Proceedings of the 13th International Symposium and 5th Conference on Lameness in Ruminants

11th - 15th February 2004, Maribor, Slovenija

Session 8 - Nutrition and Claw Health

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Nutritional management continues to be a major focal point in the attempt to reduce lameness in dairy cattle (Nocek, 1997). Lameness is a multifactorial disease resulting from an array of factors inherent to dairy operations (Lischer and Ossent, 1994). Factors affecting lameness and locomotion include nutrition, feeding strategies, wetness, abrasive or slippery floor surfaces and health events causing production of poor quality horn (fever, age, off-feed, metabolic disturbances, toxins/mycotoxins). A considerable body of evidence is available for the impact of protein, carbohydrates, non-forage fiber, fiber length, and various other macro nutritional management factors pertaining to ruminal function and performance of the dairy cow during the transition period. However, for a long time less emphasis has been placed on the role of hormones, vitamins, minerals, and trace elements and the roles they play in development of quality claw horn and keratin formation.

The objective of this paper is to summarize the processes involved in formation of quality claw horn. Special emphasis is placed on the nutritional and hormonal factors that affect claw keratin formation during the periparturient period and their potential role in production of inferior horn tissue resulting in increased incidence of lameness.

Transition period challenges

Many physiological changes occur in late gestation and early lactation of the dairy cow which affect nutrient uptake and flow. Despite the tremendous quantity of research conducted on nutrition and physiology of transition cows, the transition period remains a problematic area on many commercial dairy farms, and metabolic disorders continue to occur at economically important rates (Burhans et al., 2003). Godden et al. (2003) reported that approximately 25% of cows that left dairy herds in Minnesota from 1996 to 2001 did so during the first 60 DIM, with an uncertain percentage leaving by the end of the lactation as an end result of difficulty during the transition period. These findings are supported by summary data from California indicating approximately 30% of culled cows were leaving dairy herds by 60 DIM (Overton, 2003). Many of these cows suffer from claw abnormalities which occur in early lactation (Green et al., 2002) and may be partly due to the result of nutritional deficiencies or hormonal changes occurring during the periparturient period.

Hormonal Control of Horn Growth. An interesting area of developing research relates to the hormonal control of horn protein production and how changes at parturition may affect potential for future lameness. One of the primary physiological adaptations of transition cows is the need to synthesize and direct glucose to the mammary gland. The cow accomplishes this by concurrently increasing hepatic gluconeogenesis (Reynolds et al., 2003) and decreasing oxidation of glucose by peripheral tissues (Bennick et al., 1972). Vermunt and Greenough (1994) suggested that overfeeding during the dry period, which gives rise to hyperinsulinemia and hyperglycemia (two classic signs of insulin resistance) in early lactation, appeared to predispose cows to laminitis. Green et al. (2002) reported that incidence of first lameness was highest three months after calving, suggesting that factors affecting horn growth during the dry period and in early lactation result in production of inferior horn and subsequent lameness in early lactation.

In research to investigate keratinization control, Hendry et al. (1999) demonstrated that insulin binding was detected in both the epidermal and the dermal layers of explanted bovine hoof tissue. In the early lactating dairy cow, there is a decrease in insulin sensitivity (Cowie et al., 1980) and an inverse relationship between circulating insulin and animal productivity (Hart et al., 1978). Therefore, a decrease in insulin sensitivity, and or concentration, in early lactation could compromise production of claw horn keratin due to depressed uptake of glucose and amino acids (Hendry et al., 1999). It is conceivable that this could be exacerbated if the living (horn producing) epidermis in the claw shares the post-receptor insulin resistance shown by other tissues during the periparturient period (Vernon, 1988).

Epidermal growth factor. Epidermal growth factor (EGF) was reported to have potent mitogenic and anti-differentiative effects in epithelial tissues other than the claw (alimentary and uterine tracts), yet was bound more locally than insulin, being found only in the differentiating epidermal layer (Hendry et al., 1999). During the process of keratinization, epidermal cells rely upon the dermal layers for supply of nutrients. This supply must be provided entirely via diffusion from blood vessels in the underlying dermis because the epidermis is an avascular tissue.
Hendry et al. (1999) reported that EGF may impact keratin formation and result in formation of inferior horn production. EGF stimulated protein synthesis in bovine hoof tissue explants (Hendry et al., 1999), while EGF was reported to decrease keratin expression in healthy equine tissue (Grosenbaugh et al., 1991). Steroid hormones elevated in pregnancy down-regulate local production of EGF in a number of tissues (Plaut, 1993). If this also occurs in the claw the result would be an inhibition of keratin synthesis (Hendry et al., 1999).

**Prolactin.** Another hormone of particular interest during the periparturient period is prolactin. The major lactogenic hormone prolactin may also influence EGF-dependant keratin deposition (Cowie et al., 1980). Hendry et al. (1999) found hoof explant culture stimulation of protein synthesis by EGF was antagonized to a modest degree by prolactin. Although prolactin itself did not influence hoof protein synthesis, its ability to decrease EGF-stimulated protein synthesis in hoof tissue cultures may be another factor in reducing keratin synthesis during lactation (Hendry et al., 1999).

**Glucocorticoids.** Goff and Horst (1997) reported that periparturient dairy cows are often subjected to stress with a subsequent increase in cortisol. Glucocorticoids are thought to have an impact on maturation of keratinocytes through regulation of protein synthesis as cortisol affects the metabolism of glucose, protein, and fats (Goff and Horst, 1997). Hendry et al. (1999) found that hydrocortisone inhibited keratin protein synthesis in bovine hoof tissue explants. Epidemiologists have yet to identify a causative relationship between systemic glucocorticoid concentration and laminitis in dairy cows. Yet, it is notable that highly productive herds, which have a greater incidence of laminitis (Nocek, 1997) also have higher glucocorticoid levels (Johnson and Vanjonack, 1976). Milne (1985) reported that steroid treatment of horses exacerbates laminitis. Stress and subsequent elevation of cortisol during the periparturient period and during lactation (Goff and Horst, 1997) may predispose dairy cows to claw disorders resulting from production of inferior claw horn.

**Required Nutrients for Keratinization**

**Amino Acids.** The amino acids Cys, His and Met play key roles in establishing the structural integrity of the keratinocyte (Eklafk, 1990; Eklafk et al., 1990). Fraser and MacRae (1980) reported that the formation of disulfide bonds between Cys residues was an integral step in the final stage of keratinization and in cornification and establishment of the cellular envelope providing cell wall rigidity and high resistance against a variety of proteolytic enzymes (Elias, 1981). Grosenbaugh and Hood (1993) reported that cultured explants preferentially incorporated 35S-Cys into partially keratinized epidermal lamina as opposed to the uptake of 35S-Met, thus supporting the requirement for Cys in formation of the keratin rich cornified hoof wall.

**Zinc.** Zinc has been identified as a key mineral in the processes of keratinization (Smart and Cymbaluk, 1997; Mülling et al., 1999; Mülling, 2000). The ubiquitous distribution of Zn among cells, coupled with Zn being the most abundant intracellular trace element, points to very basic functions. While Zn is a component of over 200 enzyme systems, it has a role in three key functions in the keratinization process: catalytic, structural and regulatory (Cousins, 1996). Catalytic roles are found in enzymes such as RNA nucleotide transferases, RNA polymerase, alkaline phosphatase, carboxypeptidase, alcohol dehydrogenase and the carbonic anhydrases (Cousins, 1996; NRC, 2001). As indicated earlier, the presence of ribonucleic and deoxyribonucleic acid, ascorbic acid, free alde-


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Zinc also plays a key role in the development of the structural proteins during the keratinization process. Zn-finger proteins are involved in functions requiring protein to protein interactions, most of which are thought to affect cellular differentiation or proliferation (Cousins 1996). Two examples are the transcription factors of retinoic acid and calcitriol (1,25-dihydroxycholecalciferol) receptors (Cousins, 1996).

