A Longitudinal Field Trial of the Effect of Biotin on Lameness in Dairy Cows

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ABSTRACT

A longitudinal prospective intervention study investigated the effect of biotin supplementation on the incidence (new cases per day) of visible lameness in milking cows and heifers on five commercial farms in Gloucestershire, United Kingdom. The trial lasted from June 1997 to April 1999. Each farm participated in the trial for 18 mo. Within each herd the cows were randomly allocated to either receive a supplement of 20 mg of biotin per day or not. All cows were run as one herd on each farm. When a lame cow was identified, the farmer called one of six veterinarians to examine and treat the affected animal; findings were recorded on a standard form. A veterinarian also carried out a bimonthly locomotion assessment to ensure that all lame cows were diagnosed. There were a total of 900 cows, 1120 cow years, in the trial. The overall incidence rate of lameness (per 100 cows per year) was 68.9, with a range of 31.6 to 111.5 per farm. The incidence rates of the four most frequently reported causes of lameness were sole ulcer, 13.8; white line separation, 12.7; digital dermatitis, 12.0; and interdigital necrobacillosis, 7.1 per 100 cows per year. There was a significant difference in the incidence rate of these four lesions between supplemented and unsupplemented cows on two of the five farms, with a significant decrease in lameness in the cows supplemented with biotin. When all the farms were pooled, the risk of lameness caused by white line separation in cattle supplemented with biotin was approximately halved (Cox proportional hazard survival analysis hazard ratio = 0.57).

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Abbreviation key: DD = digital dermatitis, HR = hazard ratio, IN = interdigital necrobacillosis, SU = sole ulcer, WLS = white line separation.

INTRODUCTION

Most clinical horn lesions are the result of, or are associated with, poor hoof horn quality (Greenough, 1991). The biomechanical properties of hoof horn are determined by its structural characteristics. These characteristics, which include intra- and extracellular biochemical composition and arrangement of horn cells, are determined during keratinization and cornification. Any disturbance of this process such as interruption of nutrient supply because of circulatory abnormalities or essential nutrient deficiency may adversely affect horn structure and horn quality (Mülling et al., 1999). Claw horn is a modified derivative of skin and contains significant quantities of the structural protein keratin. Biotin, a B vitamin, is an essential nutrient in keratin synthesis and lipogenesis, the two major metabolic pathways in keratinization (Sarasin, 1994; Whitehead, 1988). Previous authors have reported that biotin influences proliferation and differentiation of the epidermis, also necessary for normal keratinization (Fritsche, 1991; Saracin, 1994).

Results from histological and biochemical studies have indicated that there are improvements in the inter- and intracellular ultrastructure of horn as a result of dietary biotin supplementation (Hochstetter, 1998). Biotin supplementation created a more defined and cohesive structure (Fritsche, 1990; Johnston, 1990).

Certain hoof disorders in horses and pigs are responsive to biotin supplementation (Comben et al., 1984;
Kornegay 1985). This has also been observed with specific hoof lesions in dairy cows (Bergsten et al., 1999; Campbell 1996; Distl and Schmid, 1994; Midla et al., 1998). Biotin supplementation has also been reported to increase the rate of lesion healing in cows: Lischer et al. (1996) and Koller (1998) found a positive influence on the structure and quality of new horn during the healing process of sole ulcers (SU).

Microorganisms in the rumen synthesize biotin and other B vitamins, and an absolute biotin deficiency has not been demonstrated in ruminants (Roberts and Baggott, 1982). However, there is evidence that acidic rumen conditions can reduce biotin synthesis (Da Costa Gomez et al., 1998). Such conditions can occur in the periparturient period and in early lactation (Blowey et al., 2000; Da Costa Gomez et al., 1998; Roberts and Baggot, 1982). Midla et al. (1998) reported a decrease in plasma biotin levels of dairy cows 25 DIM, returning to constant levels from 100 DIM until the end of lactation. Roberts and Baggot (1982) reported that lame cows had lower plasma biotin levels when compared with cows with no history of lameness, and studies have shown that orally administered biotin raises plasma (Zinn et al., 1987) and milk (Frigg et al., 1993) biotin levels in dairy cows. So it is hypothesized that biotin supplementation may benefit cows during the period of high demand on the cow, around calving, and during early lactation.

