treatment formulations on lesion size, lesion score, pain response, and evidence of lameness were evaluated in organically managed dairy cows.

Materials and methods

Cows and housing

This study was conducted in a commercial USDA certified organic dairy herd in Northern Colorado. The Institutional Animal Care and Use Committee at Colorado State University (Protocol number 17-7052A; Approval date, 28 February 2017) approved all the protocols prior to the initiation of the study. The protocol indicated that post-procedural monitoring should be performed on days 3, 12, 28, and 120 after treatment. This would assess pain, lesion scoring, and lesion stage. Treatment would be repeated if no improvement was

observed at d12 examination. Control cows would be subject to early escape (prompt removal) if their clinical status worsens to a defined level (increasing 1 point in lameness score). Rescue treatment considered the application of therapy with copper sulphate and iodine. In addition, the whole herd (including control cows) went through preventive footbath with acidified 5% copper sulfate solution twice weekly.

The study population consisted of 70 multiparous Holstein cows identified as having early DD lesions between April and June 2017. Cows did not have access to pasture during the study period. The dairy milked approximately 3,500 Holstein cows three times daily, with a rolling herd average of 8,600 kg/cow. All cows were housed in free stall barns with sand bedded stalls and had free access to a contiguous dry lot. Cows were fed a total mixed ration three times a day to meet or exceed the nutritional requirement for a lactating Holstein cow producing 30 kg/d of milk with 3.5% fat and 3.1% true protein according to NRC (National Research Council, 2001). Throughout the study period the diet consisted of corn silage (14 to 17.5%); wheat silage (13 to 20%); a premix containing soybean, soy hulls, whole cottonseed, corn, wheat, and minerals and vitamins (47.5 to 50.5%); sorghum silage (3.0 to 4.5%); alfalfa hay (12 to 16%); and grass hay (0 to 3%).

Lameness management on the farm

The herd was regularly monitored for lameness by screening for lame cows by a trained hoof trimmer. Cows showing any sign of lameness were moved to the trimming chute and examined for the presence of specific conditions. The whole herd was surveyed for lameness scores at least once every three months and all cows received trimming at least once every six

months. The herd had a history of lameness cases associated with DD with an average prevalence of 10%, which was managed without the use of any antibiotics. The whole herd went through preventive footbath with acidified 5% copper sulfate solution twice weekly.

Experimental procedures

A total of 70 cows were enrolled in the study. Cows were screened in the milking parlor using a flashlight and a mirror attached to a spatula to identify DD lesions. Additionally, cows were screened while in the headlocks, looking for pain in the area around the interdigital cleft of the hind feet. Cows with lesions suggestive of DD (n = 155) were taken to the trimming chute and further examined. Subsequently, DD lesions were categorized according to the M-scoring system (Berry et al., 2011): M0, normal digit skin without signs of DD; M1, early, small, circumscribed red to grey lesions less than 2cm 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 or proliferative filamentous or scab like mass; M4.1, chronically affected foot that displays both M4 and M1 stages. Only cows with M1 and M2 stage DD lesions in one hind foot were included in the study. If any other concurrent lesion was diagnosed during the initial checking, the cow was excluded from the trial. On day 0, before application of treatment, all cows were evaluated for pain and lesion size and received a locomotion and a lesion score. Pain scoring was based on pressure algometry (Millman, 2013) defined as the cow's response to firm pressure with one thumb in the center of the lesion (approximately 50 N/cm^2; Hernandez et al., 1999; Holzhauer et al., 2008). The responses were recorded as a number that ranged from 0 to 2 (0, no signs of pain; 1, signs of mild pain; 2, signs of severe pain). Lesion size (cm) was measured as a continuous

variable using a standard measuring ruler to report the largest diameter of the lesion (Hernandez et al., 1999). Cows were evaluated for locomotion with a scoring system (Sprecher et al., 1997) on a scale from 1 to 5 (1, stands and walks normally with a level back; 2, stands with flat back but arches when walks, abnormal gait; 3, stands and walks with an arched back and short strides; 4, arched back standing and walking, favors one or more limbs but can still bear some weight on them; 5, pronounced arching of back, reluctant to move, complete weight transfer off the affected limb. In addition, the type of lesion (early vs. mature) was recorded for all cows; early lesions were round to oval, flat or concave, raw, moist red-yellow to gray, and had tufted or granular strawberry like surfaces, whereas mature lesions were raised, with surfaces covered by small filiform papillae (Read and Walker, 1998).

Two treatments were prepared at the beginning of the experiment. A total of 30 ml 7% Tincture Iodine (Triodine-7, Aspen Veterinary Resources) was mixed with 940 g copper sulfate to prepare a formulation that was applied to the copper sulphate iodine (CUI) group. Similarly, 30 ml of iodine were mixed with 570 g of honey (Raw and Unfiltered Honey, Rice's Lucky Clover Honey) to be applied to the honey iodine (HOI) group. The formulations were dispensed using standard measuring spoons. Each cow received an amount equivalent to 10 ml of the formulation in a clean paper towel that was applied topically in the lesion after thorough cleaning of the site with water and drying. Treatment paper towels were fixed in place using a foot wrap. The CON group had the lesion cleaned with water and, after drying, received a bandage without any treatment formulation along with regular after milking footbath containing acidified 5% copper sulfate solution. The treatment groups (CUI and HOI) received the treatment formulations wrapped by a bandage for 3 days along with the regular post-milking footbath twice

weekly.

