

## Influence of Transition Diets on Occurrence of Subclinical Laminitis in Holstein Dairy Cows\*

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### ABSTRACT

Pre- and postpartum diets varying in energy and fiber were studied for effects on subclinical laminitis in Holstein cows. Also, ruminal acidosis was examined relative to sole hemorrhages and ulcers. Cows ( $n = 98$ ) were assigned to a  $2 \times 2$  factorial arrangement of treatments in a randomized complete block. Diets high in net energy of lactation ( $NE_L$ ) and low fiber were classified as high  $NE_L$ , whereas low  $NE_L$  and higher fiber were defined as low  $NE_L$ . Two diets were fed for 3 wk before calving and 2 others fed for 3 wk postpartum resulting in 4 treatment combinations: high  $NE_L$ , low  $NE_L$ ; high  $NE_L$ , high  $NE_L$ ; low  $NE_L$ , low  $NE_L$ ; and low  $NE_L$ , high  $NE_L$ . Levels of  $NE_L$  (Mcal/kg DM), percentage of acid detergent fiber, and percentage of neutral detergent fiber for low  $NE_L$  vs. high  $NE_L$  prepartum diets, were 1.51, 30.2, 47.2 vs. 1.65, 23.4, 39.8, respectively, and 1.70, 22.4, 36.8 vs. 1.77, 17.5, 31.4 for low  $NE_L$  vs. high  $NE_L$  lactating diets, respectively. A single diet was fed after 21 d in milk (DIM). Measures of hoof discoloration, hemorrhage, and/or ulcer formation were done at about 45 d before calving, and near 28 and 70 DIM. Rumenocentesis was performed 14 d before calving and at 8, 22, and 70 DIM. Hoof scores among treatments were similar at 28 DIM. The low  $NE_L$ , high  $NE_L$  group had less desirable hoof scores than high  $NE_L$ , high  $NE_L$ , or low  $NE_L$ , low  $NE_L$  groups from 55 to 75 DIM. Rumen pH did not differ by treatment before calving. The lowest postpartum rumen pH was significantly lower and rates of ruminal acidosis ( $pH \leq 5.8$ ) at 8 and 22 DIM were higher for groups fed high  $NE_L$  after calving. No direct relationship between low postpartum rumen pH and hoof scores on individual cows was found. Low  $NE_L$  before calving and high  $NE_L$  right after calving may

increase risk of subclinical laminitis if not carefully managed.

(**Key words:** subclinical laminitis, dairy cow, transition diets)

**Abbreviation key:** **HS0**, **HS1**, **HS2** = hoof scoring at means of 45 d before (**HS0**) and 30 (**HS1**) or 78 d (**HS2**) after calving, **SL** = subclinical laminitis.

### INTRODUCTION

Healthy hooves are an important prerequisite for the welfare and productivity in dairy cows (Bergsten and Herlin, 1996). Lameness in dairy cows is a costly health problem for both the individual producer and the dairy industry. For the producer, lame cows contribute to monetary losses through treatment costs, decreased performance, increased culling and reduced cull value of lame animals (Hernandez, et al., 2001) and for the industry, lameness is an important welfare issue. Reported rates of lameness in dairy cattle, usually measured as an annual, lactational, or seasonal incidence, are extremely variable (Peterse, 1985) partially due to information being obtained from multiple sources including veterinary treatment records, farm manager survey, or direct measurement of lameness in small groups of cows. Nonetheless, within a specific geographical region, and between reports that measure occurrence of lameness by the same method, the overall problem appears to be increasing (Clarkson, et al., 1996). Lameness due to hoof lesions is one of the most common diseases reported in modern dairy production (Bergsten and Herlin, 1996). Many researchers have suggested a correlation between laminitis (*Pododermatitis aseptica diffusa*) and hoof lesions such as sole ulcers (*Pododermatitis circumscripta*), white line disease (*Pododermatitis zona alba*), and abscesses in the subsole (*Pododermatitis septica*) (Peterse, 1982; Bradley, et al., 1989; Greenough and Vermont, 1991). In the Netherlands, several forms of laminitis are considered to be responsible for the majority of lameness problems in dairy herds (Feenstra, et al., 1983).

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Laminitis in the bovine has been well described (Nilsson, 1963; Nocek, 1997). In particular, the acute form has often been associated with nutritional imbalances, both because a reproducible alimentary model has been developed in the equine (Garner, 1975) and because laminitis is most common in postpartum dairy cows when nutritional and metabolic diseases are common in the cow (Nilsson, 1963). Dietary rumen soluble carbohydrate, starch, or protein in excess of that which is recommended in diets of cattle has been correlated with laminitis (Moser and Divers, 1987; Manson and Leaver, 1988a, 1988b). The subclinical form of laminitis (SL) is less well described, and certainly less understood. Cattle show no outward signs of disease, but experience a low-grade recurrence of laminitic events that impair hoof horn growth and quality. Moderate ecchymotic to severe hemorrhages on the cleaned sole of cattle is considered to be an indicator of SL (Nilsson, 1963; Peterse, 1979; Greenough and Vermunt, 1991). Multiple studies have reported SL in periparturient, confinement-housed dairy cows (Peterse, 1982; Bergsten, 1994; Smilie, et al., 1999), as well as in replacement dairy heifers (Bradley, et al., 1989; Vermunt and Greenough, 1996).

Both acute and subclinical forms of laminitis are thought to have environmental and nutritional components. Some controlled experiments have found a correlation between periparturient nutrition and SL in first lactation cows (Peterse, 1982; Livesey and Fleming, 1984; Greenough and Vermunt, 1991), whereas other reports did not observe a dietary effect (Bergsten and Frank, 1996; Olsson, et al., 1996). The most prevalent theory postulated for the nutritional pathogenesis of laminitis involves rumen acidosis causing release of vasoactive substances as a result of bacteriolysis and rumen epithelial irritation (Suber et al., 1979; Manson and Leaver, 1988a; Nocek, 1997). Histamine and endotoxin are thought to be the main vasoactive substances that are absorbed and cause vascular changes within the dermal capillary beds of the corium. These vascular changes cause pooling of blood in the corium that leads to ischemia, inflammation, and necrosis of the corium-epidermal junction. Ultimately these changes lead to hemorrhage and impaired function of keratin producing cells resulting in sole hemorrhages, and discoloration and reduced quality of horn in the sole. Controversy still exists whether sole hemorrhages are primary manifestations of a nutritional upset in the gastrointestinal tract or just bruising due to abnormal concussion on the hoof sole (Murray, et al., 1993).

Studies that examine nutritional influences on SL in the periparturient dairy cow have not been conducted in the United States, particularly under the subtropical conditions of the southeastern region using feedstuffs typically found there. The objectives of this research

were to characterize the response of the sole of each claw of dairy cows that were fed pre- and postpartum diets that varied in the levels of rumen-soluble carbohydrate, total energy, and fiber. A secondary objective was to monitor the level of subacute ruminal acidosis in cows fed these periparturient diets to determine whether a correlation between ruminal acidosis and hemorrhages in the sole could be documented.

