The Journal of Veterinary Medical Science

FULL PAPER

Surgery

Combination effect of allyl isothiocyanate and hoof trimming on bovine digital dermatitis

Takumi YAMAMOTO[1)], Hiroyuki MANABE[2)], Naoaki MISAWA[3)], Wataru YAMAZAKI[3)], Masahiro TAKAHASHI[1)] and Keiji OKADA[1)]*

[1)]Cooperative Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan

[2)]MH Limited, 12-12, Nishi 21, Minami 4, Obihiro, Hokkaido 080-2471, Japan

[3)]Department of Veterinary Science, Faculty of Agriculture, University of Miyazaki, 1-1 Gakuenkibanadai-Nishi, Miyazaki 889-2192, Japan

J. Vet. Med. Sci.

80(7): 1080–1085, 2018

doi: 10.1292/jvms.18-0097

Received: 27 February 2018

Accepted: 21 April 2018

Published online in J-STAGE: 9 May 2018

ABSTRACT. Digital Dermatitis is a localized infectious dermatitis caused by *Treponema*-like spirochetes. Antibiotics, such as lincomycin, are currently used for treatment, but their use imposes a withdrawal period. This study investigated the therapeutic effect of topical application of the natural component allyl isothiocyanate, in combination with maintenance hoof trimming, on bovine Digital Dermatitis. Study cows were divided into two groups, the Trimming Group and Non-Trimming Group. The day when allyl isothiocyanate was applied, along with hoof trimming, was set as Day 0. Lesion scores, pain, and the presence of *Treponema*-like spirochetes on the surface of hooves and in biopsy samples of the tissues were evaluated until Day 6. Both groups showed improvement of lesion scores and improved elimination of *Treponema*-like spirochetes from within the tissues. The presence of *Treponema*-like spirochetes on the surface of lesions was significantly higher in the Non-Trimming Group by Day 6. These results suggest that allyl isothiocyanate has therapeutic effects on Digital Dermatitis, when combined with hoof trimming, and may prevent a relapse of dermatitis and a re-infection of *Treponema*-like spirochetes.

KEY WORDS: allyl isothiocyanate, cow, Digital Dermatitis, hoof trimming, *Treponema*-like spirochete

Digital Dermatitis (DD) is a localized infectious dermatitis in dairy cows that is spreading worldwide. Since numerous *Treponema*-like spirochetes have been isolated in DD lesions, DD is considered to be a mixed infection [3, 9]. Numerous spirochetes have been observed among the epithelial cells in the prickle cell layer, but they do not invade further into the dermis. Antibiotics, such as lincomycin and tetracycline, are used for treatment, but their efficacy is limited to a 50–70% cure rate [2, 6, 12, 13, 18]. Although DD can spread through an entire herd, the use of antibiotics is restricted due to the withdrawal period for milk and meat. Surgical removal also provides a low cure rate, and there are no effective vaccines. Therefore, there is a strong need for a new, preventive/therapeutic non-antibiotic option.

Allyl isothiocyanate (AITC) is a natural substance with antibacterial properties. AITC is a strong irritant from the plant family *Brassicaceae*, which also includes wasabi, horseradish, and mustard [10, 21]. It is used as a food additive in Japan due to its wide antibacterial spectrum. AITC inhibits the biosynthesis of bacterial macromolecules in the exponential growth phase [13], and its amphiphilic chemical structure provides antibacterial activity toward gram-negative bacteria, such as *Salmonella Montevideo* and *E. coli* O157:H7 [13, 15]. Therefore, AITC may reduce the viability of *Treponema* species in DD lesions and then sterilize them [15]. Chiba *et al.* reported a high therapeutic efficacy of AITC on DD, similar to that of lincomycin [4].

However, hoof trimming alone can improve lameness and also prevent/treat hoof diseases [16, 20]. According to Manske *et al.* [17], the topical treatment of DD with oxytetracycline is more effective than trimming alone, but hoof trimming alone improved 34% of cows with DD. This suggests that hoof trimming has a positive impact on DD.

The objective of this study was to investigate the efficacy of AITC, in combination with hoof trimming, for the treatment of DD.

