Effects of Biotin Supplementation on Performance and Claw Lesions on a Commercial Dairy Farm

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ABSTRACT

A controlled 14-mo field trial was conducted to evaluate the effect of biotin supplementation on hoof lesions, milk production, and reproductive performance in a commercial dairy herd. One hundred seventy cows were studied and supplemented with either 0 or 20 mg/d of biotin by computer feeder. All were housed in the same free-stall facility with the same environment, base diet, and management. The feet of 99 cows were trimmed three times at 6-mo intervals, and hoof health was evaluated. Milk production and fertility data were captured monthly by the Dairy Herd Improvement Association. At the final hoof trimming, sole hemorrhages were significantly higher in control (50%) vs. biotin-supplemented animals (24%). The incidents of cows affected with double soles, hoof wall grooves, and heel horn erosion did not differ between control and biotin-supplemented animals. Biotin supplementation of trimmed cows resulted in 878 kg more milk than control cows when compared with previous lactation yield (n = 46 biotin supplemented, n = 48 control cows). At the end of the study, for both trimmed and untrimmed animals, biotin supplemented cows (n = 81) produced 481 kg more milk and 25 kg more fat than the controls (n = 81). There was no interaction between biotin supplementation and hoof trimming on milk production. There were variations in the response of fertility to biotin between age groups. First lactation heifers fed supplemental biotin had significantly fewer days from calving to conception and required fewer inseminations per pregnancy than controls of the same parity.

(Key words: biotin, claw lesions, milk production, fertility)

INTRODUCTION

Laminitis and the diseases associated with laminitis are among the most common causes of lameness in dairy cattle (Murray et al., 1996). Sudden changes in nutrition, particularly sudden increases in rapidly fermented carbohydrate and/or a drop in the quality and quantity of fiber in the diet are usually indicated as the major causes of laminitis ( Livesey and Fleming, 1984). The subsequent deterioration of the attachment of the bone inside the hoof capsule is considered to be the precipitating cause of rotation and/or sinking of the claw bone (Ossent and Lischer, 1998; Lischer et al., 2002b; Tarlton et al., 2002), which causes secondary lesions of the sole horn, such as sole ulcer, sole hemorrhages, white line disease, double sole, and possibly, heel erosion. Reduced claw horn quality contributes to the development of these lesions and the integrity of the white line is of special concern as it is the weakest part of the hoof capsule.

Biotin is a water-soluble vitamin in the B complex also called vitamin H. Biotin is naturally present in plants and thus in the diets fed to dairy cows. Biotin is also synthesized in the rumen in varying amounts depending on the composition of the diet (forage:concentrate ratios; Abel et al., 2001). In cattle, biotin is an essential cofactor in enzymes required for gluconeogenesis, lipogenesis, and protein synthesis. These properties are highly significant for hoof horn production since keratin is the major structural compound of the hoof epidermis. Biotin has been identified as an essential factor of the intracellular cementing substance, bonding together the keratin leaflets of hoof horn (Mülling et al., 1999). Several studies have shown that biotin can increase horn quality and promote horn formation (Hochstetter, 1998; Higuchi and Nagahata, 2001) and thus increase resistance against (Midla et al., 1998; Campbell et al., 2000; Fitzgerald et al., 2000) and improve healing (Lischer et al., 2002a) of claw lesions in cattle. Furthermore, studies have reported that cows supplemented with biotin produced more milk (Midla et
al., 1998; Zimmerly and Weiss, 2001) and had improved fertility (Bonomi et al., 1996). The improved performance may be a direct effect of intermediary metabolism, an indirect effect of improved claw health, or a combination of these.

The objective of this study was to evaluate the effect of supplemental biotin on claw health, milk production, and fertility with a controlled design in a commercial Holstein herd.

### MATERIAL AND METHODS

#### Animals and Management System

The year-round calving herd was chosen with regard to the use of computer feeding stations and the operator's willingness to cooperate. All milking cows were managed under equal conditions in one group.