## MATERIALS AND METHODS

### Animals and Experimental Diets

Transition Holstein dairy cows from the University of Florida Dairy Research Unit were assigned to dietary treatments in a continuous randomized complete block design with a  $2 \times 2$  factorial arrangement of treatments. Sample size calculations based on expected 20% difference in hoof scores between dietary treatments indicated that 24 cows per treatment would be needed. Expecting some attrition from the study, 108 primiparous and pluriparous cows were assigned over a 5-mo period (August to December). Treatments were blocked based upon expected calving date and comprised of 2 prepartum transition diets and 2 postpartum diets (Table 1). Cows were randomly assigned to 1 of 2 prepartum diets 21 to 28 d before expected parturition. After calving, cows were again randomly assigned one of two fresh cow diets that were fed until 21 to 24 DIM. Cows were removed from the trial if they calved less than 7 d after being assigned to a prepartum diet.

Experimental diets were formulated to represent diets fed to cows on commercial dairy farms in Florida. The overall goal in diet formulation was to provide 2 different pre- and postpartum diets that, in combination, might produce a measurable, observable difference in incidence of subacute ruminal acidosis and subsequent subclinical laminitis. Levels of energy and fiber that were far above or below NRC recommendations were not used in order to mimic diets actually fed to dairy cows in the Southeastern United States and to avoid occurrence of serious secondary digestive and metabolic diseases such as acute acidosis, acute laminitis, hepatic lipidosis, and displacement of the abomasum (Elam, 1976; Correa et al., 1990).

In each of the 2 production periods, dry and fresh cow, 2 TMR were formulated comparing those with high  $NE_L$  accompanied with low fiber to diets with moderate  $NE_L$  and high fiber (Tables 1 and 2). Designation of high and moderate levels of  $NE_L$  was based upon commonly accepted requirements for high-producing dairy cows (NRC, 1989; van Saun, 1991). High energy, low fiber diets were designated high  $NE_L$  and moderate energy, high fiber diets were called low  $NE_L$ . Cottonseed hulls were used to balance fiber values where needed. Prepar-

**Table 1.** Summary of target and actual nutrient composition of pre- and postpartum diets fed to study cows based upon analysis of individual ration ingredients.<sup>1</sup>

|                            | Low NE <sub>L</sub> <sup>2</sup> |              | High NE <sub>L</sub> <sup>2</sup> |              | Neutral <sup>4</sup> |
|----------------------------|----------------------------------|--------------|-----------------------------------|--------------|----------------------|
|                            | Target <sup>3</sup>              | Actual ± SEM | Target                            | Actual ± SEM |                      |
| Prepartum diets            |                                  |              |                                   |              |                      |
| NDF %                      | 45.2                             | 47.2 ± 0.8   | 39.8                              | 39.8 ± 0.7   |                      |
| ADF %                      | 30.0                             | 30.2 ± 0.3   | 24.0                              | 23.4 ± 0.5   |                      |
| NE <sub>L</sub> (Mcal/kg)  | 1.52                             | 1.51 ± 0.01  | 1.64                              | 1.65 ± 0.01  |                      |
| CP %                       | 15.5                             | 15.3 ± 0.0   | 15.5                              | 15.4 ± 0.0   |                      |
| UIP % (% CP)               | 36.0                             | 34.9 ± 0.1   | 36.0                              | 37.0 ± 0.05  |                      |
| DCAD (Meq/kg) <sup>5</sup> | -100                             | -126 ± 14    | -100                              | -122 ± 10    |                      |
| NDF %                      | 36.3                             | 36.8 ± 0.5   | 30.4                              | 31.4 ± 0.4   | 34.2                 |
| ADF %                      | 22.6                             | 22.4 ± 0.4   | 17.6                              | 17.5 ± 0.2   | 20.6                 |
| NE <sub>L</sub> (Mcal/kg)  | 1.71                             | 1.70 ± 0.02  | 1.78                              | 1.77 ± 0.01  | 1.73                 |
| CP %                       | 17.4                             | 17.3 ± 0.1   | 17.4                              | 17.4 ± 0.1   | 17.3                 |
| UIP % (% CP)               | 40                               | 39.1 ± 0.7   | 40                                | 41.0 ± 0.4   | 40.9                 |

<sup>1</sup>All nutrients reported on dry matter basis.

<sup>2</sup>Diet containing low energy and high fiber (low NE<sub>L</sub>) relative to diet with high energy and low fiber (high NE<sub>L</sub>).

<sup>3</sup>Target nutrient composition set by authors.

<sup>4</sup>Neutral postpartum diet fed during the first 3 to 5 d postpartum and subsequent to experimental diets (after 21 DIM).

<sup>5</sup>Dietary cation-anion difference of prepartum diets.

tum diets were formulated to maintain a dietary cation-anion difference of -10 to -15 mEq/100 g of DM using anionic salts and the formula [(Na + K) - (Cl + S)] (Horst et al., 1997). Fresh cow diets were balanced for energy and fiber levels to be higher or lower than the standard herd diet fed to the remainder of the cows in the herd. The standard herd diet was also fed to all experimental cows for the first 3 to 5 d postpartum. Diets within production periods (dry and lactating) were formulated to be identical in crude protein, rumen-degradable protein and minerals.

### Animal Housing, Management, and Feeding

At assignment, pregnant cows were moved from a large pasture to one of two 1-ha grass paddocks with a minimum of 0.6 m of feedbunk per cow during the most crowded periods. The prepartum paddocks were made by dividing a 2-ha paddock into 2 equal segments. All attempts were made to provide identical environment for cows in the 2 treatment groups. A TMR was provided ad libitum once a day at approximately 0900 h and orts collected just before this feeding; fresh water was

**Table 2.** Ingredient composition of experimental diets as percentage of dietary DM.

|                                | Prepartum diets     |                      | Postpartum diets    |                      |         |
|--------------------------------|---------------------|----------------------|---------------------|----------------------|---------|
|                                | Low NE <sub>L</sub> | High NE <sub>L</sub> | Low NE <sub>L</sub> | High NE <sub>L</sub> | Neutral |
| Corn silage                    | 39.0                | 35.2                 | 29.4                | 27.3                 | 27.8    |
| Alfalfa hay                    | —                   | —                    | 9.4                 | 8.1                  | 10.9    |
| Peanut hay                     | 16.1                | 14.1                 | —                   | —                    | —       |
| Hominy                         | 3.3                 | 20.1                 | 18.7                | 34.5                 | 20.9    |
| Distillers grain               | 8.2                 | 7.6                  | 4.9                 | 5.1                  | 5.4     |
| Soybean meal                   | 9.0                 | 7.4                  | 5.9                 | 8.2                  | 8.3     |
| Cottonseeds                    | 6.6                 | 7.6                  | 11.1                | 8.9                  | 13.0    |
| Cottonseed hulls               | 10.7                | 1.4                  | 1.0                 | 0.5                  | 6.4     |
| Prolak <sup>1</sup>            | —                   | —                    | 1.6                 | 1.9                  | 2.2     |
| Springer mineral <sup>2</sup>  | 7.0                 | 6.8                  | —                   | —                    | —       |
| Lact. Mineral mix <sup>3</sup> | 0.02                | 0.02                 | 4.3                 | 4.6                  | 4.6     |
| Trace min. salt                | 0.01                | 0.01                 | —                   | —                    | —       |
| Limestone                      | —                   | —                    | 0.6                 | 1.1                  | 0.7     |

<sup>1</sup>Protein supplement (H.J. Baker & Bro., Inc., Atlanta, GA); nutrient composition (DM basis) NDF 0%, ADF 0%, NE<sub>L</sub> 1.67 Mcal/kg, CP 66.7% UIP 60.0%.