After initial evaluation on d0, cows were randomly assigned into one of the three treatment groups to receive a one-time treatment on the trimming chute, along with the functional hoof trimming by a trained hoof trimmer. The randomization was performed by random allocation of one of the three treatments to the first three animals brought to the study chute using Microsoft Excel (Microsoft Excel, Microsoft Inc.). Subsequently, the enrolled animals received their treatment following the same initial sequence. As the specific animals to be included in the study were not known in advance, a priori randomization of subjects into each treatment group was not possible.

To maximize masking during the follow-up procedures, follow-up sheets did not include any information about the individual's treatment group. In some cases, complete masking of the follow-up procedure was not possible due to the nature of the treatment (e.g. traces of blue color from copper sulphate in the CUI treatment). However, the follow-up protocol included objective variables that would be less influenced by the potential presumption of the treatment group. Cows were evaluated for pain and lesion size and received a locomotion score and a lesion score on d3, d12, d28, and d120 by one author that was masked to the treatment groups. Throughout the study period, all treatment groups were maintained under the same management and housing conditions and were fed identical rations. A subsample of 45 cows was followed up until d120, as some cows were not available at the time of assessment. All cows that completed the study remained on the farm after the trial was concluded. A total of 22 cows were moved to other units within the farm during the study for reasons unrelated with this research or their foot conditions.

Overall, three cows left the herd for reasons not associated with their foot condition or adverse conditions related to this trial.

Clinical cure rate was defined as the transition from active to non-active stages (M1/M2 to M0 or M4) on each follow up day (Holzhauer et al., 2011; Jacobs et al., 2018). The difference in lesion size relative to d0 was defined as an outcome variable that provided an evaluation of the lesion progression relative to the day of enrollment to account for different proportions of M1/M2 lesions in each treatment group at enrollment.

Statistical analysis

Data were entered into a spreadsheet (Microsoft Excel, Microsoft Inc.) and all analyses were conducted using statistical software (SAS Version 9.4, SAS Institute Inc,). Due to low number of cows in some response variable categories, categories were collapsed into binary outcomes; either presence or absence of pain (pain scores ≥ 1 considered painful [1], pain scores = 0 considered not painful [0]) and either lame or non-lame cows (locomotion scores > 2 considered lame [1], locomotion score ≤ 2 considered not lame [0]). The FREQ procedure in SAS was used to compare proportions of categorical outcomes by treatment group, using chi squared test and Fisher's exact test. Repeated measures analyses using MIXED procedure was used to evaluate continuous responses of lesion size and lesion difference. GENMOD procedure was used to model the categorical responses of pain and lameness. Repeated measures analyses using MIXED procedure were used to evaluate continuous responses (lesion size and lesion size difference), considering the effect of treatment group and the effect of follow up days. Logistic regression (GENMOD procedure) was used to analyze the categorical responses of cure and

presence of pain and lameness, including the effects of treatment group and of follow up days.

Results

Baseline comparisons

A total of 70 cows were enrolled and allocated into CUI (*n* = 24), HOI (*n* = 23), and CON (*n* = 23) groups. Overall, 55% of the lesions were on the right rear foot and cows in CUI, HOI, and CON had 54%, 64% and 48% of the lesions in their right rear foot, respectively. Days in milk for cows in CUI, HOI, and CON were on average (±SD) 105±72 days, 128±57 days and 126±53 days, respectively. Cows averaged 4.5 years old and were in 2nd parity (54.7%), 3rd parity (20.3%), and in parity ≥ 4 (24.9%). No significant differences were determined for DIM, age, and parity across treatment groups (Table 1). All cows started the experiment demonstrating pain. Percentages of cows determined lame at enrolment were 16.7%, 13%, and 13% in CUI, HOI, and CON, respectively.

Response to treatment

The proportion of cows with M1 and M2 lesions decreased through the follow up period, as early lesions at the start of the study progressed to more mature lesions in all treatment groups. Lesions classified as M4.1 stage appeared on d28 in cows in HOI group, whereas in CON group M4.1 lesions appeared only on d120 (Fig 1). Remarkably, on d120 HOI group had a greater proportion of early than mature lesions (Fig 2).