In total, 170 animals were studied from March 1996 to May 1997. Fifty-one animals were in their first lactation, and 47, 30, 17, 13, 6, 4, and 2 animals were in their second to eighth lactation, respectively (Table 1). During the study, 26 animals were culled and replaced with 23 first-calving heifers. Only the 99 cows in lactation at the commencement of the study received claw evaluations and trimming. None of the replacements introduced during the study were trimmed, and no animals on the culling list were included. No lameness problem was observed in the herd, and previous recording of claw lesions had not been made. A footbath was not used in the operation. The rolling herd average the year preceding the study was 32.2 kg/d (~9,800 kg/305 d). All milk production and fertility data were based on monthly test day data gathered from DHIA (Provo, Utah).

Lactating cows were housed in the main uninsulated building with open walls and a common feeding platform. The 2 × 2 rows of free stalls (total = 150) on each side of the feed bunks had a clay base, and sawdust was used for bedding in all stalls. All gateways were concreted, and four computer feeding stations were placed together, outside, in the alley leading to the milking parlor and could be reached from the free stall area. The concrete alleys were tractor scraped twice daily. During the grazing period from April through September, all animals were grazed in close proximity to the barn and had access to the computer feeding stations. The cows were milked twice daily in a 2 × 6 herringbone parlor. Dry cows and heifers were transferred to the milking herd about 3 wk before calving. The same person at each of two visits (first and second) evaluated BCS. The herd was bred on observed estrus and did not have fixed voluntary waiting periods.

#### Biotin Supplementation and Allotment into Groups

The cows were assigned to control or biotin treatments according to the ear tag number given chronologically at birth; 84 cows that were to be fed biotin had identification tags ending in an even number, whereas the tags of the 86 control cows ended in an uneven number. The rationale for this was to have a quasi-random distribution of cows, and also to simplify the cow identification and assignment to the computer feeding stations. As a result, the animals were evenly distributed between the groups with regard to age at calving, calving date, lactation number, and BCS at the start of the study (Table 2). The animals in the respective groups were fitted with transponders, which activated either the supplemented or unsupplemented feed in the computer feeding stations.

#### Diets

Diets were formulated to meet or exceed nutrient requirements for lactating dairy cows (NRC, 1989) and were designed to be identical except for the presence or absence of 20 mg/d of supplemental biotin. Alfalfa hay was fed throughout the 1-yr experiment, whereas corn silage was fed during the fall and winter and pasture during spring and summer (April to September). Forages were fed to appetite in feed bunks or as pasture.
Grain was fed according to nutritional requirements via the computer feeder. The herd grain mix was fed in the milking parlor at the rate of 4.54 kg/d per cow, in two equal portions. Any additional grain required, including the biotin-supplemented grain mix, was fed via the computer feeder. A single grain mix formula (Table 3) was fed during the experiment with varying amounts according to forage source, forage quality, and cow nutrient requirements. The grain mix was prepared by a commercial feed mill in two versions: with and without supplemental biotin. The biotin-supplemented grain mix was formulated to supply 20 mg of biotin in 2.27 kg of the grain mix. Cows assigned to receive supplemental biotin were fed 2.27 kg of biotin-supplemented grain mix via the computer feeder, with the remaining grain allotment supplied by the unsupplemented grain mix. Thus, cows assigned to receive supplemental biotin could be fed 20 mg/d at a constant rate. Biotin supplementation was begun just after the initial hoof trimming and evaluation in March 1996 and continued until after the final hoof trimming and evaluation April 1997 (exact dates below). Biotin was supplemented only to lactating cows throughout this period.

Rations were balanced based on calculated nutrient requirements in a manner typical of commercial dairy management. Rations were formulated based on a range of milk production and parity (Table 4). Rations were reformulated periodically during the experiment based on changes in forage analysis and forage source. Allotments of grain for individual cows were controlled by computer feeder access.