Dairy cow lameness impacts on dairy cow health, productivity, and welfare and is a major reason for early culling. (Boettcher et al., 1998; Distl, 1995; Greenough and Weaver, 1997). Lameness is a multifactorial disease and is influenced by risk factors such as environment, disease, nutrition, and management. The purpose of this intervention study was to test the impact of a supplement of 20 mg of biotin per day on clinical lameness in adult dairy cows on commercial farms, cows that are exposed to a diverse section of risk factors.

**MATERIALS AND METHODS**

**Trial Design**

A longitudinal prospective within farm intervention study was run from 12 June 1997 to 19 April 1999 (22 mo). Five farms from Gloucester, United Kingdom, were selected on the basis of their attending veterinary practice, their willingness to participate in the trial, farmer interest, the practice of autumn calving, and an overall herd size of greater than 90 cows. Each farm participated in the study for 18 mo.

It was estimated that 752 cow years in two groups of 376 would give the study 80% power to detect a relative risk of 1.7, with 95% confidence interval excluding unity, assuming a lameness incidence of 42 cases per 100 cows per year (Epi Info 6.0; Dean et al., 1991).

Cows that were lame at the start of the trial or had a long-term history of lameness were excluded from the study. Cows that had recovered from a previous lameness were included. Study cows were stratified by farm to avoid between-farm variation confounding results, calving date (±4 wk), and whether they were a cow or heifer at the start of supplementation.

On each farm, all of the lactating cows remained together and were managed as one herd. In each milking parlor there was a header tank containing the biotin solution (ROVIMIX H-2, F. Hoffmann-La Roche Ltd., Basle, Switzerland), held at a preset temperature; the solution was continuously circulating in a ring pipeline, with down-pipes to each feeding station. These delivered a 25-ml dose (10 mg of biotin) to the in-parlor feed of the supplemented cows at each milking (twice a day). The dairyman, who used an electronic keypad to dispense the dose of biotin, operated this system. Supplemented cows were identified with two leg bands, one on each hind leg. A computer database logged the number of doses administered at each milking. Each system was also fitted with protection devices on all farms to prevent damage caused by power surges.

Heifers were sorted by expected calving date and randomly allocated either to receive the 20 mg of biotin supplement or not by tossing a coin for the first heifer and alternating subsequent heifers to receive supplementation or not. Cows and heifers that entered the trial at a later date were also randomly allocated by this technique. Supplemented and unsupplemented dry cows and heifers were kept in separate groups. Each day they were fed 0.5 kg of dry cow feed (pellets measuring approximately 1.5 cm diameter and 4 cm long) and their usual ration. Both feeds were made to the same specification with the exception that there was an addition of 20 mg of biotin per day to the feed to the supplemented group. Heifers started supplementation up to 3 mo before their predicted calving date and dry cows received the feed for the whole of their dry period, approximately 56 d.

When a farmer observed that one of his cows was lame, he contacted his veterinary surgeon (six veterinary surgeons took part in the trial). The veterinarian examined the lame cow and photographed and recorded the cause of lameness, the location of all the lesions observed, and the treatment administered on a standard form. This visit and examination were not charged to the farmer. Once every 2 mo, the veterinarian observed the whole herd’s locomotion to ensure that no lame cows were left undiagnosed.
Meetings were scheduled at regular intervals throughout the trial. First the veterinarians and research team members met, and they were joined later by the farmers involved in the intervention study. At the veterinarians’ meeting, the progress of the trial, concerns about lesion nomenclature, and study management were discussed. At the farmers’ meeting there were presentations on the progress of the trial. No results of the effect of biotin were released to either the veterinarians or the farmers throughout the trial. The meetings were an opportunity for farmers to raise queries or problems and to contribute their opinions. These have been successful in other studies (Green et al., 1994).

**Trial Monitoring and Sampling**

The consistency of the biotin supplementation was monitored throughout the trial. In the parlor, the system was checked for faults, and the level of solution in the tank was measured and recorded before and after refilling once each week. Each supplementation point was calibrated once each month, and the recorded number of doses administered at each milking was downloaded from the computer in the parlor. Power cuts and malfunctions of the biotin dispensing system were recorded. The consumption of dry cow and heifer compound feed was recorded once a week and cross-checked with the number of cattle receiving each feed during that week.