On day 3, the highest proportion of cows categorized as M0 was observed in CUI, followed by HOI and CON (27, 25, and 5%, respectively). The proportion of M0 steadily

decreased in HOI on consecutive follow up days (15, 13, and 11% on days 12, 28, and 120, respectively), indicating recurrence of the DD lesions in the cured cows. The proportion of M0 lesions decreased on d12 in both CUI and CON, but we observed an increase in proportions of M0 lesions on day 28 and day 120 in both CUI and CON, indicating a greater percentage of cure in these groups. For both CUI and CON, the proportion of M4 lesions increased with follow up days until d28. However, at d120 a lower proportion of M4 lesions was determined (43 vs. 63 and 53 vs. 64% in CUI and CON groups, respectively), possibly due to healing of the M4 lesions to M0 stage. In the HOI group, we observed a similar trend until d28; however, the proportion of M1 and M2 lesions was increased, indicating the reversion of these lesions from the M4 stage.

All cows started the experiment demonstrating pain. When evaluating the pain response on d3, a greater proportion of cows evidenced pain in CON followed by HOI and CUI ($P < 0.01$). However, on d12 a numerically greater proportion of cows in HOI group demonstrated pain, followed by CON and CUI. This trend continued until d28 and the difference among treatment groups was significant on d120 ($P < 0.01$; Table 2). The logistic regression analyses indicated that on d3, the odds (95% CI) of pain for cows in CUI were 0.04 (0.01-0.46) times the odds of pain for cows in CON. This odds ratio changed to 0.11 (0.01-0.95) on d3 follow up. Cows in HOI group had greater odds of pain than cows in CON starting on d28. On comparing the two topical treatments, CUI demonstrated lower odds of pain than HOI on all follow up days (Table 2).

A significant effect for the interaction between treatment and day on the size of the lesion was observed ($P < 0.01$). The average (± standard error, SE) lesion diameter for cows in CUI

was 2.38 (±0.22) cm on the day of enrollment (d0) and decreased to 2.25 (±0.23) cm on d3, 2.04 (±0.22) cm on d12, 2.27 (±0.23) cm on d28, and 1.19 (±0.28) cm on d120 (P< 0.01). Cows in HOI group started the study with 2.02 (±0.22) cm lesion size on average and the lesion changed to 1.69 (±0.23) cm, 1.79 (±0.24) cm, 2.23 (±0.23) cm, and 2.02 (±0.22) cm on d3, d12, d28, and d120, respectively. Cows in CON group had average lesion size of 1.7 (±0.22) cm on d0. The lesion size initially increased to 2.25 (±0.23) cm on d3, to change to 2.21 (±0.23) cm on d12, 1.97 (± 0.23) cm on d28, and 1.27 (±0.29) cm on d120 (Fig 3).

Accordingly, the difference in lesion size relative to d0 was associated with both treatment group and follow up day (P < 0.05). For CUI group the average change in lesion size was -0.25 cm on d3, -0.40 cm on d12, -0.09 cm on d28 and -1.19 cm on d120. For the HOI group, the average reduction in lesion size was -0.39 cm on d3, -0.23 cm on d12, but size increased on average by 0.19 cm on d28 and by 0.76 cm on d120. For CON group, the average change in lesion size was 0.59 cm on d3, 0.57 cm on d12, and 0.33 cm on d28 whereas the lesion decreased by -0.39 cm on d120 (Fig 4).

Both HOI and CON groups evidenced an increase in the percentage of lame cows relative to enrolment. On d3, the greatest percentage of cows experiencing lameness was in CON, followed by HOI and CUI groups (P = 0.03). A similar trend was observed on d12 (P = 0.14), and d28 (P = 0.03), whereas on d120 HOI had greater proportion of cows demonstrating lameness, followed by CON and CUI (P = 0.01). On d3, the odds of lameness among cows in CUI were 0.22 (0.07-0.75) times the odds of lameness in CON group (Table 3).

The cure rate of DD lesions, defined as the transition from active to non-active stages (M1/M2 to M0 or M4) on the day of follow up, was greatest in CUI on all follow up days, followed by HOI on d3 and d12 and CON on d28 and d120 (Table 4). On d12, the odds of cure for CUI cows were 4.13 (1.19-14.4) times the odds of cure for CON cows. However, on day 120, the odds of cure for cows in HOI were 0.14 times the odds of cure for cows in CON group (Table 4).

Discussion

Our results indicated that the non-antibiotic formulations tested in this study had variable effects on the treatment of DD lesions. Treatment group CUI had the smallest percentage of individuals experiencing pain until d12. However, this was a short-term effect and, by d28 and d120, the numerically small percentages of cows demonstrating pain were in CON. On the contrary, when compared to CON, HOI did not perform well in decreasing the pain response.

When the regression of lesions was analyzed relative to d0, CUI performed better than HOI and CON. Lesion size differences for CUI were always negative, indicating that lesions on all follow up days were smaller than during enrolment. The average lesion size in CUI decreased after d0 across the follow up days except on d28, whereas in HOI group average lesion size decreased on d3 and increased on follow up d12 and d28. On the other hand, in CON group, average lesion size increased on d3 to start constantly decreasing on d12, likely due to the aforementioned augment in size evidenced in this group. However, in this group the difference in lesion size relative to d0 was negative only on d120.

In CUI, a continued decrease in lesion size corresponded with negative lesion differences on all follow up days. In HOI, decrease in lesion size corresponded with a negative lesion difference on d3 but increased lesion sizes on d12, d28, and d120. Similarly, in CON, increased lesion sizes on d3 and d12 corresponded with positive lesion differences and decreased lesion size son d28 and d120 corresponded with smaller or negative lesion differences.