**Claw Records**

The feet were measured and observed before and after trimmings that occurred at the beginning of the study, at the mid-point, and at the end of the study on March 28, 1996, October 12, 1996, and April 12, 1997, respectively. A professional hoof trimmer using a tilt table and an electric grinder with a cutting wheel was hired for the paring. The feet were cleansed of dirt, then toe length and diagonal (Figure 1) were measured on the left lateral hind and left lateral fore claws. At the first examination, a hole 4 mm in diameter and 4 mm in depth was drilled in the hoof wall of the left lateral rear claw and left lateral fore claw at a point 30 mm from the skin/horn junction. The hole was filled with bath chalk to increase visibility. At each examination, the location of this point relative to the skin/horn junction was measured before trimming to evaluate horn growth. Furthermore, the distance from the skin/claw junction to any horizontal groove of the hoof wall (hardship grooves) was measured. This was done to ascertain the date on which a stress on horn growth may have occurred.

Hardness of the left lateral rear claw was measured in SHORE UNITS with a Rex Durometer (DIN 53 505, Amsler Otto Wolpert-Werke GmbH, Ludwigshafen, Germany). The average of four measures was recorded for each of the following three points: on either side of the hole drilled in the wall, 10 mm from the apex of the sole, and 10 mm into the sole from the abaxial groove (Vermunt, 1990).

All lesions were drawn on a chart to record their magnitude and location. However, no severity scoring or localization of the lesions were used, and the following most prevalent claw lesions at the third trimming

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**Table 2.** Average figures on age at calving (months), calving date, lactation number, and BCS by treatment group at the start of the study.

<table>
<thead>
<tr>
<th>Item</th>
<th>Age at calving</th>
<th>Calving date</th>
<th>Lactation no.</th>
<th>BCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>48.5</td>
<td>96/05/14</td>
<td>2.64</td>
<td>3.0</td>
</tr>
<tr>
<td>Control</td>
<td>48.5</td>
<td>96/05/30</td>
<td>2.64</td>
<td>3.2</td>
</tr>
</tbody>
</table>

**Table 3.** Calculated nutrient composition of base grain mix (% DM).

| Nutrients      | NEL (1989), mcal/kg | CP, % | Crude fat, % | Crude fiber, % | Ca, % | P, % | Na, % | Cl, % | K, % | Lys, % | Met, % | Mg, % | S, % | Cu, mg/kg | Co, mg/kg | Fe, mg/kg | I, mg/kg | Mn, mg/kg | Zn, mg/kg | Se, mg/kg | Vitamin A, IU/kg | Vitamin E, IU/kg | Biotin, mg/kg |
|----------------|---------------------|-------|--------------|----------------|-------|------|-------|-------|------|--------|--------|-------|------|---------|-----------|-----------|---------|-----------|-----------|-----------|-----------|---------------|---------------|-------------|
| NEL (1989), mcal/kg | 1.91                | 20.3  | 5.23         | 5.87           | 0.98  | 0.67 | 0.73  | 0.56  | 1.96 | 0.96   | 0.32   | 0.56  | 0.26 | 44.6     | 2.6       | 183      | 1.81      | 169       | 200       | 0.88       | 19,968      | 58         | 0.3        |
Table 4. Ration formulation and nutrient composition for cows in second or greater lactation.

<table>
<thead>
<tr>
<th>Item</th>
<th>Pasture season, April to September</th>
<th>Confinement, October to March</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk, kg</td>
<td>54.5</td>
<td>45.5</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>14.1</td>
<td>15.9</td>
</tr>
<tr>
<td>Grain mix</td>
<td>50.2</td>
<td>47.9</td>
</tr>
<tr>
<td>Pasture</td>
<td>35.7</td>
<td>36.2</td>
</tr>
<tr>
<td>Corn silage</td>
<td>19</td>
<td>19.3</td>
</tr>
<tr>
<td>Total, % DM</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>DM, %</td>
<td>32.4</td>
<td>32.1</td>
</tr>
<tr>
<td>CP, %</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>4.5</td>
<td>4.4</td>
</tr>
<tr>
<td>Ca %</td>
<td>0.86</td>
<td>0.87</td>
</tr>
<tr>
<td>P, %</td>
<td>0.48</td>
<td>0.47</td>
</tr>
<tr>
<td>NE&lt;sub&gt;l&lt;/sub&gt;, Mcal/kg</td>
<td>1.66</td>
<td>1.65</td>
</tr>
<tr>
<td>ADF, %</td>
<td>18.8</td>
<td>19.3</td>
</tr>
<tr>
<td>K, %</td>
<td>1.57</td>
<td>1.61</td>
</tr>
<tr>
<td>Mg, %</td>
<td>0.45</td>
<td>0.47</td>
</tr>
<tr>
<td>Na, %</td>
<td>0.48</td>
<td>0.47</td>
</tr>
<tr>
<td>Cl, %</td>
<td>0.45</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Figure 1. Claw measurements with a reference point for growth and sites for hardness appreciation in the wall and sole. Claw lesions used in the analysis; sole hemorrhages, white line hemorrhages (WL), heel horn erosion, double sole, and horizontal grooves.
were dichotomized as present or not present: hemorrhages of the sole and/or the white line, double sole, heel horn erosion, and horizontal grooves of the hoof wall. All claw measurements and claw records were made by P. R. Greenough, who was blind to treatment group.