*Correspondence to: Okada, K.: keiji@iwate-u.ac.jp

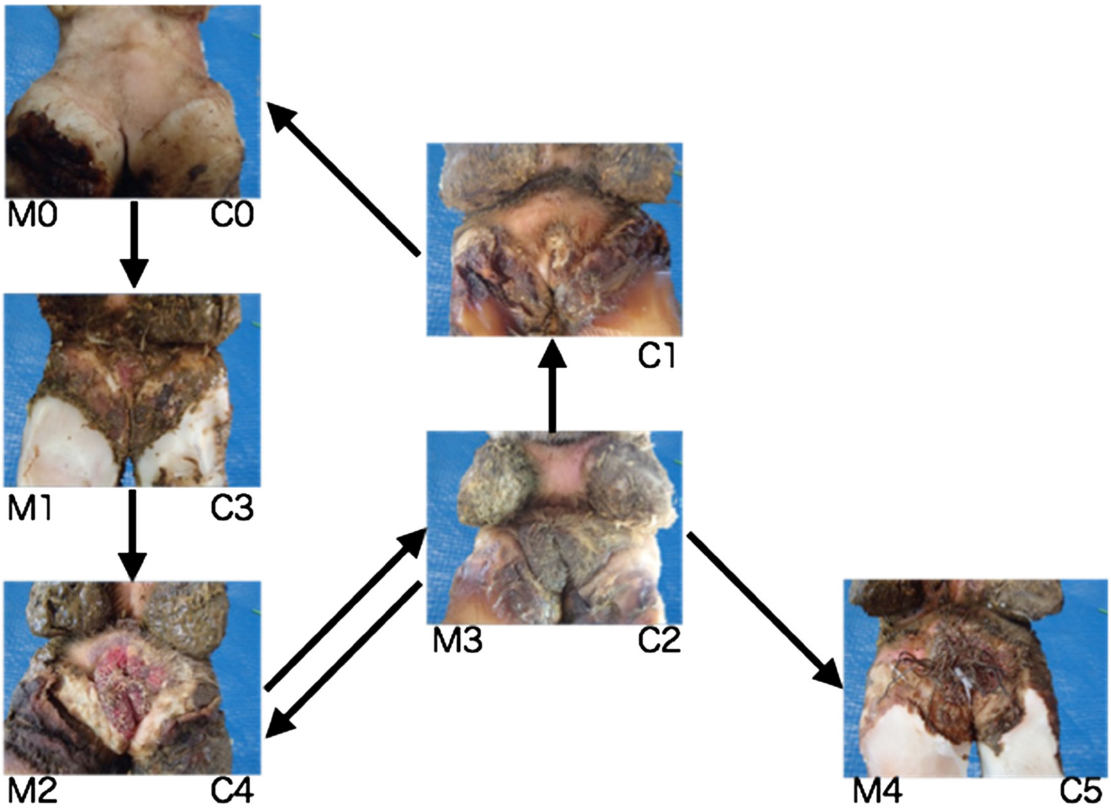

Fig. 1. Changes in lesion scores of DD. Lesion classification by Döpfer (M0−4) is based on the sequential progression of lesions. This study adopted a scoring based on severity of clinical symptoms (C0−5) in order to assess the effect of treatment. Lesion score C0 (M0) is the normal state. Cows chosen for this study had acute ulcerative lesions C4 (M2). Lesion score C4 (M2) changes to score C2 (M3), characterized by a dark scar formation, and then undergoes a healing process or progresses to a chronic lesion with a score of C5 (M4).

Table 1. Association between lesion score and Döpfer classification

Lesion score	Döpfer classification	Description
C5	M4	Chronic lesion. Lesion is covered with numerous filamentous papillae. This kind of lesion does not respond to parenteral administration of penicillin or ceftiofur, and it recurs even after surgical removal.
C4	M2	Acute ulcerative lesion (bright red) with a diameter of 20 mm or larger. Observed around coronary bands or dew claws.
C3	M1	Small early stage ulcerative lesion (less than 20 mm in diameter), observed prior to M2.
C2	M3	Healing process observed 1–2 days after topical treatment. Acute lesion is covered by dark and firm scab-like material.
C1	-	Skin after shedding of scars. Mild proliferation is present.
C0	M0	Normal digital skin without signs.