Treatment group CUI had the smallest percentage of lame cows on all follow up days and it was the only treatment demonstrating statistical evidence of an advantage relative to CON early after treatment (d3). However, the greatest long-term reduction on lameness was observed in CON group.

Digital dermatitis lesions in bovines heal by second intention with granulation tissue formation (Shearer et al., 2015). Previous studies using one-time treatment with topical formulations have associated the bandage with a significant decrease in DD lesion score (Krull et al., 2016). Other studies have used gel paint (Holzhauer et al., 2011), and spray (Jacobs et al., 2018) as treatment application methods. The technique used in our study, including the application of a bandage provided some protection from contamination and from treatment pharmaceuticals washing away, as reported in a previous study (Moore et al., 2001).

Copper sulfate is a bacteriostatic agent that acts by reaction of Cu^{++} with protein thiol groups of pathogens and should be applied in a dilution to minimize the risk of burning the skin of cows (Epperson and Midla., 2007). We combined copper sulfate with iodine, which is an antiseptic agent that in itself provides free iodine radicals with bactericidal properties (Durani et

al., 2008). The antibacterial benefits of honey are related to its low pH, high osmolarity, and high peroxide activity (Oelschlaefel et al., 2012; Shearer et al., 2015). Since antibacterial activity in honey is primarily due to hydrogen peroxide that is produced by the action of glucose oxidase in honey, a higher concentration of honey causes cellular and protein damage by giving rise to oxygen radicals (Bang et al., 2003). To minimize this effect, we used the treatment formulation containing a combination of honey and iodine. Shearer and Hernandez (2000) tested a modified product that contained 2% of original peroxide concentration, 75% of the original concentration of copper and 200% of original cationic concentration. They reported this product to be more effective than commercial products and oxytetracycline in treating DD lesions. Commercial non-antibiotic pastes containing copper and zinc sulfates, and sodium chlorides as an active ingredient have frequently been reported to be effective in the treatment of DD (Britt et al., 1996; Shearer and Hernandez., 2000; Moore et al., 2001).

In another study conducted in 70 DD hooves (Oelschlaefel et al., 2012), the honey treated group demonstrated greater cure of lesions (40%) than the group that did not receive any treatment (8%). The study also concluded that using the honey product was associated with faster healing of lesions. This result is in agreement with our results, where we observed a similar cure rate for HOI (50% on d12). However, we observed a greater (31%) proportion of cured lesions in the CON group, which may be due to the footbath treatments that were not used in the study by Oelschlaefel et al. (2012).

Clinical cure rate was defined as the transition from active to non-active stages (M1/M2 to M0 or M4; Holzhauer et al., 2011; Jacobs et al., 2018), as opposed to a complete lesion

healing that implies foot skin returning to an unaffected state (M0). Resolution to healthy foot skin is difficult to obtain (Krull et al., 2016); therefore a treatment option should be able to achieve a manageable state of disease by limiting the presence of an active lesion (Döpfer, 2009). Consequently, the transition of the lesion score and the reduction of the lesion size should be considered relevant outcomes of DD control strategies.

In a previous study by Shearer and Hernandez (2000), other non-antimicrobial formulations have been found to perform equally well compared to the antibiotic options. Apley (2015) reported that 5% copper sulfate solution healed 20% of the lesions. This formulation was received by CON in this experiment through the use of footbath, and the d28 outcome revealed 73% cured lesions in our study. This discrepancy may be due to the difference in definition of healing and curing. Another factor could be the potential acidification of the footbath containing copper sulfate solution used in our study herd, leading to a higher cure rate. Iodine used in our study has previously been tested in the treatment of DD lesions as a spray with no effect on the prevalence of DD (Esch et al., 2000). However, we applied the formulation directly on the lesion that was subsequently covered with a bandage, which may have resulted in better outcomes in our study. Similar to the observations made in this study, Moore et al. (2001) reported that 28 days after a single treatment with CUI, there were significantly reduced scores for pain and lesion size.

Most of the conventional DD treatment studies commonly use tetracycline and oxytetracycline and consider a positive control group. Britt et al. (1996) and Shearer and Hernandez (2000) used an antibiotic treatment as standard therapy for comparison of the efficacy

of non-antibiotic formulations. However, as this study was conducted in a USDA certified commercial dairy herd, there was no opportunity for a positive control group. Britt et al. (1996) also investigated a placebo group treated with tap water spray, but due to ethical and cow welfare regulations, we provided the regular footbath to all treatment groups. Although our study was conducted in an organic dairy farm, up to 76% cure rates were achieved over 28 days. These results are similar to improvement rates obtained for either chlortetracycline or comparable oxytetracycline treatments (58% to 87%; Berry et al., 2010).