<sup>2</sup>Anionic salt mixture for prevention of periparturient hypocalcemia.

<sup>3</sup>Lactating cow mineral package.

available all times. Cows were permitted to calve in the paddock and then removed to a hospital pen for 3 to 5 d.

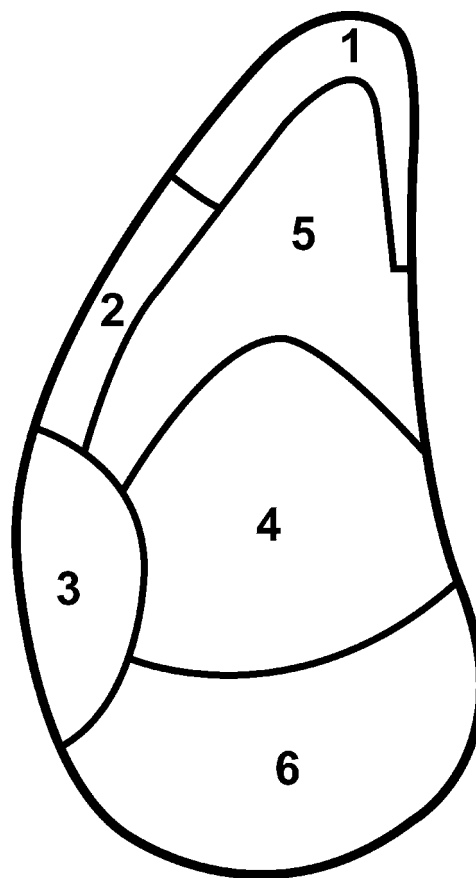
Lactating cow groups were housed on either side of an open-sided, open-ridge free-stall barn, with an east-west orientation. The east-west orientation minimizes variability in sun exposure between the two groups. Free stalls were bedded with sand and cleaned daily. Alleyways were flushed twice a day with recycled wastewater. Lactating cows were fed TMR once per day and orts were removed immediately before each morning feeding. Cows were fed their respective experimental lactating diets for approximately 21 d after which they were commingled with similar age herd cows and fed a neutral 'herd' diet for the remainder of the study. Corn silage DM was evaluated once a week and feeding levels were adjusted accordingly. With the exception of the mineral supplements, all feedstuffs were analyzed by wet chemistry monthly by commercial laboratory (Dairy One, Ithaca, NY). Diets were rebalanced based upon these analyses as necessary to stay within target ranges for crude protein,  $NE_L$ , ADF, and NDF.

Cows were milked 3 times per day at 0600, 1400, and 2200 h. Both postpartum treatment groups were exposed to the same grooved concrete walking surfaces to and from the milking parlor. Alleyways in holding pens were cleaned by flush system 3× daily. Springer cows were housed on pasture other than during periods of sample collection, when they walked up a lane to a stanchion barn.

### Hoof and Locomotion Scores

Before assignment of cows, the feet of all study cows were trimmed by a professional trimmer, thus, all cows had somewhat similar length and shape of hooves at the beginning of the study. Cows were selected for trimming and initial hoof scoring (**HS0**) based on being 4 to 8 wk before expected calving, and they did not exhibit signs of lameness; mean actual time at **HS0** was 45 d before calving. The soles of all 8 claws per cow were scored for hemorrhages and sole ulcers using an evaluation system described by Greenough and Vermunt (1991). The soles were divided into 6 zones (Figure 1) and after 1 to 2 mm of horn from area of the sole was removed, any observed hemorrhages were recorded using a 5-point scale (0 = no hemorrhages or discoloration, 1 = slight discoloration or yellow staining, 2 = moderate ecchymotic hemorrhages, 3 = severe hemorrhages or secondary horn disintegration, and 4 = exposed corium / sole ulcer). In addition, any other hoof or soft tissue abnormalities were noted and treated as needed.

Subsequent hoof scoring was performed by the same professional hoof trimmer when trial cows finished the experimental lactating diet at 3 to 5 wk postpartum



### Key for Zones

**Zone 1. White zone [line] at the toe**

**Zone 2. Abaxial white zone [line]**

**Zone 3. Abaxial wall-bulb junction**

**Zone 4. Sole-bulb junction**

**Zone 5. Apex of the sole**

**Zone 6. Bulb [of heel]**

**Figure 1.** Zones of the sole as recommended by the VIth Symposium on Diseases of the Ruminant Digit, Liverpool, 1990 (Greenough and Vermunt, 1991).

(**HS1**), and again after having been housed and fed with the herd cows at 10 to 12 wk postpartum (**HS2**). The hoof trimmer was unaware of dietary treatment and

**Table 3.** Locomotion scoring system.<sup>1</sup>

| Score | Description  |
|-------|--|
| 1.0   | Minimal abduction/adduction, no unevenness of gait, no tenderness. SOUND.      |
| 2.0   | Abduction/adduction present, uneven gait, perhaps tender.                      |
| 3.0   | Slight lameness, not affecting behavior.                                       |
| 4.0   | Obvious lameness, difficulty in turning, behavior patterns affected.           |
| 5.0   | Extreme difficulty in rising and walking, adverse affects on behavior pattern. |

<sup>1</sup>From Manson and Leaver, 1988a.

no attempt was made to trim overgrown hooves. Mean DIM at HS1 and HS2 were 30 and 78, respectively.

Clinical lameness was evaluated using the locomotion scoring system described by Manson and Leaver (1988a; Table 3). Ten locomotion scores were collected on each cow at d -45, -24, -14, -8, 1, 4, 7, 11, 14, 21, 28, 70, and 78 relative to calving. One observer with knowledge of treatment groups evaluated and scored lameness by observing each cow walk on smooth concrete through a wide fenced lane. Bias was controlled in 2 ways. Cows from the 2 prepartum and 2 postpartum treatment groups were commingled before the evaluation. The observer did not acknowledge cow identification during locomotion scoring and the locomotion score was recorded by a study coinvestigator. Lameness was not evaluated until any lying, resting cow had risen and walked adequate distance to work out any residual stiffness from lying down.

### Rumen pH Evaluation

Rumen fluid was collected for pH determination 5 to 7 h after the morning feeding using the rumenocentesis technique described by Nordlund and Garrett (1994). Briefly, the cow was restrained and a 20-cm<sup>2</sup> area caudal to the last rib in the lower left flank was shaved and surgically scrubbed. Local analgesia was obtained using 5 ml of 2% lidocaine injected in the subcutaneous and muscular tissues. Rumen fluid was collected from the caudal sac of the rumen using a 16-gauge, 10-cm needle and 12-mL syringe. Rumen pH was determined immediately using a hand-held portable pH meter (Micro pHep3 waterproof pH tester; Hanna Instruments, Woonsocket, RI). Rumen fluid collections occurred at 14 d prepartum and at 8, 22, and 70 d postpartum. The prepartum rumen sample was not collected if the cow was determined to be pregnant in the left uterine horn.

