Testing White Line Strength in the Dairy Cow

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ABSTRACT

The tensile strength of 576 pieces of white line horn collected over 6 mo from 14 dairy cows restricted to parity 1 or 2 was tested. None of the cows had ever been lame. Seven cows were randomly assigned to receive 20 mg/d biotin supplementation, and 7 were not supplemented. Hoof horn samples were taken from zones 2 and 3 (the more proximal and distal sites of the abaxial white line) of the medial and lateral claws of both hind feet on d 1 and on 5 further occasions over 6 mo. The samples were analyzed at 100% water saturation. Hoof slivers were notched to ensure that tensile strength was measured specifically across the white line region. The tensile stress at failure was measured in MPa and was adjusted for the cross-sectional area of the notch site. Data were analyzed in a multilevel model, which accounted for the repeated measures within cows. All other variables were entered as fixed effects. In the final model, there was considerable variation in strength over time. Tensile strength was significantly higher in medial compared with lateral claws, and zone 2 was significantly stronger than zone 3. Where the white line was visibly damaged the tensile strength was low. Biotin supplementation did not affect the tensile strength of the white line. Results of this study indicate that damage to the white line impairs its tensile strength and that in horn with no visible abnormality the white line is weaker in the lateral hind claw than the medial and in zone 3 compared with zone 2. The biomechanical strength was lowest at zone 3 of the lateral hind claw, which is the most common site of white line disease lameness in cattle.

(**Key words:** white line disease, mechanical strength, biotin, bovine claw)

INTRODUCTION

Healthy bovine hoof horn provides protection and support to the inner structure of the digit. It aids in the dispersal of stress and weight put upon the foot during locomotion (propulsion and concussion) (Johnston, 1990; Vermunt and Greenough, 1990) and provides resistance to excessive abrasion (Leach and Zoerb, 1983; Douglas et al., 1996). Therefore, the most important mechanical properties of hoof horn are its hardness, toughness (resistance to crack propagation), strength, and viscoelasticity (Bertram and Gosline, 1986; Baillie et al., 2000; Bonser, 2000). These properties largely depend upon the structure and chemical composition of keratins that form the horn (Baggott et al., 1988) and the horn moisture content (Budras et al., 1996; Baillie et al., 2000).

The white line lies between the hard coronary wall horn and the more pliable sole horn and runs from the heel bulb around the abaxial claw, to the toe and then along the axial wall where it extends proximally and becomes nonweightbearing (Vermunt, 1990; Warzecha, 1993; Budras et al., 1996, 1998). It allows some elasticity and mobility within the hoof. The white line consists of terminal horn tubules embedded in intertubular horn in the inner part (next to the sole horn) and horny lamellae in the outer part (next to the claw horn). The intertubular horn consists of keratinized squame cells cemented together. Horn turnover is more rapid in the white line than in the other horny structures. This commonly results in incomplete keratinization, particularly at the axial and abaxial terminations, and therefore reduced horn quality and hardness, which leaves the structure more susceptible to damage and vascular disturbances (Vermunt, 1990; Budras et al., 1996, 1997, 1998). White line disease is a common cause of lameness in cows (Kempson, 1987; Hedges et al., 2001). It is hypothesized that when the white line is weakened, for-

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eign material may be caught in the distal surface. If foreign material ascends the white line and penetrates the sensitive tissue of the corium, infection occurs, which leads to lameness (Kempson and Logue, 1993) and further damage to the white line horn at the site of production. To date, the mechanical properties of the white line have not been examined. To describe the white line and determine whether dietary or husbandry regimens have a quantifiable effect on the white line, mechanical measurement protocols are urgently required.

In a recent study, Hedges et al. (2001) reported a 0.57-fold reduction in lameness caused by white line separation in dairy cows supplemented with 20 mg/d biotin. Further analysis by Pötzsch et al. (2003) indicated that in cows of fifth parity and above, biotin supplementation decreased lameness from white line disease 3.6-fold. Biotin is an essential nutrient in keratin synthesis and lipogenesis (Whitehead, 1988; Sarasin, 1994). In reviewing the results from Hedges et al. (2001), Mülling et al. (1999), Hochstetter (1998), Fritsche (1990), and Johnston (1990), it may be concluded that the cellular and intercellular tissue adhesion of the white line had a more defined and cohesive structure as a result of biotin supplementation and that this explained the reduction in white line disease lameness. Biotin was improving the biomechanical properties of the hoof horn.