Conclusions

Non-antibiotic treatment formulations were reasonably effective in the treatment of DD in organic dairy cows. The two non-antibiotic formulations studied resulted in earlier transition to mature lesions compared with no treatment. The CUI combination was the most effective treatment in reducing lesion size, pain, and lameness in affected cows. However, this combination had short-term efficacy, which did not persist throughout the duration of the study. The HOI combination produced only transient reduction in lesion size. Therefore, CUI could represent a valid treatment option for digital dermatitis in organic dairy farms.

Conflict of interest statement

None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Figure legends

Fig. 1. Distribution of digital dermatitis (DD) lesion score categories across different follow up days (day 0, day 3, day 12, day 28, day 120) after a single treatment with: A) formulation containing copper sulfate and iodine (CUI), B) honey and iodine (HOI), and C) non-treated control (CON). DD lesions were categorized according to M-scoring system: M0, normal digit skin without signs of DD; M, early, small, circumscribed red to grey lesions less than 2cm in diameter; M2, red or red grey, active, ulcerative lesions > 2cm 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 or proliferative filamentous or scab like mass; M4.1, chronically affected foot that displays both M4 and M1 stages.

Fig. 1

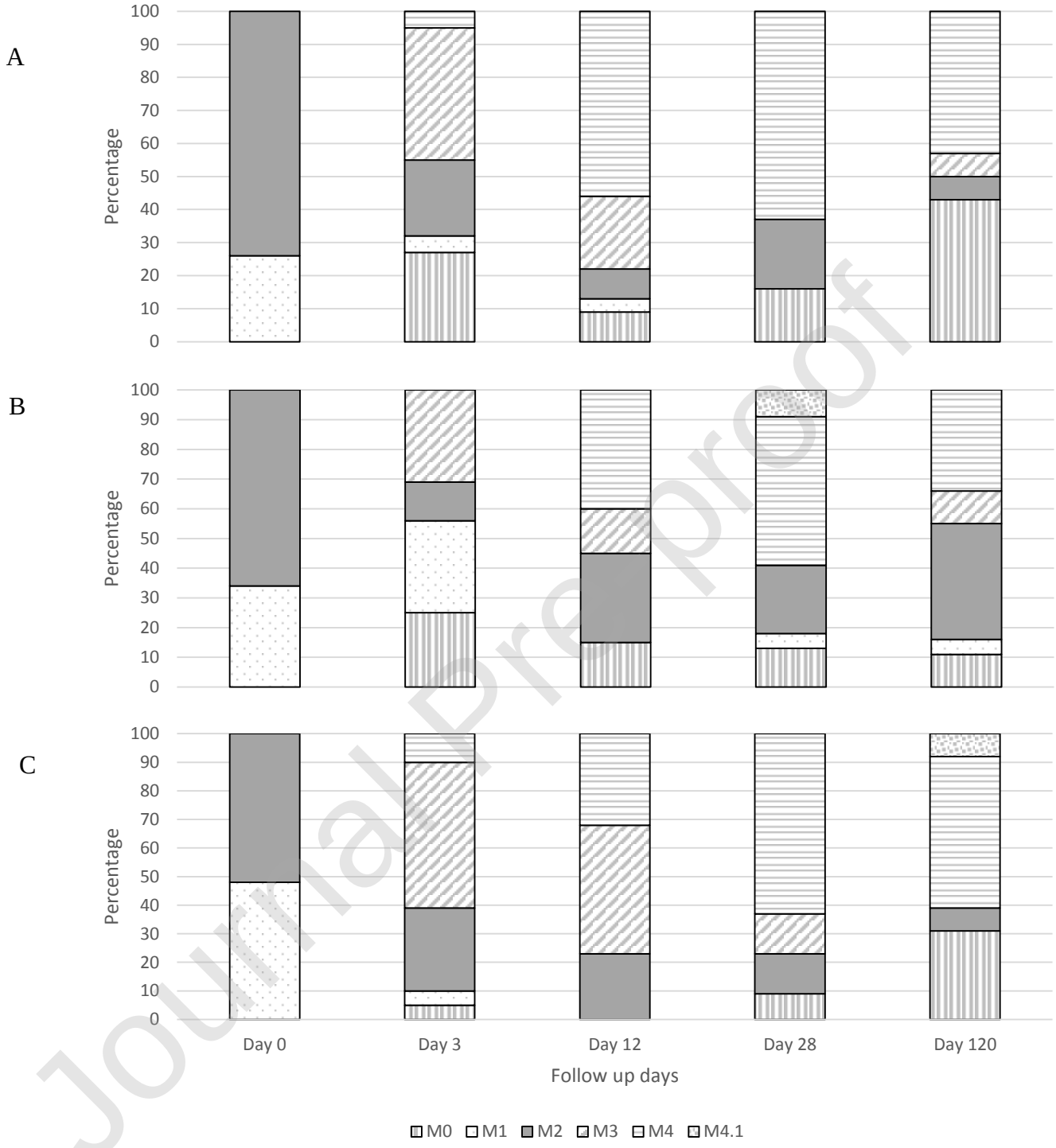

Fig. 2. Distribution of digital dermatitis (DD) lesion types across different follow up days (day 0, day 3, day 12, day 28, day 120) after a single treatment with formulation containing: (A) copper sulfate and iodine (CUI); (B) honey and iodine (HOI); and (C) non-treated control (CON). DD lesions were categorized as early and matured lesions: early lesions were round to oval, flat or concave, raw, moist red-yellow to gray, and had tufted or granular strawberry like surfaces whereas mature lesions were raised, with surfaces covered by small filiform papillae.