**Statistical Analysis**

Statistical analysis of the claw lesions was performed using $\chi^2$ tests (Statistix for Windows). The effect of biotin supplementation on milk production and performance was evaluated in two ways. First, for the 99 animals that were trimmed, the milk production before (previous to 305-d lactation yield) and after the supplementation started was compared for each animal. For cows completing their lactation within the study, actual 305-d mature-equivalent was used. For cows that did not lactate for 305 d before the study terminated, the extrapolated 305-d milk production value was used. The inclusion criterion was to achieve at least 148 DIM after the start of the trial, which was also valid for cows that lactating before the study started and dried off or were culled within the study period. Second, the milk production and fertility data were analyzed for all 170 cows present in the herd for all or part of the study period, including herd replacements, whether trimmed or not. The mature-equivalent or extrapolated 305-d milk production was used with the same criteria as described above for cows that were not trimmed.

Linear regression modeling (unweighted least squares means; Statistix for Windows) was used to analyze the difference between the control and biotin groups for their respective increases in 305-d milk production (yield and fat) from the previous lactation to the study period lactation. In the second analysis of milk production, all cows (trimmed and not trimmed) were included in the ANOVA (Minitab for Windows, release 12.21). Despite the random distribution of cows, PTA milk, and PTA fat were significantly different between treatment groups and were thus corrected for by analysis of covariance in both models. The models used in statistical analysis were as follows for the dependent variable of milk production (all effects were fixed):

$$ Y_{ijklm} = \mu + a_i + t_j + c_k + (a \ t)_{ij} + (c \ t)_{kj} + (a \ c)_{ik} + b(x_{ijkl} - \mu) + e_{ijkl} $$

where $Y_{ijklm} =$ the $ijklm$th observation, $\mu =$ general mean, $a_i =$ effect of the $i$th parity ($i = 1, 2, 3, 4$), $t_j =$ effect of the claw trimming ($j = 0, 1$), $c_k =$ effect of the biotin supplementation ($k = 0, 1$), $(a \ t)_{ij} =$ effect of the interaction between parity and the claw trimming, $(c \ t)_{kj} =$ effect of the interaction between the biotin supplementation and the claw trimming, $(a \ c)_{ik} =$ effect of the interaction between the $i$th parity and biotin supplementation, $b =$ linear regression coefficient on PTA milk or PTA fat for dependent variables milk and fat production, respectively, and $e_{ijkl} =$ random residual component.

Fertility was expressed as days to first AI, days to conception (days open), and number of inseminations per conception. Only cows that had their first insemination after the voluntary waiting period (50 d) and became pregnant within the study period were included in the analysis (76 biotin cows and 75 control cows). Animals with incomplete figures were excluded from the reproduction analysis. The model used for statistical analysis of the fertility indices data were:

$$ Y_{ijklm} = \mu + a_i + t_j + c_k + (a \ t)_{ij} + (c \ t)_{kj} + (a \ c)_{ik} + b(x_{ijkl} - \mu) + e_{ijkl} $$

where $Y_{ijklm} =$ the $ijklm$th observation, $\mu =$ general mean, $a_i =$ effect of the parity ($i = 1, 2, 3, 4$), $t_j =$ effect of the claw trimming ($j = 0, 1$), $c_k =$ effect of the biotin supplementation ($k = 0, 1$), $(a \ t)_{ij} =$ effect of the interaction between parity and the claw trimming, $(c \ t)_{kj} =$ effect of the interaction between the biotin supplementation and the claw trimming, $(a \ c)_{ik} =$ effect of the interaction between the $i$th parity and biotin supplementation, $b =$ linear regression coefficient on days in milk, and $e_{ijkl} =$ random residual component.