The third key role of Zn in differentiating cells including differentiating keratinocytes is regulatory. Zinc regulates calmodulin, protein kinase C, thyroid hormone binding and inositol phosphate synthesis (NRC, 2001). Calmodulin is responsible for binding Ca2+ and carrying Ca2+ into the cytosol of the cell when activated. This is important in the final step of the developing keratinocyte because as noted earlier, calcium activates epidermal transglutaminase. Protein kinase C (which is also calcium dependent) is responsible for phosphorylation of proteins, thus adding available energy to the differentiation process. Thyroid hormone acts to regulate the activity of calmodulin and protein kinase C. Inositol phosphate acts to increase Ca2+ by mobilizing the ion from intracellular stores, primarily from the endoplasmic reticulum.

Zinc is also required for activation of the cytosolic enzyme Cu/Zn superoxide dismutase (Cu/Zn SOD). In Cu/Zn SOD, Cu functions at the catalytic site, while Zn has a role in the 3-D structure of the enzyme (Cousins, 1996). Cu/Zn SOD is responsible for prevention of lipid peroxidation. Protection of the intercellular cementing substance is critical in maintenance of the structural integrity and biological function of the claw (horn) (Mülling et al., 1998, 1999). Mülling (2000) reported that organic Zn like other trace elements minerals and vitamins is involved in numerous biochemical pathways during keratinisation including formation of keratin protein.

British workers (Baggott et al., 1988) reported findings of lower Zn concentration in claws of lame cows than those with no history of lameness. Claws of lame cows were also softer than the non-lame individuals. This suggests an insufficiency of Zn or lack of adequate vascular supply to the developing keratinocytes. On dairies with high incidents of foot problems, cows fed 2 to 3 g/d of ZnSO4 for 70 d had fewer claw problems than cows not receiving supplemental Zn (Weaver et al., 1978). In contrast, sheep fed rations supplemented with ZnSO4 for up to 6 months did not show a reduction in claw problems (Cross and Parker, 1981). Inconsistent responses to feeding Zn in the form of ZnSO4 can be attributed to antagonists present in the diet affecting the bioavailability of the Zn (NRC, 2001). Organic sources of Zn, such as zinc methionine, have proven to be more bioavailable than Zn from inorganic sources (Wedekind et al., 1992).

Several studies have shown that complexed Zn improves claw integrity. In a year long study conducted at Illinois State University, cows fed an additional 200 mg/d of Zn from Zn Met had fewer cases of foot rot, heel cracks, interdigital dermatitis and laminitis than cows not fed Zn Met (Moore et al., 1989). Observations on ulcers and white line disease (indications of dyskeratotic structurally altered horn tissue) trended towards improvement. Of beef cattle receiving 540 mg/d of Zn from complexed Zn, 2.45% had foot rot while 5.38% of cattle not receiving complexed Zn had foot rot (Brazle, 1993). These studies indicate that feeding organic Zn complexed to a single amino acid has a beneficial influence on keratinizing tissues, thus improving hoof horn and skin integrity, resulting in improved animal well-being and performance. Zinc requirements for dairy cows vary by stage of lactation (NRC 2001). Milk production creates a significant drain on zinc stores, thus zinc requirements are highest in early lactation (NRC 2001). Insufficient supplies of bioavailable zinc, during the periparturient period and during lactation, may predispose cows to production of inferior horn tissue with a concomitant increase in lameness.

Copper. Much like Zn, Cu is instrumental in activation of enzymes. Copper is needed for activation of cytochrome oxidase enzyme involved in aerobic respiration, lysyl and thiol oxidases for structural integrity of cells, ceruloplasmin, which is essential for absorption and transport of iron for hemoglobin synthesis and superoxide dismutase which works with Zn in reducing the toxic effects of oxygen metabolites (NRC, 2001). Of greatest importance in the keratinizing horn cell is the activity of thiol oxidase (O’Dell, 1990). Copper activates thiol oxidase enzyme, which is responsible for formation of the disulfide bonds between Cys residues of keratin filaments (O’Dell, 1990). This process is essential for structural strength on the cellular level giving rigidity to the keratinized cell matrix.

Cattle suffering from a subclinical Cu deficiency are more susceptible to heel cracks, foot rot and sole abscesses (Puls, 1984). This response may be the result of insufficient cytochrome-c oxidase activity resulting in reduced respiration and oxidative phosphorylation and thus deficient energy supplies for differentiating keratinocytes (Linder, 1996). Heel cracks and abscesses may also be the result of insufficient Cu availability for activation of Cu/Zn SOD resulting in increased fragility of cell membranes. Unsaturated lipids in the cell periphery are particularly vulnerable to oxidative damage (Linder, 1996). The intercellular lipids are an integral part of the cementing substance responsible for cell-to-cell adhesion (Mülling and Budras, 1998). Therefore, any nutrient deficiency that leads to production of inferior intercellular cementing substance or predisposes it to excessive oxidative damage may potentially lead to production of dyskeratotic horn tissue with increased susceptibility to cracking and wear.

Selenium. Selenium is a constituent of the enzyme glu-
Manganese. Manganese plays an indirect role in the keratinization process. Manganese helps minimize feet problems by maintaining proper leg formation (Miller et al., 1988). Manganese is needed for activation of galactotransferase and glycosyltransferase enzymes, which are needed for the synthesis of chondroitin sulfate side chains of proteoglycan molecules (Keen and Zidenberg-Cherr, 1996; NRC, 2001). Proteoglycans are essential building blocks in formation of normal cartilage and bone. Animals suffering from a Mn deficiency will exhibit skeletal abnormalities, crooked legs and shortening of tendons as noted by knuckling over of feet (NRC, 2001).

Manganese also plays a role in activation of other critical enzyme systems, such as pyruvate carboxylase, an enzyme that catalyzes the first step of carbohydrate synthesis. This process is responsible for gluconeogenesis and production of cellular energy an essential component in production of quality horn tissue (Keen and Zidenberg-Cherr, 1996). Similar to Cu/Zn SOD, Mn plays a role in activation of Mn superoxide dismutase (Mn SOD) and the removal of superoxide free radicals. Therefore, Mn SOD may play a protective role for the lipids involved in cementing together mature keratinocytes.

Combinations of trace minerals. There are significant interactions between trace minerals and hence it is imperative that nutritionists formulate rations to maintain an appropriate balance of trace minerals in order to maximize animal performance. Research has demonstrated that supplying a combination of complexed trace minerals is more beneficial to claw integrity than supplying a sole complexed trace mineral because of synergistic effects. A two year study conducted on five commercial dairy herds in Central New York indicated that cows fed 360 mg complexed Zn, 200 mg complexed Mn, 125 mg complexed Cu and 25 mg complexed Co, in combination with inorganic trace minerals, resulted in better claw integrity than cows fed only 360 mg of complexed Zn or no complexed trace minerals (Nocek et al., 2000). Supplementation of the diet with a combination of complexed trace minerals reduced the incidents of double soles, white line disease, digital dermatitis, sole hemorrhages and ultimately, sole ulcerations (Nocek et al., 2000). In addition, three hundred cows on a large commercial dairy in Florida were fed a combination of complexed Zn, Mn, Cu and Co to evaluate claw health (Ballantine et al., 2002). Cows fed complexed trace minerals tended to have fewer incidents (P<0.15) of claw disorders than cows fed inorganic trace minerals at 75 days postpartum (23.6 vs. 34.1%) and numerically lower incidents at 250 days postpartum (10.0 vs. 17.7%). Feeding complexed trace minerals reduced incidents (P<0.15) of white line disease at 75 (9.5 vs. 14.6%) and 250 days postpartum (4.9 vs. 8.8%). Feeding complexed trace minerals during the late dry period and during early lactation improved (P<0.05) claw lesion scores. These results indicate that if cows fed complexed trace minerals did develop a claw lesion, the lesion was less severe, as measured by size and painfullness of the lesion, as compared to control cows that developed a claw lesion.