### Statistical Analyses

Statistical analyses of continuous dependent variables were performed using the general linear model procedures of SAS (SAS, 1996). These dependent variables included hoof scores, locomotion scores and rumen

pH. Within the analyses for hoof scores, initial score (HS0) was treated as a covariable.

The discrete independent variables in all analyses were treatment, parity group (1 = parities 1 and 2; or 2 = parities  $\geq$  3), season dry, season lactating, and cow. Analysis of environmental data (Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL) indicated that cool weather climate (winter) started on October 5. Days in milk was considered a continuous, independent variable throughout the analyses, using second and third degree polynomials where indicated. Cow within treatment group was used as an error term. For analysis of hoof scores, claw was also treated as an independent variable. Treatment  $\times$  DIM interaction was evaluated by using tests for heterogeneity of regression (Snedecor and Cochran, 1980).

Initial analysis of hoof score data was performed using the following hoof score dependent variables: individual zones (1 through 6), combinations of zones (e.g., zones 3 and 4), the summation of all zones per claw (claw score), and the summation of all claw scores from each cow (cow score). In the analysis of rumen pH, 2 separate models were developed, one using pH as a continuous variable and the second using dichotomized pH data, pH  $\leq$  5.8, indicating ruminal acidosis (Nordlund et al., 1995).

Final models were determined from the preliminary model by starting with all applicable independent variables and systematically eliminating independent variables with an *F*-statistic value of less than 1.0. Overall least squares means for all dependent variables were estimated. Least squares means of dependent variables were calculated at predetermined levels of DIM. Final comparison between treatments at specific levels of DIM was determined by comparing 95% confidence intervals. Significance was determined at *P* < 0.05 unless otherwise stated.

## RESULTS

### Animal Characteristics

Ninety-eight cows were assigned and successfully completed the experimental treatments and follow-up through 11 wk postpartum. An additional 10 cows were

**Table 4.** Descriptive data and least squares means (LSM) and SEM of selected variables by dietary treatment of dairy cows assigned to a study to determine the association between transition diets and occurrence of subclinical laminitis.

|                                    | Dietary treatment <sup>1</sup>                |  |  |   |
|------------------------------------|---|--|--|---|
|                                    | High NE <sub>L</sub> ,<br>low NE <sub>L</sub> | High NE <sub>L</sub> ,<br>high NE <sub>L</sub> | Low NE <sub>L</sub> ,<br>low NE <sub>L</sub> | Low NE <sub>L</sub> ,<br>high NE <sub>L</sub> |
| Cows completing study <sup>2</sup> | 25  | 23   | 25   | 25  |
| Cows removed <sup>3</sup>          | 2   | 4  | 1  | 3   |
| Parity                             | 2.6 (0.2) <sup>a</sup>                        | 2.6 (0.3) <sup>a</sup>                         | 3.1 (0.2) <sup>a</sup>                       | 2.7 (0.2) <sup>a</sup>                        |
| Days on diet <sup>4</sup>          |   |  |  |   |
| Prepartum                          | 24.9 (1.1) <sup>a</sup>                       | 25.8 (1.2) <sup>a</sup>                        | 22.3 (1.3) <sup>a</sup>                      | 24.0 (1.2) <sup>a</sup>                       |
| Immediately postpartum             | 5.5 (0.7) <sup>a</sup>                        | 5.6 (0.7) <sup>a</sup>                         | 4.2 (0.4) <sup>a</sup>                       | 5.6 (0.7) <sup>a</sup>                        |
| Postpartum                         | 16.9 (0.4) <sup>a</sup>                       | 15.9 (0.6) <sup>a</sup>                        | 17.7 (0.3) <sup>a</sup>                      | 16.3 (0.5) <sup>a</sup>                       |
| Rumen fluid pH <sup>5</sup>        |   |  |  |   |
| Day -14                            | 6.25 (0.09) <sup>a</sup>                      | 6.04 (0.15) <sup>a</sup>                       | 6.18 (0.09) <sup>a</sup>                     | 6.23 (0.12) <sup>a</sup>                      |
| Day 8                              | 6.00 (0.06) <sup>a</sup>                      | 5.82 (0.10) <sup>a</sup>                       | 6.07 (0.12) <sup>a</sup>                     | 5.93 (0.14) <sup>a</sup>                      |
| Day 22                             | 5.92 (0.10) <sup>a</sup>                      | 5.79 (0.09) <sup>a</sup>                       | 5.84 (0.06) <sup>a</sup>                     | 5.73 (0.09) <sup>a</sup>                      |
| Lowest postpartum <sup>6</sup>     | 5.84 (0.07) <sup>b</sup>                      | 5.59 (0.07) <sup>c</sup>                       | 5.78 (0.07) <sup>b</sup>                     | 5.64 (0.08) <sup>c</sup>                      |
| Incidence DD <sup>7</sup>          | 36% <sup>a</sup>                              | 39% <sup>a</sup>                               | 48% <sup>a</sup>                             | 48% <sup>a</sup>                              |

<sup>a</sup>Values within rows are not significantly different ( $P > 0.05$ ).

<sup>b,c</sup>Values within rows with different superscripts are different ( $P < 0.01$ ).

<sup>d,e</sup>Values within rows with different superscripts are different ( $P < 0.05$ ).

<sup>1</sup>Diet containing low energy and high fiber (Low NE<sub>L</sub>) relative to diet with high energy and low fiber (High NE<sub>L</sub>); first term in dietary treatment describes the prepartum diet and the second describes postpartum dietary treatment.

<sup>2</sup>Cows completing study from -60 to 77 d relative to calving.

<sup>3</sup>Cows removed from study for reasons other than lameness.

<sup>4</sup>LSM of days fed prepartum and postpartum experimental diets and days fed neutral lactation diet immediately after parturition.

<sup>5</sup>LSM of rumen fluid pH collected at d -14, 8, and 22 relative to parturition.

<sup>6</sup>Lowest rumen fluid pH among postpartum samples taken at d 8, 22, and 70.

<sup>7</sup>DD = Incidence of digital dermatitis (foot warts) as proportion of cows affected anytime during the study.

removed from the study for reasons other than lameness. Descriptive data are presented in Table 4. The majority of cows (55%) went through the trial during the winter-winter season; the first season refers to when the cow was fed the prepartum diet, and the second, when the cow was fed the experimental lactating diet. Of the remaining cows, 26 and 19% went through the trial during summer-summer and summer-winter, respectively.