**RESULTS**

Results from claw measurements of growth and hardness are presented in Table 5. Wall hardness had a poor reliability and was not included. No statistical

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Front claw</th>
<th>Rear claw</th>
<th>Apical horn</th>
<th>Abaxial horn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin, first lactation</td>
<td>29 (4.2)</td>
<td>30 (4.3)</td>
<td>40.1</td>
<td>38.1</td>
</tr>
<tr>
<td>Control, first lactation</td>
<td>24 (3.4)</td>
<td>31 (4.4)</td>
<td>41.4</td>
<td>40.8</td>
</tr>
<tr>
<td>Biotin, 2nd or &gt; lactation</td>
<td>27 (3.9)</td>
<td>28 (4.0)</td>
<td>44.6</td>
<td>39.6</td>
</tr>
<tr>
<td>Control, 2nd or &gt; lactation</td>
<td>17 (2.4)</td>
<td>22 (3.1)</td>
<td>45.8</td>
<td>38.4</td>
</tr>
</tbody>
</table>

Table 5. Net growth in millimeters per month, as measured at second trimming (7 mo) of the toe (front and rear claw) and sole horn hardness (shore units) at apical and abaxial measure point for first lactation and second or later lactation cows fed biotin and unsupplemented controls.
analysis of claw measurements and horn hardness was made. Very few claw lesions were observed at the two first examinations, and they were not statistically analyzed. At the last examination, the lesions had increased, and the results are presented in Table 6. Also observed were corns, wart growths, and corkscrew claws, but these data were not analyzed due to low prevalence. Sole hemorrhages were more (\(P = 0.014\)) common in unsupplemented animals. A trend where horizontal grooves of the hoof were more prevalent in the control group was observed (\(P = 0.072\)). Independent of treatment group, animals with sole hemorrhages had significantly higher milk production. Moreover, when the total number of lesions was taken into account, animals with more lesions had greater milk production than the healthy animals.

The net increases in milk and fat production for the biotin-supplemented and control groups were 1804, 69, 714, and 20 kg per 305-d lactation, respectively, when comparing the experimental lactation to the previous lactation of cows that were trimmed during the study. When corrected for PTA, the net increase was 878 kg (\(P = 0.005\)) in biotin-supplemented cows vs. control cows. When all the animals present in the herd during the study (both trimmed and untrimmed cows) were taken into account, the biotin group produced 481 kg more milk and 25 kg more fat than the control group (Table 7). Claw trimming or the interaction between parity and trimming, biotin and trimming, or parity and biotin did not have a significant influence on milk production (extrapolated 305-d milk and fat yields).

There were no significant differences in fertility between the groups when all animals were included, but there was an interaction with age. When the age groups were analyzed separately, the first-calving heifers fed biotin had significantly better fertility than the first-calving heifers in the control group (Table 8). The outcome of the fertility parameters varied in the other age groups but showed no statistically significant associations.

**DISCUSSION**

**Claw Lesions: Prevalence and Biotin Influence**

The prevalence of claw lesions at the first two trimmings was low, whereas it increased by the last examination at the end of the housing season the second year. The recorded lesions were considered to be subclinical (Greenough, 1985), and no cases of lameness were reported over the whole study period. In another Washington herd with similar production but grazing only the dry cows, mean prevalence of sole hemorrhages (48%), white line lesions (33%), and heel horn erosion (74%) was considerably higher (Bergsten et al., 1998). It is reasonable to suggest that there is a large variation between herds in the incidence of lameness and claw lesions, and that the awareness among different interpreters of lameness varies (Wells et al., 1993; Clarkson et al., 1996). Furthermore, there are seasonal and yearly variations and a higher incidence of lesions tends to be observed at the end of the winter housing season, as was found in present study, and a lower prevalence is usually observed during or shortly after the grazing season (Andersson and Lundström, 1981; Wells et al., 1993). Dairy cows that are housed on concrete floors and have their hooves contaminated with manure have increased risk for claw lesions and lameness (Manske, 2002). The overall high quality of management, and the fact that the cows were on grass half of the year.