A milk sample was taken from the bulk tank of each farm before the trial started and analyzed for biotin concentration. During the study, two pooled milk samples were obtained from 20 randomly selected cows, 10 samples from the supplemented and 10 from the unsupplemented cows on each farm once each month until 1 mo after the end of the trial. In addition to these samples, five individual cows from each group on each farm were randomly selected for milk sampling every 3 mo. These milk samples were sent to F. Hoffmann-La Roche Ltd., Basel, Switzerland, and the biotin concentration was determined by microbiological assay using *Lactobacillus plantarum* ATCC 8014 (VFEA, F. Hoffmann-La Roche Ltd).

**Data Management and Analysis**

Means and standard deviations were used to compare calving dates to assess the success of the random allocation within farm. Analysis of variance or chi-square analyses (Kirkwood, 1988) were used as appropriate to detect crude significant differences in lameness and in biotin supplementation and milk quality between groups within each farm and then over all the farms together.

The data were then analyzed using Cox proportional hazard survival analysis (Cox, 1972) in Egret 2.0 (Cytel Inc., Cambridge, MA) using time to failure in days (from d 1 of supplementation until the development of a specific cause of lameness) to estimate the hazard ratio \( \text{HR} \) for an exposure. The outcome variables tested in four separate models of lameness were time to failure for SU, white line separation \( \text{WLS} \), digital dermatitis \( \text{DD} \), and interdigital necrobacillosis \( \text{IN} \). All the cows that did not become lame with the specific cause under investigation were coded as right censored (Cox, 1972), i.e., they did not have that cause of lameness during their time in the trial. The farms started the trial at different dates, and the cows entered and left the trial at different dates, so the analysis used staggered entry times and was therefore a type III analysis (Collett, 1994). Because cattle were stratified by heifers and cows, not each parity, and parity is a known confounder for lameness, it was tested in the model. To test the within-herd design, farm of origin was forced as a fixed effect into the model by the inclusion of four dummy variables corresponding to farms two through five inclusive. Because of the unreliability of data from farm one, the analyses were repeated with cattle from farm one omitted. Biologically feasible interactions, i.e., farm with biotin supplementation and parity with supple-

### Table 1. Incidence (per 100 cows per year) of all causes of lameness by farm and biotin supplementation.

<table>
<thead>
<tr>
<th>Farm ID</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplemented</td>
<td>32.6</td>
<td>108.2</td>
<td>51.2</td>
<td>91.0</td>
<td>27.3</td>
<td>66.4</td>
</tr>
<tr>
<td>Unsupplemented</td>
<td>30.4</td>
<td>114.9</td>
<td>64.7</td>
<td>85.6</td>
<td>38.6</td>
<td>71.2</td>
</tr>
<tr>
<td>( \times )</td>
<td>0.70</td>
<td>0.30</td>
<td>0.57</td>
<td>0.003*</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Total incidence</td>
<td>31.6</td>
<td>111.5</td>
<td>57.9</td>
<td>88.8</td>
<td>32.5</td>
<td>68.9</td>
</tr>
<tr>
<td>Number of cattle</td>
<td>167</td>
<td>180</td>
<td>227</td>
<td>215</td>
<td>111</td>
<td>900</td>
</tr>
</tbody>
</table>

*\( P \) Significant.
Table 2. Incidence rate per 100 cows per year of the four most common causes of lameness combined, by farm and biotin supplementation.  

<table>
<thead>
<tr>
<th>Farm ID</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplemented</td>
<td>19.7</td>
<td>83</td>
<td>65.6</td>
<td>90</td>
<td>28.8</td>
<td>113</td>
</tr>
<tr>
<td>Unsupplemented</td>
<td>20.9</td>
<td>84</td>
<td>72.2</td>
<td>90</td>
<td>45.1</td>
<td>114</td>
</tr>
<tr>
<td>Overall incidence</td>
<td>20.4</td>
<td>167</td>
<td>68.8</td>
<td>180</td>
<td>36.9</td>
<td>227</td>
</tr>
</tbody>
</table>

\( P = 0.89 \) 0.04* 0.01* 0.14 0.42 0.09

* Four most common lamenesses, sole ulcer (SU), white line separation (WLS), digital dermatitis (DD), and interdigital necrobacillosis (IN), combined.
* \( P \) significant, I = Incidence rate (per 100 cows per year), No. = number of cattle.

Table 3. Incidence of white line separation lameness by farm and biotin supplementation.