Fig 2

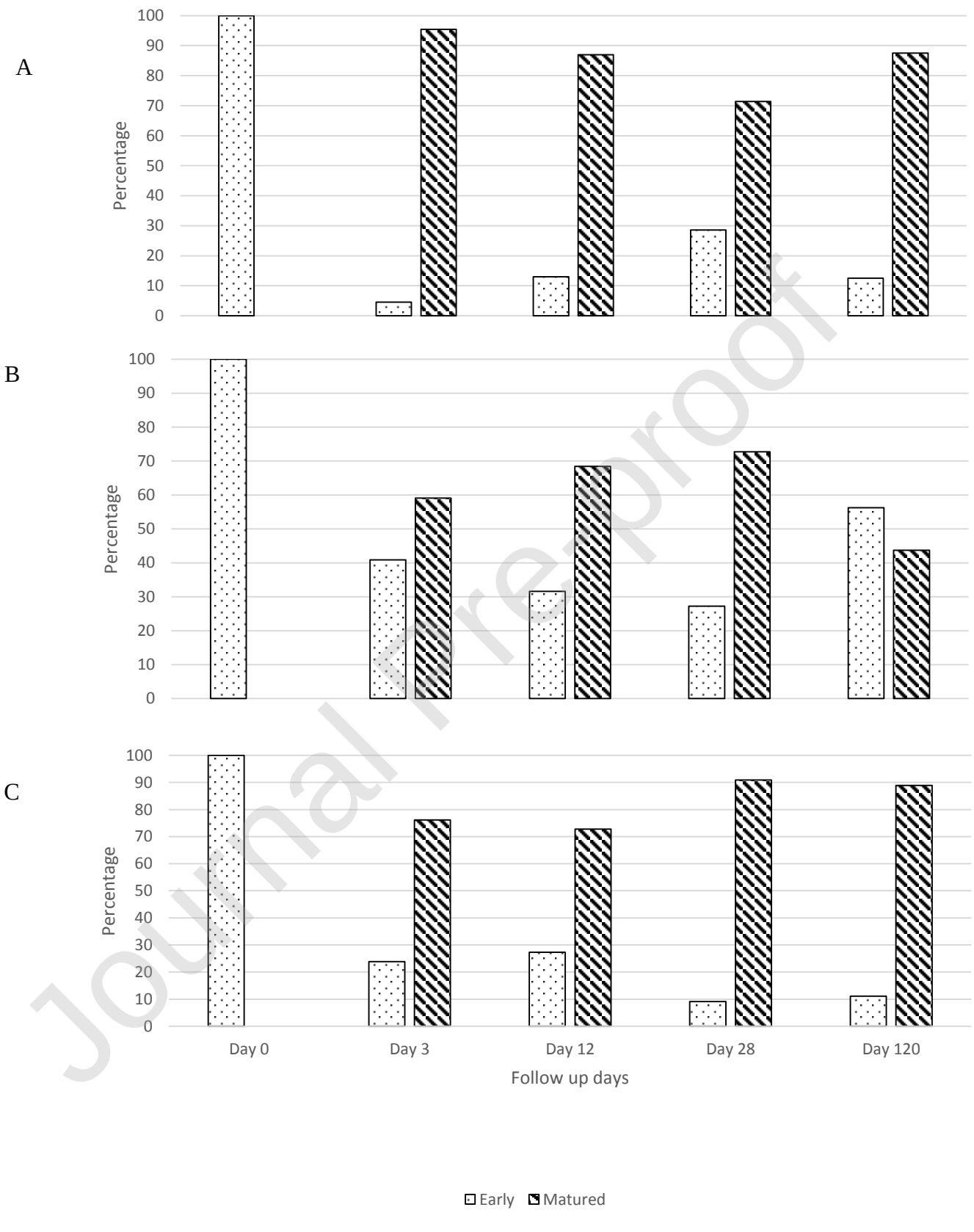

Fig. 3. Mean ± standard error lesion size (cm) by treatment group across the follow up days. The solid line represents a group that received formulation containing copper sulfate and iodine (CUI; *n* = 24); the dotted line represents a group that received formulation containing honey and iodine (HOI; *n* = 23); broken line represents non-treated control group (CON; *n* = 23). Cows were followed on day 3, day 12, day 28 and day 120 after application of treatment.

* Significantly ($P < 0.05$) different values on the day of follow up.