The study presented in this paper was a pilot study to investigate the tensile strength of the white line of 14 first- and second-lactation dairy cows with no history of white line disease. Seven cows were supplemented with biotin and 7 acted as controls to test the strength of the white line at different sites of the hoof through time. The study involved the development of novel mechanical measurement protocols that could be performed using standard materials testing machines.

MATERIALS AND METHODS

Farm and Sample Allocation

From April to November 2000 an intervention study was carried out on a commercial dairy farm in Gloucestershire, UK. The farm had approximately 60 milking cows. During the study, the cows were at pasture and walked a short distance (no more than 400 m) from the pasture on a stone track, or combination of track and tarmac, over a country lane (depending on field location), and into the concrete yard to be milked twice a day, before returning to pasture.

Fourteen first- and second-lactation cows were selected for the study. They had no history of clinical lameness, and the hoof horn of the 14 cows was visibly normal at the start of the study. The number of cows

in each group was selected to include the minimum number required to detect a significant difference in biomechanical properties. We determined that 6 cows were required per group, with repeated samples to be taken from each cow. The power of the study to detect a difference, assuming a reduced tensile strength in unsupplemented cattle, ranged from 80 to 99% depending on the strength of the autocorrelation and assuming a correlation of 1.0 to 0, respectively (Kirkwood, 1988). Seven cows per group were selected to cover the possibility of loss to follow up during the study. The 14 dairy cows were stratified by calving date and allocated randomly so that half received 20 mg/d biotin supplementation. The cattle were color-coded by group using tail tape.

Cows were kept within the main dairy herd and milked in an abreast milking system. An additional 0.5 kg of concentrate feed was given to both groups of cows at each milking (1.0 kg/d) with biotin included in the treatment ration at 20 mg/kg. Rations were made by one manufacturer and were identical with the exception of the addition of biotin to the treatment group. The 2 rations were stored in color-coded plastic bins in the milking parlor. Each bin contained a calibrated measuring tin to extract the exact amount of feed pellets. Cattle were fed the ration that matched the colored tail tape.

Milk samples were taken from the collection jars of 4 cows in each group on d 0 (before supplementation started), and again 2 and 24 wk after the start of treatment. The milk samples were stored at -20°C before being packed on dry ice and transported to F. Hoffmann-LaRoche, Basel, Switzerland, for analysis using biotin assays.

The farmer immediately reported any signs of lameness in the study cows to the researcher and the veterinarian. The veterinarian attended the animals without charge to the farmer. Each lame cow was documented with details of the date, cow identification, lesion identification and location, and any treatment applied. Four cows were culled (they had positive skin tests at the farm's annual tuberculin test) after d 90, leaving, by chance, 5 cows per group.

Samples of hoof horn were collected on d 1 (sample 1, May), 53 (sample 2, July), 90 (sample 3, August), 117 (sample 4, September), 147 (sample 5, October), and 187 (sample 6, November). At each collection, the veterinarian (RB) removed the outermost, environmentally contaminated or damaged slivers of horn by skimming the distal surface with a hoof knife. The specimens to be tested consisted of a second sliver of horn 1- to 2-mm thick taken from zone 2 and from zone 3 (Figure 1) immediately beneath the skimmed surface. Samples were taken from the lateral and medial claws of both hind feet. Thus, 8 samples were collected from each

Figure 1. A. Zones of the distal surface of the claw: Zone 1, white line region at the toe; zone 2, abaxial white line; zone 3, abaxial wall/bulb junction; zone 4, sole/bulb junction; zone 5, apex of sole; zone 6, bulb of heel (adapted from Leach et al., 1998). B. Representation of the site of the 'notch' in the horn sample. C. Electron micrograph of the white line region (Figure 1C courtesy of Christoph Mülling).

cow at each visit. The samples were placed in separate sterile containers with cotton wool soaked in demineralized water, labeled, and stored at -20°C . Soaking horn material in demineralized water has been observed to be the most effective way of mimicking the true moisture uptake that would occur in vivo (Baillie et al., 2000).