<table>
<thead>
<tr>
<th>Farm ID</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>No.</td>
<td>I</td>
<td>No.</td>
<td>I</td>
<td>No.</td>
</tr>
<tr>
<td>Supplemented</td>
<td>2.0</td>
<td>24.6</td>
<td>7.9</td>
<td>7.5</td>
<td>5.5</td>
<td>10.0</td>
</tr>
<tr>
<td>Unsupplemented</td>
<td>3.8</td>
<td>33.6</td>
<td>17.5</td>
<td>7.7</td>
<td>11.3</td>
<td>15.4</td>
</tr>
<tr>
<td>Total</td>
<td>2.9</td>
<td>29.1</td>
<td>12.5</td>
<td>7.6</td>
<td>8.1</td>
<td>12.7</td>
</tr>
</tbody>
</table>

\( P = 0.68 \) 0.13 0.04* 0.83 0.39 0.01*

* \( P \) significant.
and unsupplemented groups began after approximately 130 d (indicated by an arrow) and became more pronounced thereafter.

There was no significant difference in lameness caused by SU ($P = 0.8$), DD ($P = 0.6$), and IN ($P = 0.8$) in supplemented and unsupplemented cattle.

A significant difference in biotin concentration in the milk was observed between cattle supplemented with biotin and those left unsupplemented. The milk biotin concentration in supplemented cows was similar to levels seen in cows supplemented with 20 mg of biotin in a previous study (Kluenter et al., 1993). The mean values for the pooled samples on all farms over the trial period were 186.4 nmol/L in the unsupplemented cows and 453.2 nmol/L in the supplemented cows ($P < 0.01$). In the individual samples over all farms for the total trial period, mean values were 172.6 nmol/L in the unsupplemented and 409.3 nmol/L in the supplemented cows ($P < 0.01$).

**DISCUSSION**

In this trial, supplementation of 20 mg per cow per day biotin significantly reduced the incidence of lameness caused by WLS.

The trial was designed to observe all lameness in the feet of dairy cows beyond a period of complete horn renewal (approximately 15 mo), so the trial was carried out for 18 mo on each farm. Toe horn grows from the corium at a rate of approximately 5 to 6 mm/mo (Hahn et al. 1978; 1986; Schmid, 1995), and sole horn grows marginally slower, total renewal of sole horn occurs after approximately 3 to 4 mo (Schmid, 1995). The cattle in this study had a reduced risk of lameness caused by WLS after 130 d of supplementation. This corresponds to the time required to supplement the whole of the white line with horn supplemented with biotin.

There is evidence that biotin supplementation improves both the cellular and intercellular structure of horn. The reduction in white line lameness in cows supplemented with biotin may have occurred because the white line horn was well keratinized in these cows and soft horn (Budras et al., 1996) was prevented. It may be, however, that improved horn structure is not sufficient in itself to prevent certain other lesion pathologies. Sole ulcer, for example, is commonly considered to be a result of pinching between the sole and distal phalanx. Lischer et al. (2000) examined pathological specimens and identified the involvement of the suspensory apparatus and fat cushioning in SU pathology. An improvement in horn hardness or quality may not prevent this pathology and may therefore explain why biotin supplementation did not reduce the incidence rate of SU.

We had a high compliance from the farmers in this study because they were not charged for the examination and any treatment that their cows received and because they had regular contact with the key research worker (VJH), the veterinarians, and the whole research team at the evening meetings. Ideally we would have had a blind trial with a placebo; however, this was not feasible. Previous intervention studies on farm have indicated that farmers do not usually attempt to influence the results (Green et al., 1996). Pragmatically, farmers are busy people and care for all their animals in a similar way.