Fig. 3

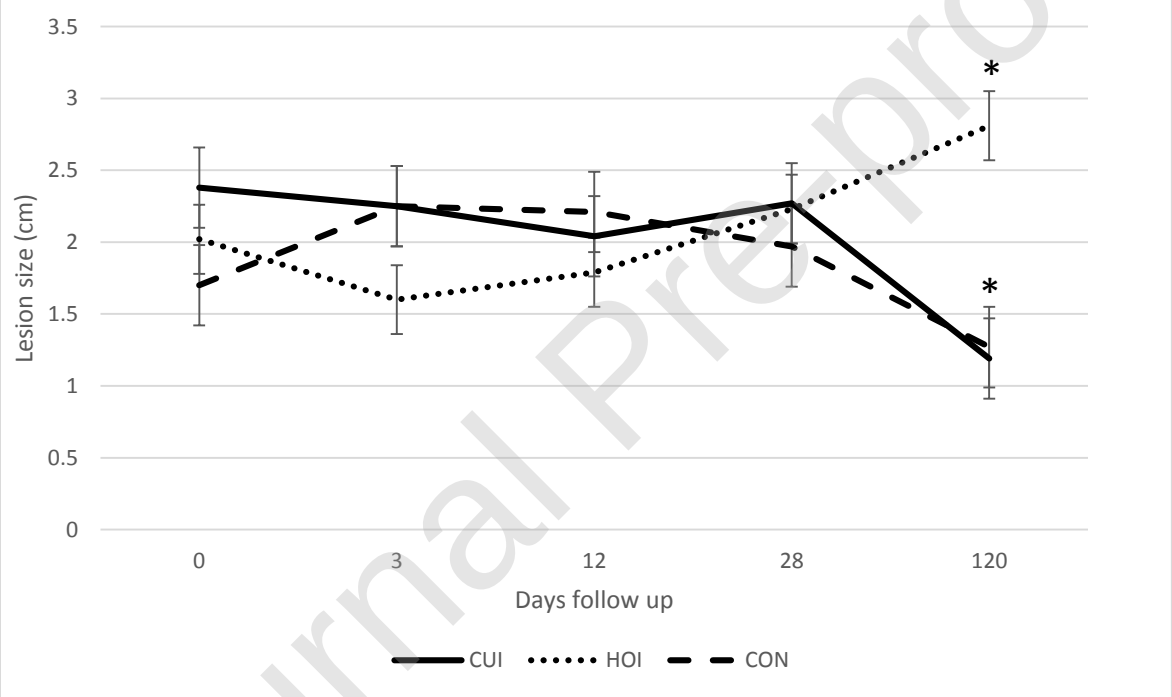

Fig. 4. Mean ± standard error difference in size of lesions from the day of enrollment by treatment groups across the follow up days. The solid line represents a group that received formulation containing copper sulfate and iodine (CUI; *n* = 24); the dotted line represents a

group that received formulation containing honey and iodine (HOI; *n* = 23); broken line represents control group (CON; *n* = 23). Cows were followed on day 3, day 12, day 28 and day 120 after application of treatment. * Significantly ($P < 0.05$) different values on the day of follow up.

Fig. 4

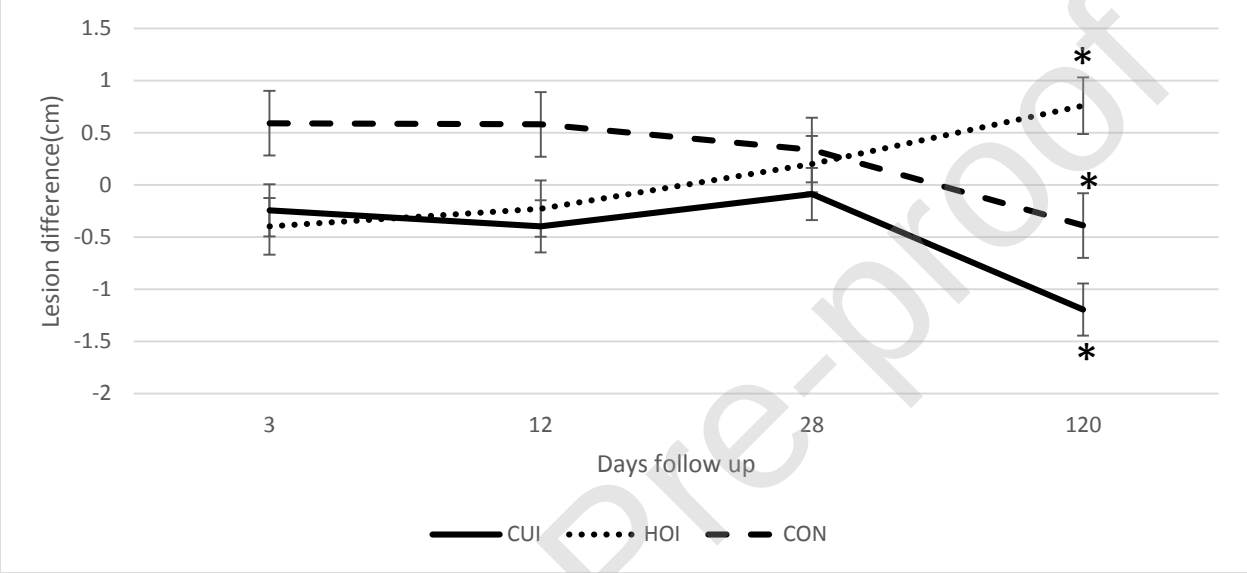

Table 1

Demographics and lesion location at the time of study enrollment (baseline) for 70 Holstein cows that received a formulation containing copper sulfate and iodine (CUI), honey and iodine (HOI) and no treatment (CON).