Role of vitamins in horn production/formation

Vitamin A. Vitamins also play an integral role in developing the structure and quality of keratinized horn tissue. Vitamin A is needed for cell differentiation (Olson, 1996). Differentiating cells have specific binding sites for vitamin A and once bound can both stimulate or inhibit gene expression. Vitamin A is needed for normal growth and development and for maintenance of skeletal and epithelial tissues (NRC, 2001). The role of vitamin A in keratinizing cells is tied to its action in gene expression (NRC, 2001).

Vitamin D. One of the most important biological regulators of calcium metabolism is vitamin D (synonym calciferol) (NRC 2001). Derived from cholesterol, a Mn dependant process, vitamin D is responsible for minute-by-minute calcium and mineral homeostasis. In its bio-
LOGICALLY ACTIVE FORM 1,25(OH)2D3, VITAMIN D IS REQUIRED FOR CONTROL OF Ca2+ RE-ABSORPTION, ABSORPTION AND MOBILIZATION/ACCRETION FROM BONES (NORMAN, 1996). BECAUSE THE BODY CAN ENDOGENOUSLY PRODUCE VITAMIN D3 AND BECAUSE IT IS RETAINED FOR LONG PERIODS OF TIME IN VERTEBRATE TISSUES, IT IS NOT LIKELY THAT DAIRY ANIMALS WOULD BE DEFICIENT IN VITAMIN D. HOWEVER, WITH INCREASED CONFINEMENT AND REDUCED EXPOSURE TO DIRECT SUNLIGHT, DAIRY ANIMALS LACKING SUFFICIENT SUPPLEMENTATION COULD SUCCEMBER TO MINOR VITAMIN D DEFICIENCIES. THEREFORE, ANY LACK OF VITAMIN D WILL CERTAINLY IMPACT CALCIUM METABOLISM AND THUS AFFECT THE KERATINIZATION PROCESS.


FUNCTIONING RUMINANTS ARE ABLE TO PRODUCE BIOTIN IN THE RUMEN. HOWEVER, HIGH GRAIN (> 50% OF DM) RATIONS REDUCE RUMINAL SYNTHESIS OF BIOTIN IN-VITRO (DACOSTA-GOMEZ ET AL., 1998). THIS RESPONSE MAY BE DUE TO AN INSUFFICIENT CONVERSION OF LACTATE TO PYRUVATE. MOCK (1996) REPORTED THAT BIOTIN DEFICIENCY WAS TIED TO INSUFFICIENT PYRUVATE CARBOXYLASE ACTIVITY RESULTING IN CELLULAR LACTIC ACIDOSIS. IT MAY BE POSSIBLE THAT RUMINANTS RECEIVING PROPORTIONATELY HIGH GRAIN DIETS LACK SUFFICIENT BIOTIN IN THEIR RUMEN TO CONVERT LACTIC ACID TO PYRUVATE AND THEN OXALOACETATE, THEREBY PREDISPOSING THEM TO LACTIC ACIDOSIS. NOCEK (1997) REPORTED LACTIC ACIDOSIS AS ONE OF THE POSSIBLE CONTRIBUTING FACTORS IN LAMENESS OF DAIRY COWS. RECENT WORKS (FITZGERALD ET AL., 2000; HEDGES ET AL., 2001; WEISS AND ZIMMERLY, 2000) INDICATE THAT DAIRY COWS RESPOND FAVORABLY (IMPROVED CLAW INTEGRITY AND REDUCED LAMENESS) WHEN PROVIDED SUPPLEMENTAL BIOTIN (20 mg/hd/d) FOR A PERIOD OF GREATER THAN 6 MO. IN A STUDY OF FIVE DAIRIES WITH A TOTAL OF 900 CATTLE, PÖTZSCH ET AL. (2003) REPORTED BIOTIN SUPPLEMENTED AT 20 mg/d FOR LONGER THAN 6 mo REDUCED WHITE LINE DISEASE IN MULTIPAROUS COWS BY 45% TO 8.5 CASES PER 100 COW YEARS. HOWEVER, THE EFFECT OF BIOTIN IN PRIMIPAROUS COWS WAS NOT SIGNIFICANT. THESE STUDIES INDICATE THAT BIOTIN REDUCES THE INCIDENTS OF WHITE LINE ABNORMALITIES IN PARTICULAR AND OTHER CLAW DISEASES SUCH AS SOLE HEMORRHAGE, SOLE ULCERS, DIGITAL DERMATITIS, AND HEEL EROSION.

MÜLLING ET AL. (1999) PROPOSED THE ANALOGY OF BUILDING A BRICK WALL TO THE EFFECTS OF SUPPLEMENTS SUCH AS BIOTIN ON HOOF KERATIN FORMATION. ZINC IS NEEDED FOR ACTIVATION OF THE ENZYME SYSTEMS NEEDED FOR FORMATION OF SOUND CELLULAR STRUCTURE, WHILE BIOTIN IS NEEDED FOR PRODUCTION OF THE INTERCELLULAR CEMENTING SUBSTANCE. THE TWO TOGETHER ALLOW THE KERATINIZING SQUAMOUS CELLS TO GENERATE STRONGER HORN WITH GREATER INTEGRITY THAT WILL BETTER WITHSTAND ENVIRONMENTAL STRESSES. IT IS THIS ABILITY TO WITHSTAND ENVIRONMENTAL STRESS THAT ULTIMATELY DETERMINES THE PRODUCTIVITY AND POTENTIAL PROFITABILITY OF THE ANIMAL.

CONCLUSIONS

FORMATION OF KERATIN PROTEINS IS AN ESSENTIAL/CRUCIAL PART OF A SYSTEMATIC PROCESS OF CELLULAR CHANGES THAT TRANSFORM LIVING, HIGHLY METABOLIC ACTIVE LIVING EPIDERMAL CELLS INTO DEAD STRUCTURAL HORN CELLS WITH NO METABOLIC ACTIVITY. THIS DIFFERENTIATION OF EPIDERMAL CELLS IS VERY COMPLEX AND VERY SENSITIVE TO HORMONAL CONTROLS, NUTRIENT FLOW AND ENVIRONMENT. IT IS THE PROCESS OF NUTRIENT FLOW, AS IMPACTED BY HORMONAL CONTROLS THAT PLAYS AN IMPORTANT ROLE IN DETERMINING THE QUALITY AND INTEGRITY OF KERATINIZED TISSUES OF THE HORN. WHEN NUTRIENT SUPPLY TO KERATIN FORMING CELLS IS COMPROMISED OR COMPLETELY ALTERED, INFERIOR KERATINIZED TISSUE IS PRODUCED. INFERIOR TISSUE INCREASES THE POTENTIAL FOR DEVELOPMENT OF CLAW DISEASE AND MAY ULTIMATELY LEAD TO LAMENESS. CALCIUM, Zn, Cu, Mn, VITAMINS A, D & E AND BIOTIN ALL PLAY IMPORTANT ROLES IN PRODUCTION AND MAINTENANCE OF HEALTHY KERATINIZED TISSUES. INCREASING THE BIOAVAILABILITY OF TRACE MINERALS, ESPECIALLY Zn, Cu AND Mn IMPROVES THEIR UTILIZATION AND THUS CONtributes TO IMPROVED INTEGRITY OF KERATINIZED TISSUES SUCH AS SKIN AND
claw. Integrity of claw horn is one prerequisite for claw health which in turn is the prerequisite for overall animal well-being, productivity and potential profitability.

References

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Introduction

Ruminal acidosis is widely considered to be a primary risk factor for the development of claw horn lesions in dairy cattle. It is hypothesised that vasoactive substances resulting from disturbed rumen function lead to vascular reactivity, laminitis, hoof deformation and finally abnormal horn synthesis thereby compromising hoof health.