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**Table 6.** Number of animals (%) affected with sole (and white line) hemorrhages, heel horn erosions, double soles, hardship grooves and heel horn erosion in biotin supplemented and control cows.

<table>
<thead>
<tr>
<th>Item</th>
<th>n =</th>
<th>Sole hemorrhages</th>
<th>Double soles</th>
<th>Horizontal grooves</th>
<th>Heel horn erosion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>42</td>
<td>10 (24%)</td>
<td>13 (31%)</td>
<td>10 (24%)</td>
<td>22 (52%)</td>
</tr>
<tr>
<td>Control</td>
<td>40</td>
<td>20 (50%)</td>
<td>11 (28%)</td>
<td>17 (43%)</td>
<td>21 (53%)</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.014*</td>
<td>0.731</td>
<td>0.072</td>
<td>0.991</td>
</tr>
</tbody>
</table>

* = \(P < 0.05\); statistically significant.

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**Table 7.** Least squares means for extrapolated 305-d milk yield and standard error in ANOVA for biotin and control animals.

<table>
<thead>
<tr>
<th>Item</th>
<th>n =</th>
<th>Milk, kg</th>
<th>SE</th>
<th>Fat, kg</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>81</td>
<td>10,565</td>
<td>157.5</td>
<td>377</td>
<td>7.5</td>
</tr>
<tr>
<td>Control</td>
<td>81</td>
<td>10,084</td>
<td>156.0</td>
<td>351</td>
<td>7.5</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.032*</td>
<td></td>
<td>0.017*</td>
<td></td>
</tr>
</tbody>
</table>

* = \(P < 0.05\); statistically significant.
(and thus were off concrete), may have contributed to the less than average presence of sole lesions and lameness. The cows were also fed rations balanced for trace minerals, vitamins, and other nutrients that are required for normal hoof horn formation (Mülling et al., 1999). A trend of higher incidence of claw lesions in general and sole hemorrhages in particular are observed in herds with higher milk production since a higher metabolic load is a risk factor for subclinical laminitis (Logue et al., 1994). Lesions of the hoof horn, in terms of sole or white line hemorrhages, double sole, white line separation, sole ulcer, and white line abscess are associated with each other, as are grooves of the hoof wall (Manske et al., 2002). The general increase of these laminitis-associated lesions during the study period could probably be explained as a consequence of a generally increased milk yield in the herd, although no cause and effect of the association was established. Manske (2002) found that cows receiving a sole ulcer had, on average, 479 kg more energy-corrected 305-d milk yield than healthy cows. However, these findings were not supported in the biotin-supplemented group that had fewer lesions despite higher production. According to Greenough (1985), hoof wall grooves and sole hemorrhages may have a related etiology. This finding would justify the different lesion pattern observed between the biotin-treated and control groups. However, due to rate of horn growth and horn wear and the severity of sole hemorrhages, the lesions are only visible on the solar surface for a limited period of time (weeks), while grooves of the hoof wall can be observed for a considerably longer period (months).