	Treatment groups			
Parameters	CUI	HOI	CON	*P*
n	24	23	23	
DIM	105.2±72	127.7±57	126.2±53	0.47
Age at enrollment (years)	4.57±1.5	4.58±1.2	4.44±1.3	0.93
Lactation number	2.95±1.4	2.73±0.9	2.75±1.2	0.81
Lesion on right claw(n)	14	15	11	
Lesion on left claw(n)	10	8	12	

DIM, Days in milk subsequent to calving

Table 2

Proportion of cows with signs of pain after the application of treatment. The odds ratios for demonstration of pain by treatment group on different follow up days are presented. All cows started the experiment demonstrating pain.

	Follow up days			
Treatment	Day 3	Day 12	Day 28	Day 120
CUI (%)	4/22 (18.2%)	4/23 (17.4%)	4/21 (19.1%)	1/14 (7.1%)
HOI (%)	11/22 (50%)	10/20 (50%)	9/22 (40.9%)	10/18 (55.6%)
CON (%)	13/21 (61.9%)	10/22 (45.5%)	4/22 (18.2%)	1/13 (7.7%)
P	0.011	0.05	0.15	0.0003
Odds ratio				
CUI vs. CON	0.04 (0.01-0.46)	0.11 (0.01-0.95)	0.90 (0.09-8.39)	1.31 (0.03-61.33)
HOI vs. CON	0.91 (0.14-6.19)	3.06 (0.39-23.69)	12.21 (1.11-134.91)	102.20 (2.76 -999)
CUI vs. HOI	0.05 (0.01-0.59)	0.04 (0.01-0.49)	0.07 (0.01-0.93)	0.01 (0.01-0.43)

CUI, Cows that received formulation containing copper sulfate and iodine; HOI, Cows that received formulation containing honey and iodine; CON, Cows that received no treatment.

Table 3

Proportion of cows with signs of lameness (locomotion score≤ 2 not lame; locomotion score > 2 lame) after application of treatment. The odds ratios for demonstration of lameness by treatment group on different follow up days are presented. Percentages of cows determined lame at enrolment were 16.7%, 13%, and 13% in CUI (formulation containing copper sulfate and iodine), HOI (formulation containing honey and iodine), and CON (no treatment), respectively.

	Follow up days			
Treatment	Day 3	Day 12	Day 28	Day 120
CUI (%)	4/22 (18.2%)	5/23 (21.7%)	3/21 (14.2%)	2/14 (14.3%)
HOI (%)	6/22 (27.3%)	7/20 (35%)	7/22 (31.8%)	8/18 (44.4%)
CON (%)	11/21 (52.3%)	11/22 (50%)	4/22 (18.2%)	2/13 (15.4%)
P	0.032	0.14	0.027	0.011
Odds ratio				
CUI vs. CON	0.22 (0.07-0.75)	0.37 (0.13-1.06)	0.99 (0.23-4.26)	1.63 (0.19-14.17)
HOI vs. CON	0.35 (0.12-1.04)	0.62 (0.22-1.71)	2.45 (0.74-8.05)	5.23 (0.76-36.17)
CUI vs. HOI	0.63 (0.16-2.50)	0.59 (0.18-1.97)	0.41 (0.09-1.69)	0.31 (0.08-1.22)

Table 4

Proportion of cows evidencing cure of the lesions after application of treatment. The odds ratios for evidence of cure by treatment group on different follow up days are presented.

	Follow up days			
Treatment	Day 3	Day 12	Day 28	Day 120
CUI (%)	7/22 (31.8%)	15/23 (65.2%)	16/21 (76.2%)	11/14 (78.6%)
HOI (%)	6/22 (27.3%)	10/20 (50%)	14/22 (63.6%)	8/18 (44.4%)
CON (%)	3/21 (14.3%)	7/22 (31.8%)	16/22 (72.7%)	10/13 (76.9%)
P	0.38	0.08	0.64	0.005
Odds ratio				
CUI vs. CON	2.77 (0.61-12.59)	4.13 (1.19-14.38)	1.26 (0.31-5.07)	0.62 (0.08-4.58)
HOI vs. CON	2.28 (0.49-10.57)	2.01 (0.57-7.03)	0.65 (0.18-2.33)	0.14 (0.02-0.30)
CUI vs. HOI	1.21 (0.33-4.43)	2.06 (0.59-7.06)	1.93 (0.50-7.43)	4.33 (0.95-19.63)

CUI, Cows that received formulation containing copper sulfate and iodine; HOI, Cows that received formulation containing honey and iodine; CON, Cows that received no treatment.