Calculation of lesion scores is based on the number variable of the scores.

MATERIALS AND METHODS

From a herd of Holstein dairy cows kept in free stall barns, 14 cows with stage M2 DD lesions on their hind hooves, based on Döpfer classification, were selected [8]. The cows were randomly divided into two groups as follows: those with hoof trimming (Trimming Group, n=6), and those without hoof trimming (Non-Trimming Group, n=8). A master class of cattle hoof trimmer, certified by the Japanese Association of Hoof Trimmers, conducted the trimming. After trimming, evaluation of lesion score and pain, preparation of direct smear specimens from the surface of the lesions and the interdigital clefts, biopsy of lesion tissues, and treatment with AITC (Wasaouro Powder; Mitsubishi-Chemical Foods Corp., Tokyo, Japan) were conducted. The day treatment started was defined as Day 0. Observation and sampling were also performed on Day 2, 4 and 6.

The scoring of lesions consisted of six stages based on the clinical severity of the lesions, according to the disease classes described by Döpfer [8]. Table 1 shows the criteria of the scores. An example of each score is shown in Fig. 1.

doi: 10.1292/jvms.18-0097

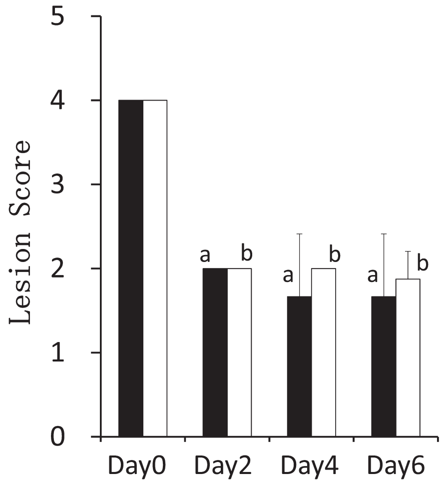

Fig. 2. Time course changes in lesion scores (mean ± s.d.). Comparison among sampling dates within the Trimming Group (n=6: ■) and the Non-Trimming Group (n=8: □), with the day of treatment as Day 0. Letters "a" and "b" represent significant differences (*P*<0.05) from Day 0 within the same group. Both groups showed a significant decrease in lesion scores after treatment.

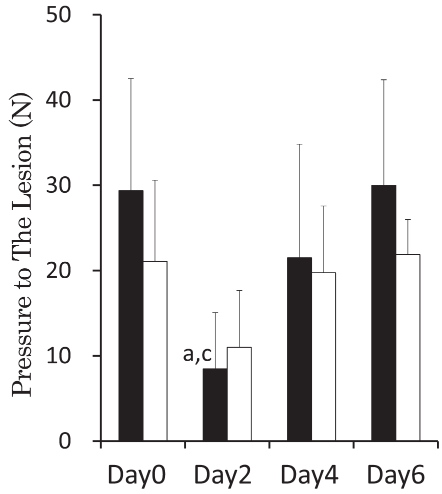

Fig. 3. Time course changes in pain (mean ± s.d.). Comparison among sampling dates within the Trimming Group (n=6: ■) and the Non-Trimming Group (n=8: □), with the day of treatment as Day 0. Letter "a" represents a significant difference from Day 0, and letter "c" indicates a significant difference from Day 6 within the Trimming Group. Pressure when cattle started feeling pain was significantly lower on Day 2 than on Day 0 in the Trimming Group.

Assessment of pain was based on a Force Gauge (AD-4932A-50N: A&D Co., Ltd., Tokyo, Japan) when applying pressure to the lesion. The pressure level was recorded when the cow started showing a pain response.

Treponema-like spirochetes isolated from the surface of the lesions and the interdigital areas were confirmed by Giemsa staining of direct smear specimens. On Day 0 and Day 6, an 8-mm diameter specimen was obtained from each cow using a Biopsy Punch (Biopsy Trepan: Kai Industries Co., Ltd., Tokyo, Japan) from the surface of the lesion through the dermis. A pathological specimen was prepared from a piece of the tissue obtained, following standard procedure. The presence of *Treponema*-like spirochetes was evaluated after staining the specimen with Warthin-Starry stain.