The tensile strength of the white line was measured by testing it as an adhesive layer. Frozen specimens were thawed at room temperature and maintained in 'wet' condition during preparation. The thawed hoof horn was cut to a standard width using a specially designed cutting instrument and 'waisted' or 'notched' by a second cutter in the center of the white line region in the cranial/caudal plane (Figure 1). This ensured that the failure properties of the white line rather than

adjacent wall or sole tissue were measured. The cutters controlled the relative notch width to remove the possible confounding influence of notch-sensitivity in the material (Vincent, 1992). The thickness of each specimen was measured using vernier calipers. Each sample was examined for visible damage within the white line, i.e., brown/black amorphous debris, blood, or defects.

Aluminum strips, approximately 0.5-mm thick and 2-mm wide, folded in half, were attached around both ends of the wall and sole material either side of the notched white line using cyanoacrylate adhesive. This gave a site of attachment for the clamps of the test machine, with sufficient grip to prevent slipping. To ensure maximum hydration before testing, the prepared samples were returned to demineralized water for a further 24 h at room temperature.

The samples were tested blind in sampling date order and samples for each cow were tested in a single batch.

Tensile Strength Testing

A Davenport-Nene T10 test frame (Davenport-Nene Ltd., Wellingborough, UK) was used to perform tensile strength tests. The machine was fitted with a 260-N capacity load cell and tensile test grips. Tests were conducted to failure at a test speed of 10 mm/min. The peak load during the test and the final load (when the white line failed completely) were divided by the cross-sectional area of the waisted portion of the test specimen to give the peak stress of the white line in MPa (N/mm^2). All samples were treated and tested using an identical protocol, regardless of the sampling period and the supplement, to prevent the introduction of bias into the analysis.

Data Analysis

Data were analyzed only after all the mechanical tests had been completed. The data were logged and checked for normality. Initial comparisons were made using 2 sample *t*-tests (Kirkwood, 1988), comparing left and right hind feet, lateral and medial claws, zones 2 and 3, supplemented vs. unsupplemented horn, and the impact of white line damage on the peak failure rate for each time period and ignoring time. Then the peak failure was analyzed in a 2-level model (Rasbash et al., 1999) to adjust for repeated measures within cows over time. White line damage, zone, claw, foot, biotin supplementation, and sample day number were fitted as fixed effects. This analysis provided adjusted effects of all the variables, accounting for autocorrelation.

RESULTS

Only one cow became lame in the study. She had white line disease; the white line was separated and could not be included in the analysis. The feet of all cows in the study were visibly normal at the start of the study. A small proportion of horn samples had visible white line damage at the start of the study when the horn slivers were examined (Figure 2). The proportion of damaged white line increased to 20% and then decreased again toward the end of the study.

The mean value for sample thickness at the notched portion was 1.147 mm, SD 0.47, SE 0.02.

There was a significant effect of sample day on the tensile strength of the white line (Figure 3). Of the unexplained variance, 5% was attributed to cows and 95% to residual error (Table 1), indicating that cows had little autocorrelation. Having accounted for the

Figure 2. Percentage of damaged white line material by biotin supplementation and sample day; this was correlated with stage of lactation.

clustering of data, the peak tensile strength was significantly weaker in visibly damaged white line specimens compared with normal-appearing specimens (2.35 vs. 4.45 MPa), in the lateral compared with the medial claw (3.64 vs. 4.75 MPa), and in zone 3 compared with zone 2 (3.11 vs. 5.27 MPa).

Cows supplemented with biotin had a greater concentration of milk biotin compared with cows that were not supplemented (513.7 ± 40.9 and 162.7 ± 10.2 nmol/g, respectively; $P < 0.01$) (Table 2). However, there was no significant difference in tensile strength between supplemented and unsupplemented cattle. Cows fed the biotin supplement did have slightly elevated tensile

Figure 3. Mean peak tensile strength (MPa) by claw (medial or lateral), zone (2 or 3), and sample day (1 to 6).

Table 1. Multilevel model of peak tensile strength of the white line (log likelihood -41.68; n = 571).

