Combination Effect of Allyl Isothiocyanate and Hoof Trimming on Bovine Digital Dermatitis

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Running Head: AITC AND HOOF TRIMMING FOR DIGITAL DERMATITIS
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 spirochetes
INTRODUCTION

Digital Dermatitis (DD) is a localized infectious dermatitis in dairy cows that is spreading worldwide. Since numerous \textit{Treponema}-like spirochetes have been isolated in DD lesions, DD is considered to be a mixed infection \cite{3, 8}. 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 \cite{2, 11, 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 \textit{Brassicaceae}, which also includes wasabi, horseradish, and mustard \cite{9, 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 \cite{13}, and its amphiphilic chemical structure provides antibacterial activity toward gram-negative bacteria, such as \textit{Salmonella Montevideo} and \textit{E. coli} O157:H7 \cite{13, 15}. Therefore, AITC may reduce the viability of \textit{Treponema} species in DD lesions and then sterilize them \cite{15}. Chiba \textit{et al.} reported a high therapeutic efficacy of AITC on DD, similar to that of lincomycin \cite{4}.

However, hoof trimming alone can improve lameness and also prevent/treat hoof diseases \cite{16, 20}. According to Manske \textit{et al.} \cite{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.
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 [7]. 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 Corporation, Tokyo, Japan) were conducted. The day treatment started was defined as Day 0. Observation and sampling were also performed on Day 2, Day 4, and Day 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 [7]. Table 1 shows the criteria of the scores. An example of each score is shown in Fig. 1.

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 Corporation, Japan) 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**

1) **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).

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).

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 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).

4) 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 [10], pain [2], smear specimens, and the histopathology of tissues [2, 10] 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.

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

REFERENCES


FIGURE LEGENDS

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).

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.

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

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.

Table 1 Association between Lesion Score and Döpfer Classification

<table>
<thead>
<tr>
<th>Lesion Score</th>
<th>Döpfer Classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5 M4</td>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>C4 M2</td>
<td></td>
<td>Acute ulcerative lesion (bright red) with a diameter of 20 mm or larger. Observed around coronary bands or dew claws.</td>
</tr>
<tr>
<td>C3 M1</td>
<td></td>
<td>Small early stage ulcerative lesion (less than 20 mm in diameter), observed prior to M2.</td>
</tr>
<tr>
<td>C2 M3</td>
<td></td>
<td>Healing process observed 1-2 days after topical treatment. Acute lesion is covered by dark and firm scab-like material.</td>
</tr>
<tr>
<td>C1 - M0</td>
<td></td>
<td>Skin after shedding of scars. Mild proliferation is present.</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimming Group</td>
<td>83.3</td>
<td>0 a</td>
<td>0 a</td>
<td>0 a</td>
</tr>
<tr>
<td>Non-Trimming</td>
<td>87.5</td>
<td>25.0</td>
<td>12.5</td>
<td>50.0  *</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trimming Group</strong></td>
<td>16.7</td>
<td>0</td>
<td>0</td>
<td>16.7</td>
</tr>
<tr>
<td><strong>Non-Trimming Group</strong></td>
<td></td>
<td>*</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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.
Fig. 1. Changes in Lesion Scores of DD
Fig. 2. Time course change in lesion score (mean +/-s.d.)
Fig. 3. Time course Changes in Pain (mean +/-s.d.)
Fig. 4. Treponema-like spirochetes in a direct smear specimen stained with Giemsa
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Fig. 6. Keratin layer on Day 6