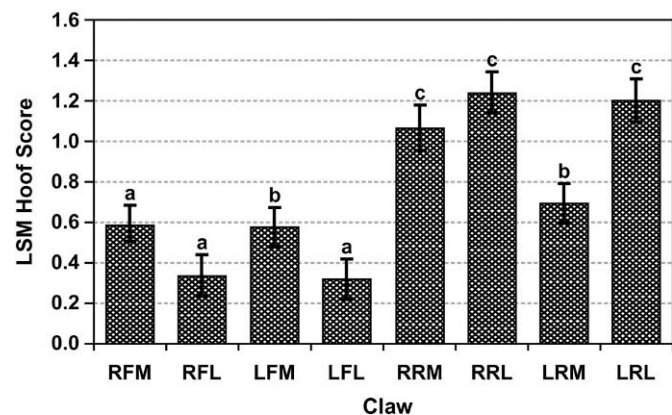
The mean number of days on prepartum and lactating diets, and the period of time spent in the hospital pen immediately after calving were similar among treatment groups ( $P > 0.10$ ). Diets varied minimally during the trial; however, the overall goal of having them different within each time period (prepartum and postpartum) was met and they remained so throughout the study (Table 1).

## Hoof Scores

Two distinct patterns of hoof scores by individual claw were seen (Figure 2). The highest hoof scores were seen in the rear claws. In the forelimbs, the medial claws were significantly higher than the lateral claws.

In the rear limbs, the lateral and right rear medial claws were higher than the remaining claw.

In the analysis of claw scores (sum of all zone scores per claw) and cow scores (sum of all zone scores per



**Figure 2.** Least-squares means (LSM  $\pm$  SEM) of sum of hoof scores at zones 3 and 4 by claw. The first term denotes left (L) or right (R), the second term denotes front (F) or rear (R), and third term denotes lateral (L) or medial (M). <sup>a,b,c</sup> = LSM with different letters are significantly different ( $P < 0.05$ ).

**Table 5.** Summary ANOVA tables for models of hoof scores at zones 3 and 4.

| Variable                            | Model and $P \leq$ |            |           |
|-------------------------------------|--------------------|------------|-----------|
|                                     | All 4 limbs        | Hind limbs | Forelimbs |
| Treatment <sup>1</sup>              | 0.05               | 0.05       | 0.11      |
| Parity group <sup>2</sup>           | 0.08               | 0.08       | NS        |
| Treatment $\times$ parity           | NS <sup>5</sup>    | NS         | NS        |
| Cow (Treatment $\times$ parity)     | 0.001              | 0.05       | 0.001     |
| Season dry <sup>3</sup>             | 0.06               | 0.05       | NS        |
| Claw                                | 0.001              | 0.01       | 0.01      |
| HS0 for zones 3,4 <sup>4</sup>      | 0.01               | 0.05       | 0.05      |
| HS0 for zones 3,4 $\times$ Claw     | 0.01               | 0.05       | NS        |
| DIM                                 | 0.001              | 0.001      | 0.09      |
| DIM $\times$ DIM                    | 0.001              | 0.001      | NS        |
| DIM $\times$ Treatment              | 0.001              | 0.001      | 0.01      |
| DIM $\times$ DIM $\times$ Treatment | 0.001              | 0.001      | 0.05      |
| R <sup>2</sup>                      | 0.34               | 0.44       | 0.33      |

<sup>1</sup>Dietary treatment; diet containing low energy and high fiber (low NE<sub>L</sub>) relative to diet with high energy and low fiber (high NE<sub>L</sub>); first term in dietary treatment describes the prepartum diet and the second describes postpartum dietary treatment.

<sup>2</sup>Parity group: 1 = parity 1 and 2; 2 = parity  $\geq$  3.

<sup>3</sup>Season while on experimental prepartum diet; summer = 1 August to 5 October, winter = 6 October to 31 December.

<sup>4</sup>Sum of initial hoof score at zone 3 and zone 4 (before feeding dietary treatments).

<sup>5</sup>NS = not significant,  $P > 0.10$ .

cow), treatment was not significant either as a simple effect or as an interaction with DIM. In each analysis, DIM was significant to the second order and the effect of claw was highly significant ( $P < 0.01$ ). Analysis of individual hoof scores from 6 zones per claw, and 8 claws per cow did not show any significant effect of treatment. Zones 3 and 4 produced very similar models and suggested an association between dietary treatment and hoof scores. The sum of the numerical hoof scores of these 2 zones per claw resulted in a model with the lowest random error and the highest value for the coefficient of determination ( $R^2$ ), therefore the majority of the discussion on the hoof-score data will use the combination of hoof scores from zones 3 and 4.

The summary of models describing lesions at zones 3 and 4 is presented in Table 5. Independent variables that were significant included treatment, parity, individual cow nested within the interaction of treatment and parity, claw, prepartum diet fed in winter, initial hoof score (HS0) at zones 3 and 4, and DIM. Variables tested in the preliminary models but not significant were days on feed for either experimental diet, days in the hospital pen, presence of digital dermatitis, and lactating diet fed in winter.

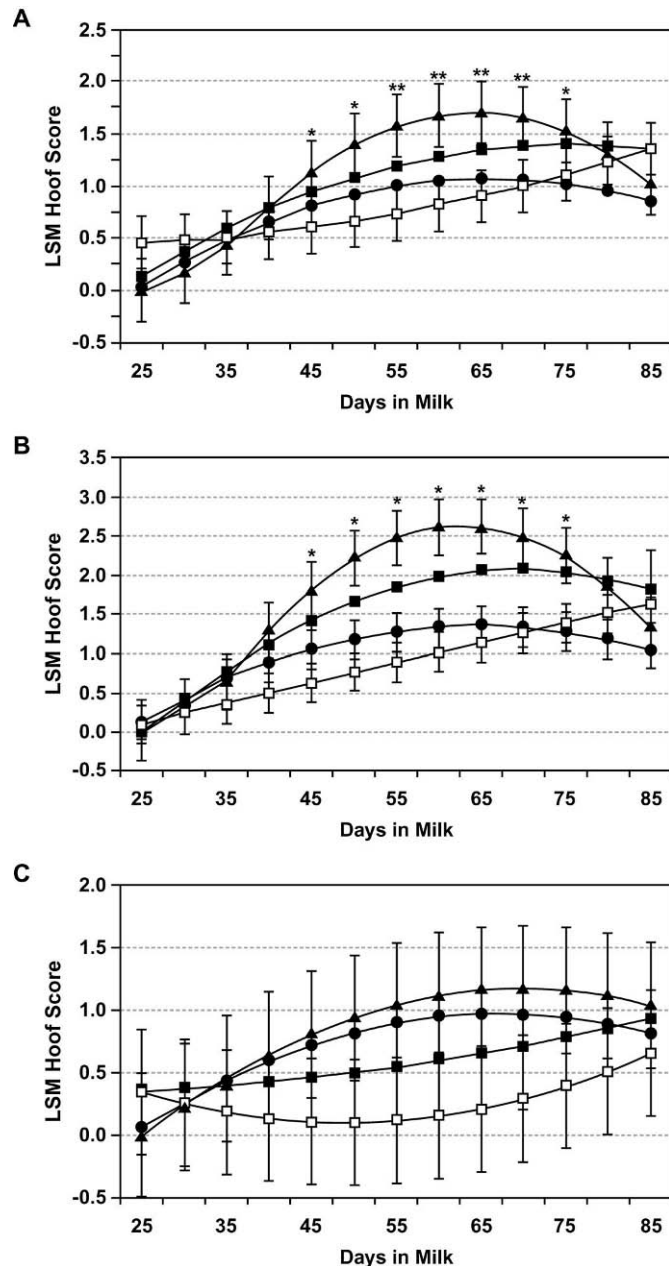
A significant interaction between treatment and DIM was observed when using data from either all 4 limbs or the hind limbs only. Claw, HS0 for zones 3 and 4, and the interaction of HS0 and claw were also significant. Season on the prepartum diet and parity group both showed trends towards significance ( $P = 0.06$  and  $0.08$ ,