Materials and methods

Claw horn lesion development was observed in a group of 22 spring calving first lactation Holstein Friesian heifers. These animals were part of an observational lameness study looking at the effect of training heifers to use cubicles before calving on hoof health after calving (Logue et al., 2004). All but 2 were housed in a lactating group when they were accidentally overfed concentrates within a TMR (almost twice ration) early in their first lactation (on average 11 weeks post calving, range 4 - 15 weeks). The exact amount of concentrate given to the animals could not be identified but the error occurred because the normal farm mixer wagon (with limited sensitivity of + or - 60 kg) was used to weigh 90kg of barley and 65 kg of blended concentrate for a group of 20 animals. The animals showed signs of ruminal acidosis, some more severely than others. Severely affected animals were moved to a straw yard and given veterinary attention. Rumination ceased in the majority of animals (14/22), seven of the 22 were given Vitatrace (Vetoquinol UK Ltd); five became recumbent and required intravenous fluids. All animals recovered and were returned to the cubicle house within 2 - 4 days. Since animals were already part of a lameness study detailed claw horn lesion data existed before the animals went sick. Routine examinations continued throughout lactation and again at housing (October) and 3 months post housing (January) for any long-term effects of the feeding incident.

After the feeding incident the cows were treated and for analysis purposes were split into four groups by severity of clinical signs and (treatments given) as given in Table 2.

Table 2 Definition of ‘illness score’ according to severity and numbers of animals affected.

<table>
<thead>
<tr>
<th>Illness score Definition</th>
<th>N (trained, untrained)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Still ruminating</td>
<td>6 (2,4)</td>
</tr>
<tr>
<td>2 Not ruminating</td>
<td>5 (4,1)</td>
</tr>
<tr>
<td>3 Requiring Vitatrace, not ruminating</td>
<td>4 (3,1)</td>
</tr>
<tr>
<td>4 Recumbent, requiring IV fluids and Vitatrace.</td>
<td>5 (3,2)</td>
</tr>
</tbody>
</table>

Results

The initial models applied to the data are detailed in Logue et al. (2004). For this study acidosis score was included in the model and so lesion data were modelled as a function of training regime, days relative to calving, the time of year, optionally, days on straw, and severity of the acidosis.

There was significant benefit from fully training for sole lesions (P=0.006, and P=0.012, for the models excluding and including days on straw, respectively). For line lesions it was significant for the model excluding days on straw (P=0.030) and marginally insignificant when including days on straw (P=0.091). Inclusion of the acidosis score to the models reduced the respective significance of the treatment effects to P=0.013, and P=0.015 for sole lesions and P=0.060, and P=0.144, for white line lesions.

The inclusion of acidosis score in the models slightly reduces the between cow variance component for sole lesions, but for line lesions it is increased slightly. In all cases the coefficient was positive (for line and for sole for the models excluding days on straw and for line and for sole for the models including days on straw) indicating a very marginal increase of line and sole lesions with increasing acidosis score (see figure 1). Acidosis score, however, was not statistically significant for any model considered.
Introduction

Lameness continues to be a major area of concern for dairy managers. While nutrition is often blamed for laminitic insults, it may also have a positive impact on lameness management. Previous workers have indicated that supplementing dairy cows with organically complexed trace minerals will help improve bioavailability and aid in the reduction and alleviate the severity of claw disorders in dairy cattle (Moore et al., 1989; Nocek et al., 2000; Ballantine et al., 2002). The objective of this study was to evaluate the effects of supplementing metal specific amino acid complexes on the incidence and severity of claw disorders in dry and lactating dairy cows.

Materials and Methods

Animals. A total of 157 pregnant Holstein cows were blocked by parity, milk production and season of calving and randomly assigned within block to one of two dietary treatments: daily supplementation with 443 mg of Zn, 444 mg of Mn, 261 mg Cu, and 25 mg of Co as inorganic salts (control) or a combination of inorganic salts and complexed trace minerals (treatment, 14 g of 4-Plex/day, containing 360 mg Zn from zinc methionine, 200 mg Mn from manganese methionine, 125 mg Cu from copper lysine, and 25 mg Co from cobalt glucoheptonate, Eden Prairie MN, USA). Trace mineral supplementation was equal between treatments. Diets were fed from -60 d from projected calving date through 250 d postcalving. Dry cow diet composition on a % of dry matter basis: corn silage (32.1%), grass hay (30.3%), mixed haylage (5%), and a grain mix containing the treatment supplement (32.6%). Lactating cow diets on a % of dry matter basis contained; corn silage (32.2%), mixed haylage (12.8%), dry cow grain treatment supplement (11.8%), corn grain (7.9%), citrus pulp (8.1%), whole cottonseed (5.7%), soy hulls (5.9%), protein/mineral mixture (15.6%). To be included in the study, cows had to be on...
the precalving ration for at least 60 days prior to calving.

**Housing and Management.** During both the pre and post-calving periods, cows were housed in cubicles (free stalls) lined with a surface of chopped rubber covered with a nylon mesh sheet bedded with sawdust. Cows were milked 2 X/day. All feed ingredients were weighed and mixed together into a TMR and fed once a day prior to and after calving. Mineral supplements were combined with corn grain and pelleted. Diets were formulated to NRC (2001) standards for energy and protein for late gestation, nonlactating cows and lactating cows producing 30 or 40 kg of milk, depending on stage of pregnancy and lactation.

**Claw Examination.** Examination of claws and soft tissues surrounding the claws was made by a trained clinician at -60 d, 30 d, and 250 d relative to calving. After cleaning, claws and soft tissues around the claws and interdigital area were examined for lesions. Lesions were classified according to Toussaint Raven (1989) based on macroscopic examination of claw and tissues. Lesions were classified as involving the keratinous tissue or soft tissue. The keratinous lesions were classified as follows: dorsal wall ridges, erosion of the heel bulb, abaxial wall lesions, double sole, white line separation, sole abscess, sole hemorrhage, sole ulceration or sole erosion. The lesions of the soft tissues surrounding claws were classified as: digital dermatitis, pododermatitis of the digit or interdigital area, interdigital fibroma, or hairy heel warts. Lesions were mapped for location on the claw and surrounding soft tissues.

**Statistical analysis.** Claw lesions were analysed as repeated observations with PROC GENMOD (SAS, Raleigh NC, USA) and subject = cow (claw) treated as the nested term for the covariance matrix. Claw and tissue were analyzed using the logistic link function. Multiple lesions were analyzed using the cumulative logistic link function and the multinomial distribution.

Pearson's correlation coefficient was used to examine the correlation between lesions on the same claw and lesions on the same cow between claws and feet.

**Results**

A total of 157 cows were assigned to the study and began the experiment. Due to early calving, defined as less than 60 days on precalving diets, or health complications (abortion, mastitis, etc.) only 138 cows were included in the final analysis (73 cows on control, 65 cows on treatment diet). At thirty days postcalving, two cows were removed for health reasons, resulting in 136 cows at 30 d postcalving (73 cows on control, 63 cows on treatment diet). At 250 days postcalving, 6 cows on the control diet and 6 cows on the treatment diet were removed prior to sampling, resulting in 121 cows for sampling at 250 d postcalving.

Heel erosions were the most frequent claw lesion across all treatments and time periods (45%). The second most common lesion were abaxial wall lesions (26.7%). Only 18 (4.6%) of cow examinations across all periods had no lesions. Erosions were distributed uniformly between front and rear feet and medial and lateral claws (Table 1). Dermatitis of the soft tissue was more often observed in the rear feet (Table 1).