The prevalence of claw lesions was reduced in cows fed supplemental biotin in North America (Midla et al., 1998), Europe (Hedges et al., 2001), and Australia (Fitzgerald et al., 2000). Due to different types of claw disorders, and the multifactorial causes behind them, it is difficult to interpret the cause and effect unless there is a controlled design of the study. An explanation of the positive results in this study can be related to biotin-mediated resistance of the epidermis, both of the soft skin and the keratinized epidermis (i.e., claw horn). This was the justification for evaluating hoof hardness, although no difference was detected between supplemented and unsupplemented animals. However, an appreciation of the hardness (quality) of the white line would have been beneficial since lesions of the white line preceded lesions of the sole (Kempson and Logue, 1993). We concluded that biotin improves the quality of claw horn, which encourages the replacement of defective horn, improves healing, and makes it less likely for sole lesions to develop from laminitis in its early stages. No significant affect on claw growth rate was indicated, however, in the present study, and the growth rates were slightly lower than those reported earlier (Manson and Leaver, 1988; Vermunt, 1990). On the other hand, a more resilient, better attachment of the pedal bone into the hoof capsule together with intact hoof cushions might explain the lower incidence of sole hemorrhages and hardship grooves in the supplemented group. This hypothesis is now considered to be an interesting explanation for the pathology and development of laminitis-related claw lesions (Ossent and Lischer, 1998; Tarlton et al., 2002; Lischer et al., 2002b). For example, the role of biotin in lipogenesis, as a cofactor in acetyl CoA carboxylase, and in the synthesis of higher fatty acids, is a possible connection to both the quantity and fatty acid profile of the hoof cushion. No differentiation of hemorrhages of the white line and sole was made in the present study, although Midla et al. (1998) found significantly less white line separation and Hedges et al. (2001) found less lameness associated with white line lesions, in cows fed supplemental biotin. A distinction in scoring between sole and white line in the present study could possibly have given a better resolution of differences in the biotin effects between the two areas if sampling had been made according to calving date. However, in a cross-section study, a strong correlation was shown between sole and white line hemorrhages (Manske et al., 2002). Moreover, it is likely that biomechanics may determine where in the sole area the lesion occurs (Lischer et al., 2002b), although it was not evaluated in this study. Higuchi and Nagahata (2001) found that cows suffering from clinical laminitis had lower DM content of the sole horn and lower serum biotin than matched control cows in the same herd.

### Table 8. Least squares means for the fertility parameters days to first artificial insemination, days to conception, and number of breeding per conception in ANOVA of biotin and control first-calving heifers.

<table>
<thead>
<tr>
<th>Item</th>
<th>n</th>
<th>Days to first breeding</th>
<th>SE</th>
<th>Days to conception</th>
<th>SE</th>
<th>Breedings per conception</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>23</td>
<td>83</td>
<td>7.5</td>
<td>108</td>
<td>7.5</td>
<td>1.50</td>
<td>0.34</td>
</tr>
<tr>
<td>Control</td>
<td>23</td>
<td>76</td>
<td>7.5</td>
<td>169</td>
<td>7.5</td>
<td>2.96</td>
<td>0.34</td>
</tr>
<tr>
<td>P-value</td>
<td>0.535</td>
<td>0.016*</td>
<td>0.004*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05; statistically significant.
Performance

Milk production. Both the relative increase of milk and fat yield from previous lactation in biotin-supplemented cows and the average milk and fat production in the supplemented group were significantly higher than in unsupplemented cows. Bonomi et al. (1996), Midla (1998), and Zimmerly and Weiss (2001) reported a significant dose-dependant increase in milk and milk protein yield in cows supplemented with 10 or 20 mg/d biotin. But, due to relatively less increase in fat yield, the energy-corrected milk production did not differ significantly in the study of Zimmerly and Weiss (2001). Fitzgerald et al. (2000) supplemented pastured cows with 0 or 20 mg/d of biotin and found no significant difference in milk yield between treatment herds. The average milk production in the present study herd was above average, and it can be suggested that with higher production, the demand for biotin, required for biosynthesis of glucose, fatty acids, and protein, is increased. In the three studies in which milk production was increased in response to supplemental biotin, the average milk yield was 32 kg/d or greater. But, in the Fitzgerald et al. (2000) experiment, using lower producing cows (20 kg/d), no significant effect of biotin on milk yield was found. It can be suggested that supplementation of essential nutrients such as biotin will elicit an animal response when the supply of the nutrient is limiting in the metabolism (e.g., enzyme activity or other physiological processes of the animal). At higher levels of milk production, the activities of one or more biotin-containing enzymes may become limited by biotin supply or synthesis. Biotin is also a required for certain rumen bacteria and specifically for propionic acid synthesis (Baldwin and Allison, 1983), and may limit rumen metabolic activity under some conditions. It has also been shown that biotin synthesis by rumen organisms in continuous culture is reduced by increased proportions of grain in the ration (Abel et al., 2001). In the present study, no blood or milk samples were analyzed for biotin. In other studies, there was a significant increase in serum biotin in supplemented animals compared with those that were unsupplemented (Fitzgerald et al., 2000; Hedges et al., 2001; Zimmerly and Weiss, 2001).