respectively). Figure 3 represents the least squares means of zones 3 and 4 for each treatment at specific DIM values for each of the three statistical models. At 25 DIM, hoof scores were all very low and not significantly different. As DIM increased, hoof scores for all treatments increased. In the all-limb model (Figure 3a) hoof scores for treatment low NE<sub>L</sub>, high NE<sub>L</sub> rose significantly faster than treatments low NE<sub>L</sub>, low NE<sub>L</sub> and high NE<sub>L</sub>, high NE<sub>L</sub>, to peak at 65 DIM. Hoof scores in the remaining treatment group, high NE<sub>L</sub>, low NE<sub>L</sub>, were intermediate and not different at any point of DIM from the other 3 dietary treatments. By 85 DIM, hoof scores for all treatments were similar again, but higher than at 25 DIM.

Figure 3b and c depicts the same interaction of treatment and DIM at designated values for DIM for the hind and fore limbs, respectively. In the hind limb model, the shape and similar pattern of significance of treatment low NE<sub>L</sub> – high NE<sub>L</sub> and higher R<sup>2</sup> indicates the strong influence the hind limb had on the overall hoof score data. In the forelimb model, all hoof scores increased; however, no significant difference between dietary treatments was observed.

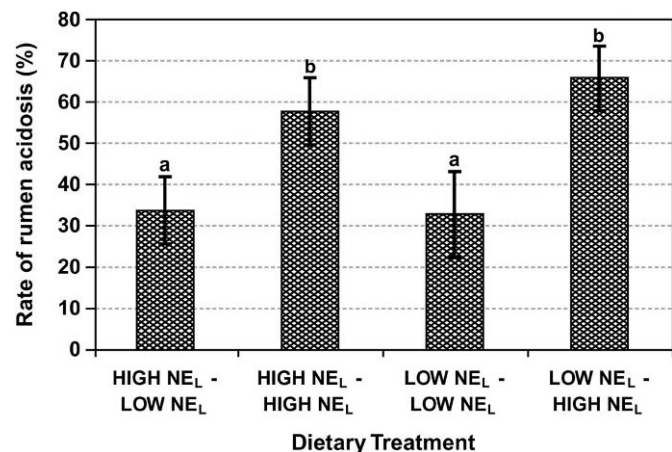
### Rumen Fluid pH

Individual cow rumen pH values varied from 4.9 to 7.8, and no cows showed signs of acute rumen acidosis. Dietary treatment and parity group were not significantly associated with rumen pH at any of the 4 sam-



**Figure 3 A.** Least squares means (LSM  $\pm$  SEM) of sum of hoof scores at zones 3 and 4 of the sole at predetermined days in milk intervals for all 4 limbs by pre- and postpartum dietary treatments<sup>1</sup>. Dietary treatments are low NE<sub>L</sub>, low NE<sub>L</sub> (□); low NE<sub>L</sub>, high NE<sub>L</sub> (▲); high NE<sub>L</sub>, low NE<sub>L</sub> (■); and high NE<sub>L</sub>, high NE<sub>L</sub> (●)<sup>1</sup>. Diet containing low energy and high fiber (low NE<sub>L</sub>) relative to diet with high energy and low fiber (high NE<sub>L</sub>); first term in dietary treatment describes the prepartum diet and the second describes postpartum dietary treatment. \*Low NE<sub>L</sub>, high NE<sub>L</sub> differs from low NE<sub>L</sub>, low NE<sub>L</sub> at specified days in milk ( $P < 0.05$ ). \*\*Low NE<sub>L</sub>, high NE<sub>L</sub> differs from low NE<sub>L</sub>, low NE<sub>L</sub> and high NE<sub>L</sub>, high NE<sub>L</sub> at specified days in milk ( $P < 0.05$ ). **B.** Least squares means (LSM  $\pm$  SEM) of hoof score at zones 3 and 4 of the sole for hind limbs only at predetermined DIM intervals by pre- and postpartum dietary treatments.<sup>1</sup> Dietary treatments were low NE<sub>L</sub>, low NE<sub>L</sub> (□); low NE<sub>L</sub>, high NE<sub>L</sub> (▲); high NE<sub>L</sub>, low NE<sub>L</sub> (■); and high NE<sub>L</sub>, high NE<sub>L</sub> (●)<sup>1</sup>. Diet containing low energy and high fiber (low NE<sub>L</sub>) relative to diet with high energy and low fiber (high NE<sub>L</sub>); first term in dietary treatment describes the prepartum diet and the second describes postpartum dietary treatment. Error bars for high NE<sub>L</sub>, low NE<sub>L</sub> treatment removed for clarity. \*LSM of low NE<sub>L</sub>, high NE<sub>L</sub> differs from high NE<sub>L</sub>, high NE<sub>L</sub> and low NE<sub>L</sub>, low NE<sub>L</sub> ( $P < 0.05$ ). **C.** Least squares means (LSM  $\pm$  SEM) estimates of hoof scores at zones 3 and 4 of the sole for fore limbs only at predetermined DIM intervals by pre- and postpartum dietary treatments. Dietary treatments were low NE<sub>L</sub>, low NE<sub>L</sub> (□); low NE<sub>L</sub>, high NE<sub>L</sub> (▲); high NE<sub>L</sub>, low NE<sub>L</sub> (■); and high NE<sub>L</sub>, high NE<sub>L</sub> (●)<sup>1</sup>. Error bars for high NE<sub>L</sub>, low NE<sub>L</sub> treatment removed for clarity. LSM of hoof scores do not differ among treatment at any days in milk interval ( $P > 0.10$ ). <sup>1</sup>Diet containing low energy and high fiber (low NE<sub>L</sub>) relative to diet with high energy and low fiber (high NE<sub>L</sub>); first term in dietary treatment describes the prepartum diet and the second describes postpartum dietary treatment.

pling periods; however, lowest postpartum ruminal pH was significantly associated with dietary treatment ( $P < 0.01$ ; Table 4). In the analysis using binomial pH data, the rate of ruminal acidosis at 8 and 22 DIM was approximately double ( $P < 0.01$ ) in cows fed postpartum high NE<sub>L</sub> diets compared with those fed postpartum low NE<sub>L</sub> diets (Figure 4). Using a more conservative cutoff of pH 5.5, the same pattern was seen with approximately 41 and 24% of cows receiving postpartum high NE<sub>L</sub> and low NE<sub>L</sub> diets, respectively, being classified as having ruminal acidosis.



**Figure 4.** Least squares means (LSM  $\pm$  SEM) of rate of ruminal acidosis (pH  $\leq$  5.8) for samples collected 8 to 22 DIM by dietary treatment. Diet containing low energy and high fiber (low NE<sub>L</sub>) relative to diet with high energy and low fiber (high NE<sub>L</sub>); first term in dietary treatment describes the prepartum diet and the second describes postpartum dietary treatment. <sup>a,b</sup>LSM with different letters are significantly different ( $P < 0.05$ ).