AITC treatment of DD was conducted on Day 0 and Day 4. AITC powder (10%, Wasaouro Powder TM: Mitsubishi-Chemical Foods Corp.) was applied to the lesions (0.6 g AITC) and interdigital clefts (0.3 g AITC), and the areas were then wrapped with an elastic bandage. Elastic bandages applied on Day 0 and Day 4 were removed on Day 2 and Day 6, respectively.

Lesion score, pain assessment, and detection of *Treponema*-like spirochetes on the surface of lesions and interdigital clefts were compared between groups. Time course changes from Day 0 through Day 6 (with two-day intervals) were analyzed using two-way repeated measure analysis with Bonferroni's correction. A *P*-value of less than 5% was considered statistically significant. Statistical analysis was conducted using the SPSS version 18.0 for Mac.

This study was conducted after approval from the Iwate University Animal Experiment Committee.

RESULTS

DD lesion scores

Lesion scores in both groups significantly decreased from 4 on Day 0 to 2 on Day 2. Both groups then maintained similar scores after Day 2, and there were no differences between the groups (Fig. 2).

Assessment of pain

The pressure when pain was noticed on Day 0 was 29.4 ± 13.2 N for the Trimming Group and 21.1 ± 9.5 N for the Non-Trimming Group. On Day 2, the pressure was 8.5 ± 6.6 N for the Trimming Group and 11.0 ± 6.7 N for the Non-Trimming Group, which shows a significant decrease in pressure for the Non-Trimming Group. After that, the pressure changes were 21.5 ± 13.3 N for the Trimming Group and 19.8 ± 7.8 N for the Non-Trimming Group on Day 4 and 30.0 ± 12.4 N for the Trimming Group and 21.9 ± 4.1 N for the Non-Trimming Group on Day 6, showing a significant decrease for the Non- Trimming Group. The Non-Trimming Group did not show significant changes during the experimental period, and differences between the groups were not significant (Fig. 3).

Detection of treponema-like spirochetes from the surface areas

One of the microscopic observations of direct smear specimens to detect spirochetes is shown in Fig. 4. These long and coiled

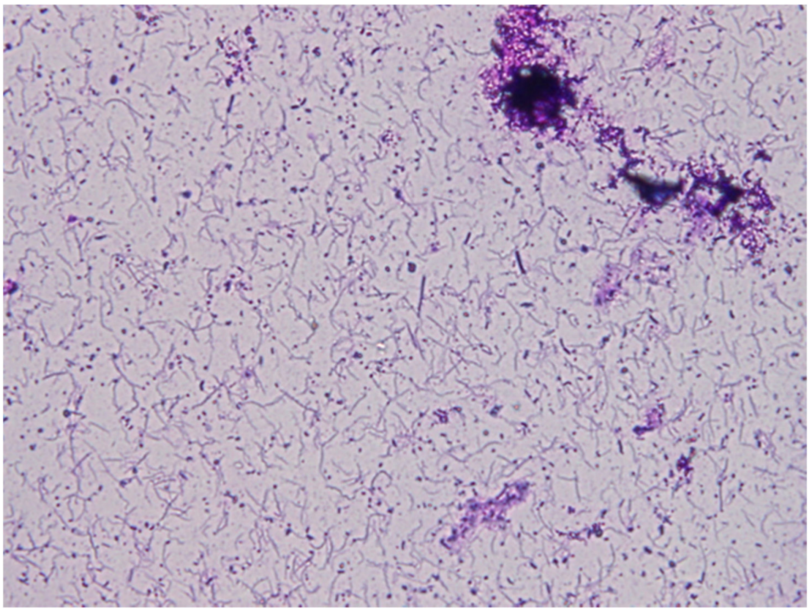

Fig. 4. *Treponema*-like spirochetes in a direct smear specimen stained with Giemsa.