Many cows had multiple lesions. A total of 3160 claw examinations were made. Claw lesions were classified as involving keratinous tissues (% of claw lesions observed) including heel erosions (44.4%), abaxial wall lesions (28.3%), sole erosion (7.9%), white line separation (3.4%), double sole (2.75%), dorsal wall ridges (1.59%), solar abscess (1.2%), sole hemorrhage (0.1%), sole ulceration (1.1%). Lesions of the soft tissues surrounding claws included digital dermatitis (12.4%), interdigital dermatitis (6.2%), pododermatitis (0.03%), hairy heel warts (0.2%) and interdigital fibroma (0%). Sixty eight percent of the claws were identified to have one or more lesions. Observations identified 39.6% of claws had one lesion, 19.2% had two lesions, 6.5% had three lesions, 2.2% had four lesions, 0.5% had five lesions, and 0.2% of claws had six or more lesions.

Overall, supplementation with complexed trace minerals did not affect claw or foot health (P<0.31), yet supplementation had an effect on reducing solar lesions and had a tendency to reduce claw lesions. The incidence of claw lesions (lesions involving the keratinous tissues) tended to be lower for the complexed trace mineral supplemented group (61.5% versus 65.2%, P<.08). This was largely due to a reduction in solar lesions at 30 days postcalving in supplemented cows. Significant factors influencing claw lesions included lactation number (P=0.002), phase (P<0.001; time relative to calving), season (P<0.001), and the interactions of treatment*phase (P=0.03) and treatment*phase*lactation (P<.001). In addition, lactation*season (P<.002) and phase*season (P<.0001) were significant factors describing claw lesions.

Results of this study indicate that supplementation of dairy cattle, beginning in the dry period, may help reduce the number of claw lesions in the subsequent lactation. However, lactation number, stage of lactation and season had a greater influence on the number and severity of claw lesions.
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Table 1. Lesions by claw and foot of cows that had at least one claw or foot with the condition from a total of 395 cow examinations from 138 cows made over three periods at -60d, 30d and 250d relative to calving.

<table>
<thead>
<tr>
<th>Claw/foot</th>
<th>Heel and sole erosion</th>
<th>Claw lesion associated with laminitis</th>
<th>Dorsal wall injury</th>
<th>Normal claw or foot</th>
<th>Infections</th>
<th>Tissue lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF inside</td>
<td>184</td>
<td>17</td>
<td>56</td>
<td>0</td>
<td>21</td>
<td>135</td>
</tr>
<tr>
<td>LF outside</td>
<td>153</td>
<td>24</td>
<td>44</td>
<td>153</td>
<td>26</td>
<td>177</td>
</tr>
<tr>
<td>RF inside</td>
<td>182</td>
<td>16</td>
<td>45</td>
<td>151</td>
<td></td>
<td>135</td>
</tr>
<tr>
<td>RF outside</td>
<td>148</td>
<td>24</td>
<td>40</td>
<td>158</td>
<td></td>
<td>177</td>
</tr>
<tr>
<td>RF foot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26</td>
<td>173</td>
</tr>
<tr>
<td>LR inside</td>
<td>168</td>
<td>16</td>
<td>55</td>
<td>135</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LR outside</td>
<td>102</td>
<td>40</td>
<td>23</td>
<td>74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LR foot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>177</td>
<td></td>
</tr>
<tr>
<td>RR inside</td>
<td>165</td>
<td>18</td>
<td>56</td>
<td>137</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR outside</td>
<td>173</td>
<td>33</td>
<td>22</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR foot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>173</td>
<td></td>
</tr>
</tbody>
</table>

a Double sole, White line separation, Solar abscess, Sole hemorrhage, and Sole ulceration
b No claw or foot lesions on any claw
c Digital dermatitis, Interdigital dermatitis, Pododermatitis, Hairy Heel Warts, and Fibroma

References available upon request.

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EFFECT OF FEEDING COMPLEXED TRACE MINERALS TO HEIFERS FROM 12 MONTHS OF AGE TO ONE MONTH PREPARTUM ON RISK OF DEVELOPING CLAW LESIONS DURING LACTATION AND LACTATION PERFORMANCE


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3Zinpro Corporation, 10400 Viking Dr., Suite 240, Eden Prairie, MN 55344

Introduction

Research has shown that lameness contributes to reduced milk production (Guard, 1997; Robinson et al., 2003) and reproductive failure (Sprecher et al., 1997; Hernandez et al., 2000; Melendez et al., 2002). Furthermore, research indicates that previously lame cattle are more prone to future recurrences (Nocek, 1997). Therefore, preventing animals from becoming lame must be a key management objective.

Supplementing cows with a combination of complexed zinc, manganese, copper and cobalt has been shown to reduce incidence (Nocek et al., 2000; Ballantine et al., 2002) and severity of claw lesions (Ballantine et al., 2002). However, the effect of complexed trace mineral supplementation during the rearing phase on claw lesion incidence has not been examined. The objective of this study was to determine the effect of feeding heifers a combination of cobalt glucoheptonate and zinc, manganese and copper amino acid complexes (Availa®4) on incidence of claw lesions both during the rearing phase and in the first lactation.

Materials and methods

Five hundred seventy-two heifers at a commercial heifer rearing facility were alternatively assigned to one of two dietary treatments: control diet (DM basis, 66 ppm Zn, 66 ppm Mn, 18 ppm Cu and 0.52 ppm Co) or control diet plus daily 360 mg zinc, 200 mg manganese and 125 mg copper from amino acid complexes and 12 mg cobalt from cobalt glucoheptonate (CTM; Availa-4, Zinpro Corporation, Eden Prairie, MN). Treatments were fed from 12 months of age until one month prepartum. Heifers originated from one of four source dairies and were returned to the source dairy at one month prepartum.

Heifers were housed in open earthen-mound lots without overhead protection and fed a total mixed ration (TMR) consisting of 98.1% forage and 1.9% concentrate. Diets were formulated to contain (DM basis) 15% CP, 30% ADF; 40% NDF, 64% TDN, 1.0% Ca, 0.36% P, 0.28% Mg, 0.22% S and 1.45 ppm Fe. Pen feed intakes were monitored daily. Diets were reformulated weekly based on DMI changes, and bimonthly based upon changes in diet ingredients.

Claws evaluations were conducted prior to initiation of treatment, at approximately one month prepartum and at two months postpartum by one claw trimmer using a clean, light grind. The claw trimmer was a graduate of the Dairyland Hoof Care Institute (Baraboo, WI) and was not informed of the heifers’ treatment assignment. Lesions were noted in the seven zones of the claw and each lesion was scored for severity on a scale of 1 to 3 (1=minor, 2=moderate, 3=severe). To assess both incidence and severity of claw lesions, a claw lesion incidence and severity (CLIS) index was calculated. This index was the average number of zones affected per cow multiplied by the average severity score multiplied by 10. After returning to the source dairy, heifers were housed in naturally ventilated free stall barns, fed similar TMR with overhead protection and fed a total mixed ration (TMR) consisting of 98.1% forage and 1.9% concentrate. Diets were reformulated weekly based on DMI changes, and bimonthly based upon changes in diet ingredients.

Results of first lactation performance were collected via production record systems on each dairy by the researchers as heifers completed or neared completion of their first lactation. Due to removal of some of the trial ID tags of heifers at one dairy after completing the claw evaluation at 2 months postpartum, lactation performance data were available from only 421 heifers.

Production data and CLIS index measurements were analysed using the MIXED procedure of SAS V8.1 (1999) with the effects of dietary treatments and source dairies as
8. Session: Nutrition and claw health

discrete, class variables, and CLIS index in phase one and its interaction with dietary treatments as continuous variables.

Result and discussion

Feeding CTM increased \( P < 0.05 \) the CLIS index for sole hemorrhages and tended to increase \( P < 0.15 \) the CLIS index for claw lesions and heel erosion at one month prepartum (Table 1).

Table 1. Effect of feeding complexed trace minerals\(^a\) to growing heifers on incidence and severity of claw lesions as measured by the claw lesion index and severity index\(^b\) during the rearing phase and subsequent lactation.