**Table 6.** Least squares means estimates of locomotion score by dietary treatment, parity group, and season dry.

| Dietary treatment <sup>1,NS</sup>             |  |  |   |
|---|--|--|---|
| High NE <sub>L</sub> ,<br>low NE <sub>L</sub> | High NE <sub>L</sub> ,<br>high NE <sub>L</sub> | Low NE <sub>L</sub> ,<br>low NE <sub>L</sub> | Low NE <sub>L</sub> ,<br>high NE <sub>L</sub> |
| 1.89 ± 0.07                                   | 2.01 ± 0.08                                    | 1.91 ± 0.10                                  | 1.82 ± 0.07                                   |
| Parity group <sup>†</sup>                     |  | Season dry <sup>3†</sup>                     |   |
| 1 and 2                                       | 3+   | Summer                                       | Winter  |
| 1.82 ± 0.05                                   | 1.99 ± 0.07                                    | 1.59 ± 0.20                                  | 2.23 ± 0.16                                   |

<sup>1</sup>Diet containing low energy and high fiber (low NE<sub>L</sub>) relative to diet with high energy and low fiber (high NE<sub>L</sub>); first term in dietary treatment describes the prepartum diet and the second describes postpartum dietary treatment.

<sup>2</sup>Parity group: 1 = parity 1 and 2; 2 = parity ≥ 3.

<sup>3</sup>Season while on experimental prepartum diet; summer = 1 August to 5 October, winter = 6 October to 31 December.

<sup>NS</sup>Not significant,  $P > 0.10$ .

<sup>†</sup> $P < 0.10$ .

## Rumen pH and Hoof Scores

A tendency ( $P = 0.09$ ) for an association between lowest postpartum rumen pH value and hoof scores at zones 3 and 4 was seen.

## Locomotion Scores

All locomotion scores were very low; no cows ever scored a 5.0 during the trial. The unadjusted mean locomotion score across all treatments was 1.97. In the ordinal scale of locomotion scores, cows were not lame until they score a 3.0 or greater (Manson and Leaver, 1988a). The effect of treatment was not significant (Table 6); however, a significant interaction between treatment and DIM was seen (Figure 5). Parity group and season on prepartum diet tended to be associated with locomotion scores ( $P < 0.10$ ). Although digital dermatitis was observed at least once in approximately 40% of the cows throughout the trial, the presence of digital dermatitis was not significant nor was it a confounder of the data.

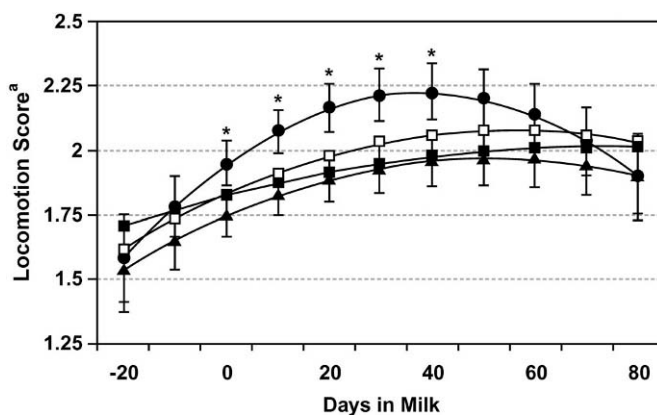
## DISCUSSION

### Hoof Score

The experimental diets used in this study were effective in inducing measurable laminitic changes in the hoof. Hoof scores in cows fed the diet combination requiring the most ruminal adaptation (low NE<sub>L</sub>, high NE<sub>L</sub>) were more than 1 score higher at peak lactation than scores in cows fed diets requiring less adjustment by rumen microflora (low NE<sub>L</sub>, low NE<sub>L</sub>). Using diets similar to those in the current study, Olsson et al. (1996) observed an increase in hoof scores postpartum, but no

specific influence of diet combinations was detected. Bergsten and Frank (1996a) found no influence of high and low concentrate diets on occurrence of SL. In another study, a significant increase in hemorrhages in the sole was reported in cows fed 6 kg/d concentrate prepartum compared with those fed 1 kg/d (Peterse, 1979).

From the current study, there may be a lag period of 30 to 60 d between the transition from dry cow to lactating cow diets and occurrence of hemorrhages in the hoof



**Figure 5.** Least squares means (LSM ± SEM) of locomotion scores for pre- and postpartum dietary treatments at predetermined days in milk intervals. Dietary treatments<sup>1</sup> were low NE<sub>L</sub>, low NE<sub>L</sub> (□); low NE<sub>L</sub>, high NE<sub>L</sub> (▲); high NE<sub>L</sub>, low NE<sub>L</sub> (■); and high NE<sub>L</sub>, high NE<sub>L</sub> (●). Error bars for low NE<sub>L</sub>, low NE<sub>L</sub> and high NE<sub>L</sub>, low NE<sub>L</sub> treatments removed for clarity. <sup>1</sup>Diet containing low energy and high fiber (low NE<sub>L</sub>) relative to diet with high energy and low fiber (high NE<sub>L</sub>); first term in dietary treatment describes the prepartum diet and the second describes postpartum dietary treatment. \*LSM locomotion score of high NE<sub>L</sub>, high NE<sub>L</sub> treatment differs from low NE<sub>L</sub>, high NE<sub>L</sub> ( $P < 0.05$ ); all other dietary treatments did not differ at any DIM interval ( $P > 0.05$ ).

of lactating dairy cows. Vermunt and Greenough (1996) suggested that hemorrhages in the sole reflect either a feeding change or trauma that occurred 6 to 8 wk before observation of the hemorrhage. Others have observed the highest incidence of hemorrhages in the sole at 2 to 4 mo post calving and suggested that the feeding change close to calving induced the subclinical laminitis (Peterse, 1982; Bergsten, 1994). Although these suggestions have been largely accepted, limited research on the relationship between periparturient dairy cow diets and hemorrhages of the sole exists.

Among researchers who have measured and analyzed hemorrhages of the sole, no one has focused specifically upon the combination of zones 3 and 4. Greenough and Vermunt (1991) and Bergsten and Herlin (1996) did combine the scores from zones 1 through 3 into the white line region, and labeled zones 4 and 5 as the ulcer zone, where sole ulcers were most likely to occur. Hemorrhages at the sole region were reportedly more severe than those in other zones of the sole (Greenough and Vermunt, 1991). In a later report, Vermunt and Greenough (1996) modified their diagram of the sole zones, so comparisons between studies are difficult. Frankena et al. (1992) measured hoof scores in female dairy calves using the 6 zones of the sole used in this study and found the prevalence of sole hemorrhages was highest (21.7%) at zone 3 irrespective of the number of affected feet, claws, and zones. The region of the sole that comprises primarily zone 4 has been identified as a common site for sole hemorrhages and ulcers (Peterse, 1982).