Variable	Mean log peak tensile strength $\pm$ SE	Geometric mean difference (MPa)	Lower 95% CI ¹	Upper 95% CI
Intercept	1.18 $\pm$ 0.06	15.14	11.48	19.95
White line damage*	-0.208 $\pm$ 0.03	-1.61	-1.85	-1.41
Lateral vs. medial claw*	-0.12 $\pm$ 0.02	-1.32	-1.45	-1.20
Zone 2 vs. zone 3*	-0.25 $\pm$ 0.02	-1.78	-1.95	-1.62
Sample 1*				
Sample 2	-0.04 $\pm$ 0.03	-1.10	-1.26	-0.95
Sample 3	0.06 $\pm$ 0.03	1.15	1.00	1.32
Sample 4	0.15 $\pm$ 0.03	1.41	1.23	1.62
Sample 5	-0.02 $\pm$ 0.03	-1.05	-1.20	-0.91
Sample 6	0.18 $\pm$ 0.03	1.51	1.32	1.74
Biotin supplementation vs. control	0.001 $\pm$ 0.03	1.00	0.87	1.15
Left vs. right hind claw	0.03 $\pm$ 0.02	1.07	0.98	1.17
Variance estimates		% Variation		
Between cow*	0.003 $\pm$ 0.001	5.4		
Residual*	0.053 $\pm$ 0.003	94.6		

¹CI = Confidence interval.**P* < 0.05.

strength compared with unsupplemented cows in all sample periods except for sample d 1 (d 53) and 6 (d 147) (Table 3).

DISCUSSION

In our study, we matched cows on calving date and restricted the study to young cows that had had 1 or 2 calves only and had no history of lameness. This was done to eliminate the impact of history, age, and stage of lactation on the biomechanical measurements. A few animals but many samples were studied. In the analysis, the estimates of significance were adjusted for the repeated measures from cows in the 2-level hierarchical model, which accounts for the correlation structure from these repeated measurements (Goldstein, 1987). All other variables—zone, claw, foot, biotin supplementation, and sample day number—were fitted as fixed effects. The results are significant, indicating adequate sample size, even though 4 cows were lost at d 90. In fact, only 5% of the unexplained variance was attributed to cows and 95% to residual error (Table 1). This is a combination of measurement and unobserved causes of error. These measurements should be re-

peated on a larger number of cattle to investigate their generalizability.

The average values observed for the tensile strength of white line (3.63 to 5.60 MPa) in this study were lower than those reported by Budras et al. (1996), who reported values of 5.1 MPa for the cap-horn and 6.9 MPa for the terminal horn. These differences are small and in our study, the comparisons are made between estimates using one machine and testing protocol. There was a clear pattern of increasing and then decreasing white line damage through stage of lactation (Figure 2), as reported by other studies (Kempson and Logue, 1993; Green et al., 2002). Visibly damaged white line was seen in 69 samples and separated white line in one (the lame cow); the former had a highly reduced tensile strength, indicating that damaged white line is indeed a site of weakness that will permit penetration of foreign matter.

This study identified a significantly lower tensile strength in the white line of the lateral compared with the medial hind claw and in zone 3 compared with zone 2 within each claw. The lateral claw and zone 3 are the sites of most white line damage (Budras et al., 1997) and disease (Midla et al., 1998; Hedges et al., 2000); our study has demonstrated that the white line at these sites is weaker than at other sites. The presence of morphological differences between the lateral and medial claws has been reported by several researchers (Toussaint Raven, 1973; Ossent et al., 1987; Vermunt and Greenough, 1996; van der Tol et al., 2003). The abaxial border of the white line of the lateral claw has the greatest weightbearing load during locomotion. Zone 3 of the white line has weaker horn than zone 2

Table 2. Mean biotin analysis of milk samples during the trial (nmol/kg).

Biotin supplement	No. of samples	Mean	SD	SE	Significance
Yes	8	513.67	119.92	40.93	***
No	8	162.69	29.47	10.23	

****P* < 0.001.

Table 3. Univariate comparison (*t*-test) of peak tensile strength (MPa) and 95% confidence interval for white line region by variable by sample day.