Table 2. Detection rate (%) of *Treponema*-like spirochetes on the surface of lesions

	Day 0	Day 2	Day 4	Day 6
Trimming Group	83.3	0 a)	0 a)	0 a)
Non-Trimming Group	87.5	25.0	12.5 b)	50.0*

Detection rate of *Treponema*-like spirochetes on the surface of lesions in the Trimming Group (n=6) and in the Non-Trimming Group (n=8). Letters "a" and "b" indicate a significant difference from Day 0 within the same group. The "*" indicates a significant difference between the groups on the same sampling date. *Treponema*-like spirochetes were not detectable on the surface of lesions after Day 2 in the Trimming Group.

Table 3. Detection rate (%) of *Treponema*-like spirochetes on the skin of the interdigital clefts

	Day 0	Day 2	Day 4	Day 6
Trimming Group	16.7	0	0	16.7
Non-Trimming Group	37.5	50*	0 a)	0 a)

Detection rate of *Treponema*-like spirochetes on the skin of the interdigital clefts in the Trimming Group (n=6) and in the Non-Trimming Group (n=8). The letter "a" represents a significant difference from Day 2 in the Non-Trimming Group. The "*" indicates a significant difference between the groups on the same sampling date. *Treponema*-like spirochetes were not detectable on the skin of interdigital clefts after Day 2 in the Trimming Group and after Day 4 in the Non-Trimming Group.

stained microorganisms are likely *Treponema*-like spirochetes related with the DD. On the surface of the lesions in the Trimming Group, the percentage of *Treponema*-like spirochete positive cows was 83.3% on Day 0, but spirochetes were not detected from Day 2 onward. On the surface of the lesions in the Non-Trimming Group, the percentage of *Treponema*-like spirochete positive cows was 87.5% on Day 0, but decreased to 25.0% on Day 2 and 12.5% on Day 4. On Day 6, it was 50%, which was significantly higher than it was on Day 4. In addition, the percentage of positive cows in the Trimming Group was significantly lower than that in the Non-Trimming Group on Day 6 (Table 2).

On the surface of the interdigital clefts in the Trimming Group, the percentage of *Treponema*-like spirochete positive cows was 16.7% on Day 0, 0% on Day 2 and Day 4, and 16.7% on Day 6, which were not significant changes. In the Non-Trimming Group, the percentage of *Treponema*-like spirochete positive cows was 37.5% on Day 0 and 50.0% on Day 2. On Day 4 and Day 6, the percentage was 0%, which was significantly lower than the percentage on Day 2. Additionally, the percentage of positive cows in the Trimming Group was significantly lower in the Non-Trimming Group on Day 2 (Table 3).

Detection of treponema-like spirochetes in biopsy samples

This factor was examined for 13 of the cows (Trimming: n=5; Non-Trimming: n=8). Photos of the DD stained with Warthin-Starry show numerous *Treponema*-like spirochetes that were detected in the keratin layer on Day 0 (Fig. 5), but were not detectable in any of the cows on Day 6 (Fig. 6).

DISCUSSION

AITC has a therapeutic effect equivalent to that of antibiotics in the treatment of DD [4]. Furthermore, regular hoof trimming is generally thought to alleviate the symptoms of DD. In this study, we investigated whether hoof trimming along with AITC application would be an effective treatment for DD. Evaluation of the lesions [11], pain [2], smear specimens, and the