Another possible explanation for the higher milk production in biotin-supplemented cows, frequently put forward in promoting claw health, is that cows with healthy feet perform better due to better mobility and higher DMI. In recent studies, lameness significantly reduced milk production, and the decrease in milk production started even before the lameness was diagnosed (Warnick et al., 2001; Green et al., 2002). In another study, animals with healthy feet were more likely to walk and compete for feed, had longer eating times, and had higher DMI than lame herd mates in free stalls (Manson and Leaver, 1989) and on pasture (Hassall et al., 1993). Leonard et al. (1996) showed that overcrowding at the feeding area resulted in more claw lesions. Although it was not possible to estimate DMI from forage or pasture in the present study, the stall design of 2 × 2 rows gave enough feed bunk space. Also, the availability of concentrates at the four computer feeders and in the milking parlor probably minimized any risk for antagonistic competition for feed.

When comparing the trimmed and untrimmed animals during the study period independent of biotin treatment, there was no difference in milk production. It is therefore possible that the positive effect of biotin on production was a direct metabolic effect. In addition, it does it appear that the positive effect of biotin on fertility in first-calving heifers could be related to improved foot conditions. Such an effect of hoof health on milk yield would likely require the presence of more severe lesions and clinical lameness (Sprecher et al., 1997; Manske, 2002).

Reproduction. Biotin supplementation reduced days from calving to conception in some previous studies, although cow numbers were limited (Bonomi et al., 1996; Fitzgerald et al., 2000; Voigt et al., 2000). In the present study, reproduction was affected by an interaction between age and biotin supplementation. Heifers fed biotin had improved fertility, which was the result of marked extension of days to conception in the control animals of the same parity. As the days to first breeding were similar in the groups, the prolonged calving interval was a result of poor conception rate and an increased number of inseminations. The explanation for this is unclear but could be explained by weaker estrus symptoms, which are less likely to be detected by the herdsman, or reduced physiological response. Collick et al. (1989) and Sprecher et al. (1997) observed a significant influence of lameness on reproduction in a dairy herd with severe lameness problems that were accelerated since claw trimming and treatment of lameness were completely neglected.

Higher capability for milk production is a risk factor for nutritional imbalance and declined fertility, reportedly seen as reduced first service pregnancy rate and increased number of services per pregnancy (Oltenacu et al., 1991; Butler, 2000). This did not seem to be the case in the present study since the first-calving cows fed biotin produced more milk than the controls and had a higher conception rate. Increased gluconeogenesis may lead to increased reproductive performance since energy is also a limiting factor in the estrous cycle. This may have been a factor of concern in the current study.
CONCLUSIONS

In the present dairy, with a rolling herd average of approximately 32 kg/d of energy-corrected milk, biotin supplementation of 20 mg/d per cow during lactation resulted in increased milk production and fat yield in all parities, as well as improved fertility in first-calf heifers. The prevalence of subclinical claw lesions was higher at the end of the study in the control group than in biotin-supplemented cows. Most likely, the effect of biotin on milk production was a direct effect on metabolism rather than an indirect effect of improved claw health since the claw lesions observed did not cause lameness and since the results were not dependent on trimming. Furthermore, despite the greater increase in milk production in the biotin-supplemented group, their claws were less affected than those of the unsupplemented group. The latter finding indicates a dual effect of biotin on performance and claw health.

This study provided a unique opportunity to study the effect of biotin supplementation in a commercial herd in which management, environment, and feeding were identical. The controlled design lent support to the results under the present conditions but might have limitations in comparison with other herds under different management.

ACKNOWLEDGMENTS

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