The pattern of hoof scores at zones 3 and 4, particularly for the low  $NE_L$ , high  $NE_L$  diet, can be explained by the occurrence of ruminal acidosis in early lactation. The low  $NE_L$ , high  $NE_L$  dietary treatment required the greatest adaptation to  $NE_L$  (from 1.51 to 1.77 Mcal/kg DM) and the postpartum diet in this treatment was marginally fiber deficient, with 17% ADF and 31% NDF. This increase in energy density, due to a higher proportion of starch and NFC in the postpartum diet, likely provided substrate for lactic acid producing bacteria. This leads to rapid decrease in rumen pH, death, and lysis of acid-sensitive rumen microbes with subsequent release of endotoxins and histamine. It is felt that these vasoactive compounds have a profound effect on the vasculature of the hoof that leads to laminitis (Nocck, 1997).

The significant increase in hoof scores at zones 3 and 4 for the hind limbs during the winter season may have been related to increased DMI during winter months, although no measurements were actually made in this trial. Heat stress has been shown to significantly decrease DMI (Collier et al., 1982). Conversely, higher feed intake, particularly when the diet is high in rapidly

degradable carbohydrates, can predispose cows to ruminal acidosis and subsequent SL (Slyter, 1976; Deluyker et al., 1991). If intakes were higher during cooler weather in prepartum cows, more incidences of ruminal acidosis may have occurred because of proportionally lower concentration of salivary buffer relative to rumen volume. Other reports linking SL to a seasonal pattern are confounded with other management changes which may also influence occurrence of laminitis, such as moving from pasture to complete diets, or housing on concrete after a summer on pasture (Peterse, 1982; Greenough and Vermunt, 1991; Bergsten, 1994).

Higher hoof scores seen in the lateral rear claws and medial claws of the forelegs were expected (Andersson and Lundström, 1981; Vermunt and Greenough, 1996); however, the results noted in the right rear medial claw were possibly an aberration. Likely, a small number of cows maintained a sole ulcer on that claw throughout the trial and thereby raising the average score for that claw.

The observed trend in this study for younger animals to have higher hoof scores has been widely observed by many researchers (Andersson and Lundström, 1981; Peterse, 1982; Greenough and Vermunt, 1991). Greenough and Vermunt (1991) specifically noted that hemorrhage of the sole in first lactation cows occurred more commonly at calving, whereas sole hemorrhages peaked in older cows at 2 to 4 mo postpartum. This disparity between age groups is likely due to multiple causes including changes in hoof health before first parturition, social and behavioral factors after parturition, and differential culling of older cows with previous or predisposing hoof pathology.

## Rumen pH

Although controversy continues, normal fiber digestion has been noted to decrease significantly when rumen pH values are below 6.0 (Slyter, 1976; Nocek, 1997). Nordlund and Garrett (1994) suggested that values below 5.8 are not ideal for lactating cows and are not normal. Others have used pH of less than 5.6 as the threshold for subacute rumen acidosis (Garrett et al., 1999; Keunen et al., 2002). Our results indicate that prepartum ruminal acidosis did not occur to any significant degree as values stayed within the range of 6.0 to 6.2 and only 4 of 45 prepartum samples had a pH less than 5.8.

In postpartum cows, rumen pH values were more variable. When lowest postpartum pH was evaluated, the two high  $NE_L$  postpartum diets produced significantly lower ruminal pH. The incidence of ruminal acidosis was also significantly higher in these two treatment groups. Nordlund et al. (1995) has suggested that

an appropriate approach to analyzing rumen pH values on a herd level is to evaluate the individual cow's rumen pH and classify the rumen as acidotic or not. This methodology prevents cows with high-normal rumen pH from masking an acidosis problem in other cows in the same group. Although defining rumen acidosis as pH  $\leq 5.8$  was a less-conservative definition than others have used (Garrett et al., 1999), such a dividing line clearly separated the diets into predictable groups based on proportions with rumen acidosis (Figure 4).

### Rumen pH and Hoof Scores

While there is evidence that our diets were effective at inducing ruminal acidosis and hoof scores reflect episodes of SL, no direct association between ruminal acidosis and SL could be found. These results are consistent with those of Brandejksy et al. (1994), who observed in Braunvieh cows a 50% incidence of mild to moderate ruminal acidosis but with no significant correlation between rumen pH values and hoof scores found.

Postpartum ruminal acidosis was observed at equal rates in both the low  $NE_L$ , high  $NE_L$  and high  $NE_L$ , high  $NE_L$  treatment groups, but the low  $NE_L$ , high  $NE_L$  treatment produced the highest rate of SL in the zone 3 and 4 regions of the sole. These results suggest that the lactating diet alone did not influence the occurrence of SL, but rather the transition diets, or the combination of prepartum and postpartum diets induced SL. The authors speculate that the rumen microflora of cows receiving the high  $NE_L$ , high  $NE_L$  diet combination had adapted better to the postpartum diet than that in the low  $NE_L$ , high  $NE_L$  treatment group. Perhaps more intensive rumen fluid sampling would have been more sensitive in identifying the magnitude and/or duration of ruminal acidosis in this study population (Keunen et al., 2002).

### Locomotion Scores

Locomotion scores for high  $NE_L$ , high  $NE_L$  treatment did differ statistically from low  $NE_L$ , high  $NE_L$  treatment in the 0 to 40 DIM interval, but this variation has little biological significance. The range in locomotion scores was 1.5 to 2.25. A locomotion score of 3.0 identifies a lame cow (Manson and Leaver, 1988b); any score less than 3.0 is thought to be more a variation on the cow's own pattern of walking, or lameness too subtle to affect behavior. Also, the increase in locomotion scores occurred at 0 to 40 DIM, while hoof scores increased at 65 to 70 DIM. No correlation of lameness scores to hoof scores was seen.

Overall, significant lameness was not observed among any diet treatments during the period of obser-

vation. This lack of significant lameness was not surprising because the goal of this study was to observe occurrence of SL. By definition, SL does not cause clinical lameness. If intensive observation of the cows had continued beyond 85 DIM, more clinical lameness might have been observed.

The authors acknowledge that there may be some potential for bias in this study because of lack of replicated pens or use of switchback design. Although attempts were made to make the environment and management within prepartum and postpartum dietary groups as similar as possible, unforeseen pen effects could be present. Therefore, results of this study should be interpreted with caution.

### CONCLUSIONS

With the exception of research by Peterse (1982), controlled studies demonstrating the influence of nutrition on SL have not been conducted. In this study, a range of diets commonly fed to dairy cows in Florida were used. While the rates of ruminal acidosis and subclinical laminitis were only moderate, a difference in dietary treatments was observed. However, a direct relationship between ruminal acidosis and SL was not found. Cows requiring the most adaptation between prepartum and postpartum diets, the low  $NE_L$ , high  $NE_L$  group, exhibited more hemorrhages and ulcers in the sole suggestive of SL. Other disease processes, including severe sole ulcers and abscesses below the sole were not apparently influenced by these periparturient dietary treatments or they occurred later in lactation, after the followup period of this study. The transition from prepartum diets to early lactation diets is challenging for all dairy cows. Large differences in fiber and net energy content between the closeup and early lactation diets challenges the adaptation of the rumen microbes and the rumen epithelium and can increase incidences of ruminal acidosis and SL.

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