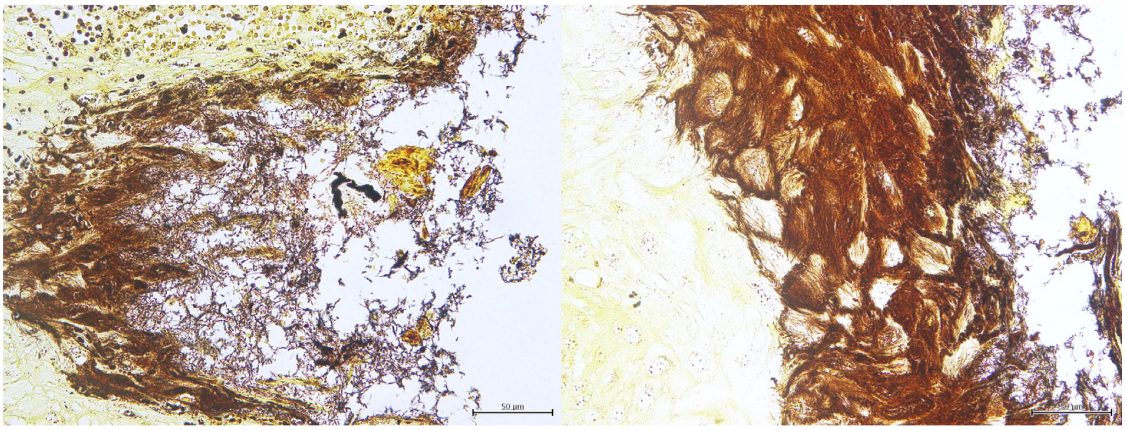

Fig. 5. Keratin layer on Day 0. Accumulation of *Treponema*-like spirochetes is present. Bar=50 μm.

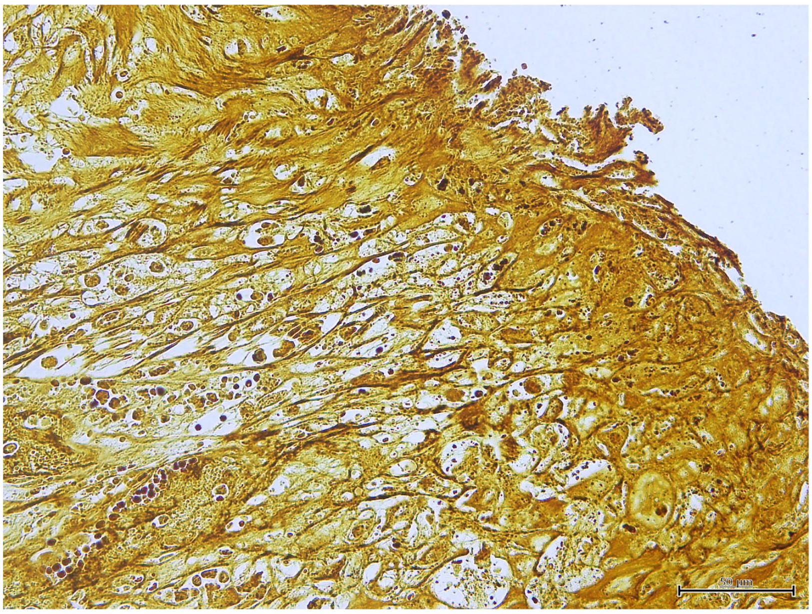

Fig. 6. Keratin layer on Day 6. *Treponema*-like spirochetes are not present. Bar=50 μm.

histopathology of tissues [2, 11] was used.

The course of lesion healing in both groups in this study was similar to the report by Mumba *et al.* [19], with a change from a red ulcerative lesion (M2, score 4) to a firm, dark scar formation (M3, score 2). Since lesion scores did not differ between groups at any sampling point, lesion scores were improved by AITC treatment alone, regardless of trimming.

According to Mumba *et al.* [19], after antibiotic were sprayed on the affected area, pain from DD disappeared two to three days later. In this study, the pressure when cows started feeling pain significantly decreased on Day 2 as compared to Day 0 in the Trimming Group. However, it returned to the same level as Day 0 on Day 6. In the Non-Trimming Group, there was also a decreasing amount of pressure when cows started feeling pain on Day 2, in contrast to the report by Mumba *et al*. [19]. The Transient Receptor Potential (TRP) family of cation channels excite nociceptors, which induce pain and inflammation by binding with various plant ingredients [5, 22]. Since TRPA1, a member of the TRP family, is involved in the sensing of pain [1, 14], increased pain on Day 2 was thought to be due to the topical application of AITC.

Since *Treponema*-like spirochetes disappeared from the lesions in the Trimming Group on Day 2, AITC treatment appeared to have completely eradicated them. However, the percentage of *Treponema*-like spirochete positive cows decreased by Day 4 in the Non-Trimming Group, but it increased on Day 6; therefore, the antibacterial efficacy alone was not sufficient. Regarding *Treponema*-like spirochetes on the surface of the interdigital cleft, they were not detected on Day 2 and Day 4 in the Trimming Group, whereas they were detected in 50% of cows on Day 2 in the Non-Trimming Group. Spirochetes were not detected after Day 4 in the Non-Trimming Group, which varies from what was observed on the surface of the lesions.