**Figure 1.** Kaplan-Meier Survival Functions for white line separation lameness by supplementation (dotted line = no supplement, solid line = supplement).
**Table 5.** Cox proportional hazard survival analysis of white line separation lameness, parity, and farm of origin excluding Farm One.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>No. cattle</th>
<th>Coefficient</th>
<th>Standard error</th>
<th>Hazard ratio</th>
<th>Lower CI</th>
<th>Upper CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin supplemented</td>
<td>Yes</td>
<td>453</td>
<td>−0.55</td>
<td>0.67</td>
<td>0.58</td>
<td>0.41</td>
<td>0.83</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Parity 2</td>
<td>200</td>
<td>0.95</td>
<td>0.48</td>
<td>2.3</td>
<td>0.95</td>
<td>5.47</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>At end of trial 3</td>
<td>123</td>
<td>1.74</td>
<td>0.48</td>
<td>5.45</td>
<td>2.33</td>
<td>12.73</td>
<td>&lt;0.01*</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>89</td>
<td>1.78</td>
<td>0.47</td>
<td>6.79</td>
<td>2.89</td>
<td>15.96</td>
<td>&lt;0.01*</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>42</td>
<td>2.13</td>
<td>0.48</td>
<td>8.98</td>
<td>3.55</td>
<td>22.73</td>
<td>&lt;0.01*</td>
<td></td>
</tr>
<tr>
<td>≥7</td>
<td>49</td>
<td>2.94</td>
<td>0.43</td>
<td>23.8</td>
<td>10.4</td>
<td>54.73</td>
<td>&lt;0.01*</td>
<td></td>
</tr>
<tr>
<td>Farm Three 2</td>
<td>227</td>
<td>−0.80</td>
<td>0.22</td>
<td>0.45</td>
<td>0.29</td>
<td>0.69</td>
<td>&lt;0.01*</td>
<td></td>
</tr>
<tr>
<td>Four</td>
<td>215</td>
<td>−1.15</td>
<td>0.27</td>
<td>0.32</td>
<td>0.19</td>
<td>0.54</td>
<td>&lt;0.01*</td>
<td></td>
</tr>
<tr>
<td>Five</td>
<td>111</td>
<td>−1.65</td>
<td>0.34</td>
<td>0.19</td>
<td>0.10</td>
<td>0.38</td>
<td>&lt;0.01*</td>
<td></td>
</tr>
</tbody>
</table>

*P significant, CI = 95% confidence interval.

Reductions in white line lesions, not lamenesses, were observed in a study carried out by Midla et al. (1998), where biotin supplementation, from 305 DIM, reduced the prevalence of lesions observed at 108 DIM. Fitzgerald et al. (2000) reported an improved locomotion score in dairy cows supplemented with biotin in the Australian tropical upland environment, although this study compared herds of cattle and was therefore prone to between-farm variation.

Our within-farm design removed the confounding effect and variability of farm and improved the validity of the study. Cows that were repeatedly lame and those lame at the start of the study were excluded, but not those that had ever been lame. Calving and age have been shown to be key factors associated with the occurrence of lameness (Bergsten, 1995; Kempson and Logue, 1993). Stratifying on calving date accounted for the effect of calving on lameness (Bergsten, 1995; Kempson and Logue, 1993). The next commonly reported risk for the occurrence of lameness is parity, particularly the risk for newly calved heifers. We therefore stratified cattle by heifer and cow. There were not enough cows within each farm to stratify by parity so the residual effect of parity was tested in the Cox Proportional Hazard model. There was a small effect; the HR for biotin supplementation altered slightly and the precision of our estimate increased (confidence intervals narrowed). The amount and causes of lameness was highly variable between farms (Tables 1 to 3). However, this had no impact on the estimate or precision of the HR for biotin supplementation and lameness caused by WLS, as can be seen when dummy variables for Farm were included in the Cox proportional hazard model (Table 4 vs. 5). The large inconsistencies in the number of cows supplemented on farm one occurred when temporary staff milked the cows. Excluding the cattle from farm one did not affect the HR for the effect of biotin on WLS lameness.

Ideally, it would have been preferable to carry out this study on many farms throughout the United Kingdom. However, generalizability was sacrificed for precision. The lack of farm effect indicated that there was no significant interaction between farm and biotin supplementation and when interaction terms for biotin supplementation and farm and biotin supplementation and parity were formally tested in the model, they were not significant. We can therefore conclude that the effect of biotin was consistent across farms and parities and that farms with a high incidence of lameness caused by WLS may benefit from biotin supplementation to their cows as one of the interventions in a herd health program.

**CONCLUSIONS**

This paper has provided evidence that in a commercial situation, where cattle are exposed to many factors that may influence the occurrence of lameness, it is possible to run a controlled intervention study and estimate the impact of biotin supplementation on the incidence of lameness. We propose that biotin supplementation may improve white line structure and strength and so reduce incidence of lameness caused by WLS. Further work that focuses on the structure and properties of the white line of cattle supplemented with biotin, and on the requirements of biotin for periparturient dairy heifers and cows would assist in defining its precise mode of action.

**ACKNOWLEDGMENTS**

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VFEA, Microbiological Analytics, F. Hoffmann-La Roche Ltd, Basle, Switzerland. Confidential assay methodology (Determination of biotin in plasma or serum and in milk).