Variable	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
	n	Mean ¹	n	Mean	n	Mean	n	Mean	n	Mean	n	Mean
Control	56	3.65 ± 0.21	54	3.60 ± 0.27	56	4.08 ± 0.28	40	4.89 ± 0.30	40	3.82 ± 0.30	39	5.51 ± 0.40
Biotin supplementation	55	3.94 ± 0.27	55	3.31 ± 0.24	56	4.30 ± 0.27	40	4.99 ± 0.33	40	3.44 ± 0.26	40	5.70 ± 0.41
<i>P</i>		0.39		0.43		0.58		0.83		0.35		0.75
Left hind	55	3.81 ± 0.27	53	3.66 ± 0.24	56	4.04 ± 0.28	40	5.14 ± 0.36	40	3.85 ± 0.26	39	6.19 ± 0.43
Right hind	56	3.77 ± 0.21	56	3.27 ± 0.27	56	4.34 ± 0.26	40	4.74 ± 0.26	40	3.41 ± 0.29	40	5.04 ± 0.36
<i>P</i>		0.90		0.29		0.43		0.37		0.27		*
Lateral claw	56	3.24 ± 0.22	53	3.10 ± 0.23	56	3.62 ± 0.26	40	4.32 ± 0.33	40	3.19 ± 0.28	39	4.72 ± 0.42
Medial claw	55	4.35 ± 0.24	56	3.80 ± 0.27	56	4.76 ± 0.26	40	5.56 ± 0.26	40	4.07 ± 0.27	40	6.47 ± 0.34
<i>P</i>		**		*		**		**		*		**
Zone 2	55	4.41 ± 0.23	55	4.50 ± 0.25	56	5.50 ± 0.18	40	6.05 ± 0.18	40	4.67 ± 0.24	40	7.02 ± 0.32
Zone 3	56	3.18 ± 0.22	54	2.40 ± 0.17	56	2.89 ± 0.23	40	3.83 ± 0.32	40	2.59 ± 0.22	39	4.15 ± 0.35
<i>P</i>		**		**		**		**		**		**
No white line damage	109	3.80 ± 0.17	95	3.62 ± 0.20	93	4.61 ± 0.19	68	5.23 ± 0.22	65	4.09 ± 0.20	72	5.90 ± 0.29
White line damage	2	2.85 ± 0.23	14	2.38 ± 0.32	19	2.15 ± 0.33	12	3.31 ± 0.62	15	1.65 ± 0.28	7	2.59 ± 0.24
<i>P</i>		0.08		**		**		**		**		**

¹Mean = Mean values and 95% confidence interval for mean.**P* < 0.05.***P* < 0.01.

because it has a higher rate of horn production and thus incomplete keratinization (Kempson and Logue, 1993; Warzecha, 1993; Budras et al., 1996). There is also evidence that greater sole and wall wear occurs in the lateral than the medial digit in the hind feet. This corresponds with the distribution of a cow's weight, which is borne more by the lateral hind claw than medial (Tranter and Morris, 1992; Warzecha, 1993; van der Tol et al., 2003). These factors may explain the accumulation of clinical problems in the lateral hind claw. Although we have demonstrated that damaged white line tends to be weaker, it is difficult to attribute the incidence of damage to a causal effect of white line weakness. It is possible that damage may weaken the white line or that the weak white line is more susceptible to damage. Clearly, the only way of testing this would be to experimentally induce lesions within the white line and measure their effect on mechanical performance.

In this study, biotin supplementation did not have a significant effect on white line tensile strength. A longer study period may have elicited a significant effect from biotin supplementation, because it takes approximately 130 d of biotin treatment before a significant difference in white line lesion lameness occurs (Hedges et al., 2001). However, a more recent study has indicated that cattle of parities 1 and 2 have low levels of white line disease and that biotin may not significantly influence its occurrence in young cows compared with older cows (Pötzsch et al., 2003). Young cattle without a history of lameness were used in the current study because it was a small study and the authors wished to avoid the confounding effects of previous white line lameness.

In an earlier study, a significant increase in tensile strength and horn quality was reported after 5 mo of biotin supplementation (Schmid and Geyer, 1994). However, that study did not use unsupplemented cattle as controls, and increased horn quality was observed before the start of the study. In the current study, the tensile strength of white line showed considerable variability by sample day. One can speculate that changes in stage of lactation, diet, and housing may have influenced this. However, because the changes occurred in supplemented and unsupplemented cattle, they are accounted for in the analysis and do not influence the estimates of white line tensile strength by foot, claw, zone, or biotin supplementation.

CONCLUSIONS

These results indicate that in visibly healthy horn, the white line is weaker in the lateral hind claw and in zone 3, and that damage to the white line impairs its tensile strength. These new biomechanical findings support previous studies on weight distribution and conformational qualities of dairy cow feet as well as identifying that the common sites for white line disease are also the mechanically weaker parts of the hoof.

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