Since the detection rate of *Treponema*-like spirochetes from the surface of the interdigital cleft was higher in the Non-Trimming

Group, the interdigital area of the Non-Trimming Group may provide a better environment for *Treponema*-like spirochetes. Since the interdigital cleft is located in proximity to the heel bulb where DD commonly occurs, residual *Treponema*-like spirochetes in the interdigital cleft after lesion treatment might induce a relapse of DD; therefore, proper sterilization is needed when treating DD.

According to Döpfer *et al.*, a spiral form of *Treponema*-like spirochete isolated from DD lesions changed to an encysted form and then back to a spiral form and became activated [6], which may account for the many relapses in cases of DD after treatment. In this study, *Treponema*-like spirochetes that had disappeared from the surface of lesions after treatment became detectable again later. However, the numerous *Treponema*-like spirochetes that had accumulated in the keratin layer on Day 0 disappeared on Day 6 in both groups.

In this study, AITC improved lesion scores both in the Trimming and Non-Trimming Groups, eliminating detectable *Treponema*-like spirochetes from the lesions. Although the *Treponema*-like spirochetes disappeared from the surface of lesions in the Trimming Group on Day 2, they were detectable in the Non-Trimming Group, and the amount was increased on Day 6. This suggested that trimming might enhance the therapeutic effects of AITC by preventing reinfection. In addition, AITC was effective against *Treponema*-like spirochetes residing not only on the surface of tissues but also in the deeper tissues.

These results suggested that hoof trimming at the time of AITC application might enhance its therapeutic efficacy against DD.

ACKNOWLEDGMENT. This study was funded by Mitsubishi-Chemical Foods Corporation and Japan Society for the Promotion of Science KAKENHI, grant number JP26450406.