<table>
<thead>
<tr>
<th>Claw Disorder</th>
<th>1 Month Prepartum</th>
<th>2 Months Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall claw lesions</td>
<td>3.8(^w)</td>
<td>4.8(^x)</td>
</tr>
<tr>
<td>Distal wall ridges</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Heel erosion</td>
<td>29.4(^w)</td>
<td>34.5(^x)</td>
</tr>
<tr>
<td>Abaxial wall fissures</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Double soles</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>White line separation</td>
<td>2.8</td>
<td>3.0</td>
</tr>
<tr>
<td>Sole hemorrhages</td>
<td>9.7(^x)</td>
<td>16.7(^x)</td>
</tr>
<tr>
<td>Sole Ulcers</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Digital dermatitis</td>
<td>1.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Interdigital dermatitis</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

\(^a\) Availa\(^®\) provided daily 360 mg Zn, 200 mg Mn, 125 mg Cu and 12 mg Co

\(^b\) Calculated using the following formula (number of zones affected per cow x average severity score x 10); Severity score ranged from 1 (minor) to 3 (severe)

\(^c\) Slope of P3 on P1 for control heifers was greater than 0 (\( P < 0.05 \)) indicating a positive relationship between the CLIS index at 12 months of age and the CLIS index at 2 months postpartum

\(^w\) Within phase, within row, means with uncommon superscripts differ \( P < 0.15 \)

\(^x\) Within phase, within row, means with uncommon superscripts differ \( P < 0.05 \)

However feeding CTM from 12 months of age to one month prepartum reduced \( P < 0.05 \) the CLIS index for heel erosion and tended to reduce \( P < 0.15 \) the CLIS index for claw lesions and sole ulcers two months postpartum.

For heifers fed the control diet, there was a positive relationship \( P < 0.05 \) between the CLIS index one month prepartum with the CLIS index two months postpartum for heel erosion, abaxial wall fissures and digital dermatitis (Table 2).

Table 2. Effect of feeding complexed trace minerals\(^a\) to heifers during the rearing phase on the regression coefficients of the relation between claw lesion incidence and severity index\(^b\) at 1 month prepartum and at 2 months postpartum.

<table>
<thead>
<tr>
<th>Claw Disorder</th>
<th>Control</th>
<th>Complexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall claw lesions</td>
<td>0.067</td>
<td>0.150</td>
</tr>
<tr>
<td>Distal wall ridges</td>
<td>-</td>
<td>-0.008</td>
</tr>
<tr>
<td>Heel erosion</td>
<td>0.164z</td>
<td>0.040</td>
</tr>
<tr>
<td>Abaxial wall fissures</td>
<td>1.311z</td>
<td>0.268</td>
</tr>
<tr>
<td>Double soles</td>
<td>-0.073</td>
<td>0.010</td>
</tr>
<tr>
<td>White line separation</td>
<td>-0.003</td>
<td>-0.000</td>
</tr>
<tr>
<td>Sole hemorrhages</td>
<td>0.234</td>
<td>0.368z</td>
</tr>
<tr>
<td>Digital dermatitis</td>
<td>0.164z</td>
<td>0.209z</td>
</tr>
<tr>
<td>Interdigital dermatitis</td>
<td>-0.001</td>
<td>-0.013</td>
</tr>
</tbody>
</table>

\(^a\) Availa\(^®\) provided daily 360 mg Zn, 200 mg Mn, 125 mg Cu and 12 mg Co

\(^b\) Calculated using the following formula (number of zones affected per cow x average severity score x 10); Severity score ranged from 1, minor to 3, severe

\(^z\) Differs from zero \( P < 0.05 \)

These results indicate that if control heifers had heel erosion, abaxial wall fissures or digital dermatitis one month prior to calving, they were more likely to have heel erosion, abaxial wall fissures and digital dermatitis two months post calving. For heifers fed CTM, there was a positive relationship \( P < 0.05 \) between the CLIS index one month prepartum and the CLIS index two months postpartum for sole hemorrhages and digital dermatitis. The regression coefficient for the relationship between presence of claw lesions during the rearing phase and milk yield in the first 60 to 90 days of lactation was greater than zero \( P < 0.15 \) for CTM heifers, indicating that if heifers had a claw lesion during the rearing phase, feeding CTM tended to increase production in the first 60 to 90 days of lactation (Table 3).

Table 3. Effect of feeding complexed trace minerals\(^a\) during the rearing phase on the regression coefficients of the relationship between claw lesions and lactation performance.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Complexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield, first 60 to 90 days of lactation</td>
<td>-90</td>
<td>20z</td>
</tr>
<tr>
<td>Fat yield, 305 Mature Equivalents</td>
<td>-5503z</td>
<td>-1901</td>
</tr>
<tr>
<td>Protein yield, 305 Mature Equivalents</td>
<td>-19.65</td>
<td>-38.80</td>
</tr>
</tbody>
</table>

\(^a\) Availa\(^®\) provided daily 360 mg Zn, 200 mg Mn, 125 mg Cu and 12 mg Co

\(^z\) Differs from zero \( P < 0.15 \)

The presence of a claw lesion two months postpartum tended to \( P < 0.15 \) reduce the 305 mature equivalent milk yields of heifers fed the control diet during the rearing phase. Results of this study indicate that feeding CTM to heifers did not reduce incidence of claw lesions during the rearing phase. However, feeding CTM to heifers during the rearing phase did reduce incidence of claw lesions at two months postpartum and helped alleviate the effects of claw lesions on lactation performance.
Introduction

Daily biotin supplementation of dairy cows has frequently improved hoof integrity (Fitzgerald et al. 2000). However, relatively little is known about changes in growth rates of hooves in intensively fed beef steers. Such animals are fed high grain-based diets for long periods (>300 days), exhibit high live weights (>700kg) and contend with periodic muddy conditions. Biotin deficiency has been associated with feeding grain-based diets to cattle by compromising ruminal biotin synthesis (Fitzgerald et al. 2000). An experiment was conducted to determine the effects of biotin supplementation at three levels (0, 10, 20mg/hd/d) on hoof chemical composition and rates of wear and growth in F1 Wagyu/Black Angus beef steers fed long term on a dry rolled wheat based ration.

Material and Methods

The experiment, conducted in Queensland, Australia began in March 2001 and consisted of twelve pens of nine F1 Wagyu/Black Angus steers per pen (live weight 410.5kg, SD 24.4). Pens were allocated to one of three biotin treatments, including nil biotin (Cont), 10mg/hd/d (B10) and 20mg/hd/d (B20) in a randomised complete block design with four replicates. Biotin was administered to pens within a mineral supplement mixed into a wheat-based ration at a rate of 250g/hd/d.

REFERENCES AVAILABLE UPON REQUEST

Results

Biotin supplementation had a significant effect on hoof growth (P=0.09) and wear (P=0.12) for MP6. B10 had a lower growth (7.0mm) compared to both B20 (20.8mm) and Cont (16.0mm). B10 tended to have a lower wear (11.2mm) when compared to B20 (29.6mm), with no difference to Cont (21.4mm). Total hoof wear (MP7) also tended to be lower (P=0.06) for B10 (52.6mm) compared to B20 (75.7mm), with no difference to Cont (64.8mm). Net growth (growth - wear) for MP7 approximated 0.5, 4.5 and -13.1mm for Cont, B10 and B20 respectively.

Irrespective of biotin treatment, rates of hoof growth and wear were not constant over MP as described in Table 1. No relationship existed between steer live weight and rate of hoof growth or wear. However, rates of hoof growth and wear coincided with rainfall events, particularly for MP5. Hoof growth rate increased by 6.4mm and wear rate increased by 15.5mm for this period. Rate of wear (18.9 and 22.0mm) exceeded rate of growth (14.5 and 14.3mm) for MP5 and 6 respectively.

Table 1: Effect of rainfall (mm) and steer live weight (kg) on hoof growth and wear rates for MP.