REFERENCES

1. Bautista, D. M., Jordt, S. E., Nikai, T., Tsuruda, P. R., Read, A. J., Poblete, J., Yamoah, E. N., Basbaum, A. I. and Julius, D. 2006. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell* **124**: 1269–1282. [Medline] [CrossRef]
2. Berry, S. L., Read, D. H., Famula, T. R., Mongini, A. and Döpfer, D. 2012. Long-term observations on the dynamics of bovine digital dermatitis lesions on a California dairy after topical treatment with lincomycin HCl. *Vet. J.* **193**: 654–658. [Medline] [CrossRef]
3. Capion, N., Boye, M., Ekstrøm, C. T. and Jensen, T. K. 2012. Infection dynamics of digital dermatitis in first-lactation Holstein cows in an infected herd. *J. Dairy Sci.* **95**: 6457–6464. [Medline] [CrossRef]
4. Chiba, K., Miyazaki, T., Sekiyama, Y., Miyazaki, M. and Okada, K. 2017. The therapeutic efficacy of allyl isothiocyanate in cows with bovine digital dermatitis. *J. Vet. Med. Sci.* **79**: 1191–1195. [Medline] [CrossRef]
5. Clapham, D. E. 2003. TRP channels as cellular sensors. *Nature* **426**: 517–524. [Medline] [CrossRef]
6. Döpfer, D., Anklam, K., Mikheil, D. and Ladell, P. 2012. Growth curves and morphology of three Treponema subtypes isolated from digital dermatitis in cattle. *Vet. J.* **193**: 685–693. [Medline] [CrossRef]
7. Döpfer, D., Koopmans, A., Meijer, F. A., Szakáll, I., Schukken, Y. H., Klee, W., Bosma, R. B., Cornelisse, J. L., van Asten, A. J. A. M. and ter Huurne, A. A. H. M. 1997. Histological and bacteriological evaluation of digital dermatitis in cattle, with special reference to spirochaetes and Campylobacter faecalis. *Vet. Rec.* **140**: 620–623. [Medline] [CrossRef]
8. Evans, N. J., Brown, J. M., Murray, R. D., Getty, B., Birtles, R. J., Hart, C. A. and Carter, S. D. 2011. Characterization of novel bovine gastrointestinal tract Treponema isolates and comparison with bovine digital dermatitis treponemes. *Appl. Environ. Microbiol.* **77**: 138–147. [Medline] [CrossRef]
9. Fahey, J. W., Zalcmann, A. T. and Talalay, P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* **56**: 5–51. [Medline] [CrossRef]
10. Gomez, A., Bernardoni, N., Rieman, J., Dusick, A., Hartshorn, R., Read, D. H., Socha, M. T., Cook, N. B. and Döpfer, D. 2014. A randomized trial to evaluate the effect of a trace mineral premix on the incidence of active digital dermatitis lesions in cattle. *J. Dairy Sci.* **97**: 6211–6222. [Medline] [CrossRef]
11. Hernandez, J. and Shearer, J. K. 2000. Efficacy of oxytetracycline for treatment of papillomatous digital dermatitis lesions on various anatomic locations in dairy cows. *J. Am. Vet. Med. Assoc.* **216**: 1288–1290. [Medline] [CrossRef]
12. Cutler, J. H., Cramer, G., Walter, J. J., Millman, S. T. and Kelton, D. F. 2013. Randomized clinical trial of tetracycline hydrochloride bandage and paste treatments for resolution of lesions and pain associated with digital dermatitis in dairy cattle. *J. Dairy Sci.* **96**: 7550–7557. [Medline] [CrossRef]
13. Holzhauer, M., Bartels, C. J. M., Döpfer, D. and van Schaik, G. 2008. Clinical course of digital dermatitis lesions in an endemically infected herd without preventive herd strategies. *Vet. J.* **177**: 222–230. [Medline] [CrossRef]
14. Jordt, S. E., Bautista, D. M., Chuang, H. H., McKemy, D. D., Zygmunt, P. M., Högestätt, E. D., Meng, I. D. and Julius, D. 2004. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* **427**: 260–265. [Medline] [CrossRef]
15. Lin, C. M., Preston, J. F. 3rd. and Wei, C. I. 2000. Antibacterial mechanism of allyl isothiocyanate. *J. Food Prot.* **63**: 727–734. [Medline] [CrossRef]
16. Manske, T., Hultgren, J. and Bergsten, C. 2002. The effect of claw trimming on the hoof health of Swedish dairy cattle. *Prev. Vet. Med.* **54**: 113–129. [Medline] [CrossRef]
17. Manske, T., Hultgren, J. and Bergsten, C. 2002. Topical treatment of digital dermatitis associated with severe heel-horn erosion in a Swedish dairy herd. *Prev. Vet. Med.* **53**: 215–231. [Medline] [CrossRef]
18. Moore, D. A., Berry, S. L., Truscott, M. L. and Koziy, V. 2001. Efficacy of a nonantimicrobial cream administered topically for treatment of digital dermatitis in dairy cattle. *J. Am. Vet. Med. Assoc.* **219**: 1435–1438. [Medline] [CrossRef]
19. Mumba, T., Döpfer, D., Kruitwagen, C., Dreher, M., Gaastra, W. and van der Zeijst, B. A. M. 1999. Detection of spirochetes by polymerase chain reaction and its relation to the course of digital dermatitis after local antibiotic treatment in dairy cattle. *Zentralbl. Veterinarmed. B.* **46**: 117–126. [Medline]
20. Phillips, C. J. C., Chiy, P. C., Bucktrout, M. J., Collins, S. M., Gasson, C. J., Jenkins, A. C. and Paranhos da Costa, M. J. 2000. Frictional properties of cattle hooves and their conformation after trimming. *Vet. Rec.* **146**: 607–609. [Medline] [CrossRef]
21. Sultana, T., Savage, G. P., McNeil, D. L., Porter, N. G., Martin, R. J. and Deo, B. 2002. Effects of fertilization on the allyl isothiocyanate profile of above-ground tissues of New Zealand-Grown wasabi. *J. Sci. Food Agric.* **82**: 1477–1482. [CrossRef]
22. Wang, H. and Woolf, C. J. 2005. Pain TRPs. *Neuron* **46**: 9–12. [Medline] [CrossRef]