<table>
<thead>
<tr>
<th>Rain</th>
<th>MP 1</th>
<th>MP 2</th>
<th>MP 3</th>
<th>MP 4</th>
<th>MP 5</th>
<th>MP 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
<td>26.8</td>
<td>10.8</td>
<td>10.8</td>
<td>158.1</td>
<td>28.0</td>
</tr>
<tr>
<td>LW</td>
<td>474</td>
<td>545</td>
<td>606</td>
<td>638</td>
<td>694</td>
<td>749</td>
</tr>
<tr>
<td>Growth</td>
<td>Overall</td>
<td>10.1 a</td>
<td>12.3 a</td>
<td>6.3 b</td>
<td>8.1 ab</td>
<td>14.5 c</td>
</tr>
<tr>
<td>s.e.m</td>
<td>0.8</td>
<td>1.3</td>
<td>1.4</td>
<td>1.0</td>
<td>1.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Wear</td>
<td>Overall</td>
<td>3.8 a</td>
<td>10.3 a</td>
<td>6.7 a</td>
<td>3.4 a</td>
<td>18.9 b</td>
</tr>
<tr>
<td>s.e.m</td>
<td>0.6</td>
<td>1.3</td>
<td>0.9</td>
<td>0.5</td>
<td>1.6</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Means within each row with different superscripts differ (P<0.05).

Biotin supplementation had no effect on white line width of the lateral claw. Whereas B10 tended to increase white line width of the medial claw (P=0.12). B10 had a
Introduction

Sulphur amino acids have an important role in the structure and function of integumental tissues. Previous studies (Galbraith et al., 2002) have described the concentration-dependent importance of methionine in regulating apparent total protein and DNA synthesis in claw tissues by explant technique. The aim of the present study was to investigate the effects of (a) inadequate and (b) approximately optimal concentrations of methionine on indices of cell proliferation under similar conditions in vitro.

Materials and Methods

Claw tissue for culture was obtained from right hind lateral claws of crossbred female cattle (age 18-20 months).
post mortem at a commercial abattoir. Explants were prepared from the solear tissue, each weighing 30-50mg, and were cultured in an atmosphere of air/CO₂ (95:5, v/v) at 37 °C in DMEM/F12, deficient in methionine, with methionine added at 1 or 30 µM. Each well of 6-well plates contained ca 300mg tissue and 2.5ml culture medium. Explants were removed from the media at 24, 48 and 72 h. Another set of explants were treated at time 0 with a pulse of 0.05 mM BrdU for 6 hours then fresh media was replaced and explants removed at 24, 48 and 72 h. Explant tissue slices were transferred into OCT embedding compound and frozen in liquid nitrogen. Cryosections were cut at 10 µm using a steel knife on a cryostat (Reichert-Jung Frigocut 2800E) and air dried onto Vectabond coated slides.

For immunohistochemistry, cryostat sections were fixed in 4% paraformaldehyde for 10 min then immersed in a 1 mg/ml trypsin solution. Endogenous peroxidase was blocked by incubating the tissue section in 3% hydrogen peroxide in methanol for 5 min. The sections were rinsed in buffer prior to incubation with dilute serum to block non-specific binding. The primary antibodies were added (monoclonal mouse anti-PCNA, 1:3000 dilution, Sigma; polyclonal rabbit anti-Bcl-2, 1:50 dilution, Oncogene; monoclonal mouse anti-BrdU, 1:1000 dilution, Sigma) and left overnight at 4 °C. The sections were then washed in tris-buffered saline and blocked with serum as before. The secondary antibody Vectastain Universal Elite ABC kit (Vector Laboratories) was used to achieve detection. Sections were then dehydrated through an ethanol series finishing with histoclear for 5 min and mounting in Styrolite (BDH) under a glass coverslip. Apoptotic cells at the basement membrane and measured following the manufacturer’s method were stained with digoxigenin-dUTP antibodies (Apoptag; Oncor Inc.). Cryostat sections for Feulgen staining were fixed and immersed in glacial acetic acid: ethanol (1:3) for 2 hours and then hydrated through 75% ethanol for 10 min followed by 50% ethanol for 10 mins. Sections were then washed and immersed in 1 M HCl for 6 min at 60 °C. Following hydrolysis, sections were immersed in Schiff’s Reagent (Sigma) for 20 min. Sections were then washed and mounted in glycerol under a glass coverslide and stored at 4 °C until examination.

Slides were viewed using a Polyvar microscope (Reichert-Jung) that was attached to a digital camera (TK-C1381, JVC). Images were captured using specialised analysis software (Image Pro-Plus Version 4.5.0.19 for Windows 98/NT/2000). The positive epidermal cells along the basement membrane of papillae were counted and compared as a function of its length (1 unit basement membrane length = 401 µm) rather than of total cells, for 18 papillae (3 sections x 3 explants x 2 animals). Data were analysed by Minitab (Release 13.3, General linear model).

Results

Epidermal cell proliferation measured by the PCNA and Feulgen techniques was greater (P<0.05) at the higher (30µM) compared with the lower (1 µM) concentration of methionine after 72 hours with non-significant trends evident at 48 hours (Table 1; Figure 1). A significantly greater number of cells expressed a positive signal for BrdU at 48 h with similar trends evident at 24 and 48h, in response to the higher methionine concentration. There was also evidence of a significantly greater expression of the apoptosis inhibitor Bcl-2 (30µM vs 1 µM, 72h).

However, tests for apoptosis by TUNEL gave time-dependent increases in positive signal but also a greater apoptotic response to the higher methionine concentration.

Table 1. Cells staining positive for various proliferation and apoptosis markers with two concentrations of methionine. Results are shown as mean ± SEM (P<0.05).

<table>
<thead>
<tr>
<th>Positive cells / basement membrane</th>
<th>Methionine concentration and time removed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td></td>
<td>(n=18)</td>
</tr>
<tr>
<td>PCNA</td>
<td>105 ±</td>
</tr>
<tr>
<td></td>
<td>4.79a</td>
</tr>
<tr>
<td>Feulgen</td>
<td>118 ±</td>
</tr>
<tr>
<td></td>
<td>6.14a</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>127 ±</td>
</tr>
<tr>
<td></td>
<td>6.30a</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>54.2 ±</td>
</tr>
<tr>
<td></td>
<td>7.48a</td>
</tr>
</tbody>
</table>

Mean values with different superscripts within rows are significantly different (P<0.05).

Discussion

The data produced evidence of the importance of methionine supply in supporting proliferative activities in basal epidermal cell keratinocytes. This result was particularly evident with increasing time of incubation and likely depletion of endogenous concentrations up to 72h. Responses were associated with the maintenance of the signal for the apoptosis inhibitor Bcl-2 at the higher compared with the lower methionine concentration. In contrast, the signal for apoptosis by TUNEL analysis suggested a greater number of apoptotic cells at the higher methionine concentration. This result, along with that for proliferation, may indicate a stimulation of epidermal cell turnover. The reasons for this are not clear but may relate...
The claw is a specialised structure of the bovine integument with function dependent on interaction between epidermis and dermis at a number of sites. These sites vary in the composition of horn-produced proteins and contribution to suspensory function. Relatively little is known about the dynamics of nutrient uptake and protein and DNA synthesis at these functionally important locations. The sulphur containing amino acids are among important nutrients that support successful horn production. The dermis is known to supply nutrients and chemical signals to support proliferation and differentiation of horn-forming epidermal keratinocytes and expression of proteins involved in cytoskeleton and intercellular adhesion. The cytoskeletal intermediate filament proteins and associated proteins contain variably high concentrations of cysteine, which, in certain tissues may be maintained by methionine via the transulphuration pathway. Methionine also has a central role in polyamine synthesis, methylation and protein synthesis both structurally and in translation. Utilisation of methionine by tissues is governed by extracellular and cellular uptake and incorporation during synthesis and metabolism.

The aim of this study was therefore to investigate at functionally important sites, (a) the uptake of methionine and its incorporation into protein in cells and (b) DNA synthesis by thymidine incorporation under conditions standardised and established previously (Galbraith et al., 2002b).

Materials and Methods

Tissue explants (ca 50 mg wet weight) from the sole, heel, mid-laminar and coronary region were prepared post mortem from hind right lateral claws of female cattle (n=6, age 18-20 months). Explants (4 per well) were incubated in DMEM/F12 culture medium and an atmosphere of air/CO2 (95:5, v/v) at 37°C. After 21 h incubation, fresh media were added supplemented with either L-[35S] methionine (1µCi/ml) or U-[14C] sucrose (0.5µCi/ml) for 30 min followed by washing in fresh media, blotting and incubation in the presence of TCA (10%, w/v) for 16 h. Radioactivity in the TCA soluble fraction was counted and intracellular uptake of L-[35S] methionine calculated as released radiolabel, applying corrections for uptake in extracellular space (U-[14C] sucrose uptake) (Shennan and McNeillie, 1994). Also after 21 h, a series of explants were incubated in fresh media supplemented with L-[35S] methionine (6µCi/ml) and [6-3H] thymidine (2.5µCi/ml) for 3 h to measure their incorporation into the TCA-insoluble, formic acid soluble fractions as measures of protein and DNA synthesis by methionine and thymidine incorporation, respectively. Data were analysed by Minitab (Release 13.3, General linear model).

Results

Measurement of intracellular accumulation of methionine as a measure of cellular uptake in tissue explants was significantly higher in the mid-laminar region compared to the sole and heel but not different from the coronary region (Table 1). In contrast, methionine (35S) incorporation in the cellular protein fraction, was significantly higher in the sole compared to the mid-laminar but not different from the heel or coronary region (Table 1). There was no difference in the incorporation of thymidine into the TCA insoluble, formic acid soluble fraction, as a measure of DNA synthesis, between the various regions (Table 2).
**Table 1.** Uptake and incorporation of methionine in four regions of the claw. Results are shown as mean ± SEM for n=6 animals.

<table>
<thead>
<tr>
<th>Region of hoof measured:</th>
<th>Methionine uptake (pmol/kg extracellular water/30 min)</th>
<th>Extracellular space (F-value)</th>
<th>Methionine incorporation (pmol/kg intracellular water/3h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sole</td>
<td>29.4 ± 3.54abc 31.6 ± 2.81ab 41.5 ± 3.18ab 38.7 ± 3.39ab</td>
<td>0.30 ± 0.02a 0.51 ± 0.01b 0.20 ± 0.02b 0.21 ± 0.02b</td>
<td>3.52 ± 0.15b 2.59 ± 0.31ab 1.59 ± 0.31a 2.63 ± 0.31ab</td>
</tr>
<tr>
<td>Heel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid laminar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean values with different superscripts within rows are significantly different (P<0.05).

**Table 2.** Incorporation of thymidine in four regions of the claw. Results are shown as mean ± SEM for n=6 animals.

<table>
<thead>
<tr>
<th>Region of hoof measured:</th>
<th>Thymidine incorporation (pmol/kg intracellular water/3h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sole</td>
<td>46.6 ± 6.46 38.8 ± 3.60 49.5 ± 7.15 52.8 ± 6.21</td>
</tr>
<tr>
<td>Heel</td>
<td></td>
</tr>
<tr>
<td>Mid laminar</td>
<td></td>
</tr>
<tr>
<td>Coronary</td>
<td></td>
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</table>

Discussion

The data for the proportion of extracellular space indicate significantly greater values for sole and heel compared with the coronary and mid-laminar region. These results may suggest a larger ratio of extracellular matrix to cells in the former than the latter explant systems studied. Results for methionine uptake and incorporation and thymidine incorporation were of a similar order to those recorded previously for solear explants (Galbraith et al., 2002b). The data for cellular uptake suggest a greater apparent transport of methionine into cells of the mid-laminar region than the sole during the time period of study. Reasons for this are not known but may relate to differences in the epidermal-dermal structures at these sites which may result in nutrient supply to the predominant epidermal cells. In contrast, the incorporation of the 35-S label into the intracellular protein fraction suggests a greater rate of protein synthesis in cells of the solear compared with the mid-laminar region. Trends were also noted which gave some evidence suggesting apparently greater cellular protein synthetic activity in cells of heel and coronary compared with that of the cells of the mid-laminar region. The absence of significant differences in apparent DNA synthesis in cells suggest a similarity in proliferation rates at the four sites. It is concluded that the characterisation of the in vitro explant system provides the first comparative description of proliferation and protein synthetic potential at the functionally important sites investigated. Future work will refine the systems to permit investigation of the regulation of these processes and how they may be influenced to produce the lesions character-istic of lameness in affected claws.

Acknowledgements

This work was funded by the EU Lamecow Project QLRT-2001-00969. The technical assistance of MJ Birnie and MA Brown is gratefully acknowledged.

References


Introduction

Hoof disease is one of the most serious problems in the dairy industry. It leads to reductions in milk yield and fertility and to shortening of productive life. Hoof disease can be caused by various factors, including housing, behavior, genetics, infective agents and nutrition. A strong and intact horn shoe (stratum corneum) is required for the protection of the claw's soft tissues and for maintenance of the biomechanical function of the claw. Lipids are a component of cell membranes and are present in the intracellular space of the claw epidermis in cattle. It is known that these lipids are important for both water retention and barrier function in the stratum corneum of human skin. Water content of the claw horn was found to be closely related to the horn hardness. Hardness is an...
important factor for the protection of soft tissue and basal membrane cell layer inside the claw against physical loads, especially body weight. Thus, water regulation mechanism is important for maintenance of the proper function of the claw horn. Ceramides are composed of sphingosine and fatty acid, and have been shown to be predominantly associated with water retention and barrier function. It has been reported that seven types of ceramide were detected in porcine and human skin. These ceramides play crucial roles in physiological function of skin, and a decrease in ceramide contents of the stratum corneum is related to atopic lesions of skin. However, ceramides in the bovine hoof horn have not been analysed in detail. In this communication, we present results of analysis of the quantity and pattern of the hoof horn ceramides from normal cows and cows with subclinical laminitis.

Materials and methods

Hoof horn samples of Holstein-Friesian cows were obtained from slaughterhouse. Cows were clinically evaluated to determine hoof health, and were identified as having sound hooves (n=13) or suffering from subclinical laminitis (n=8). The diagnosis of subclinical laminitis was made on the basis of findings of extensive yellow sole horn consisting of extensive yellow discoloration and haemorrhage. Four zones of sole horn based on the recommendation of the Sixth Symposium of Disease of the Ruminant Digit were used in our study. Two zones of wall horn were newly shown in this study (Fig. 1). Lipid extraction from hoof horn samples was performed according to the methods of Scaife et al. (2000) and Imokawa et al. (1991). Briefly, samples of bovine hoof horn were reduced to fine shavings using a rasp. Lipid extraction was performed at room temperature for 1 h in a 15-fold excess of chloroform : methanol (2:1). Ceramides of hoof horn samples were separated by thin-layer chromatography and were developed twice with chloroform : methanol : acetic acid (190:9:1). After solvent development, the chromatograms were air-dried, sprayed with 10% CuSO₄ - 8%H₃PO₄ aqueous solution, and charred on a 180° hot plate. The charred lipids were quantitated using NIH (National Institute of Health) Image software. Ceramides were measured by determining the amount on ceramides on a TLC chart of appropriate commercial standards and expressed as µg ceramide / g hoof horn. Ceramide type 1 and type 2 were used as standards for ceramides I, IIa, IIb, III, IV, V+VI and VII respectively. Reproducibility of this method was confirmed using triplicate samples from the same animal, and deviation of values was within 5%. The level of significance of the difference was calculated by Student's t-test.

Results and discussion

Ceramides I, IIa, IIb, III, IV, V+VI and VII were clearly detected in sole horn samples, but ceramides I, III, IV and VII were not detected in wall horn samples. Total ceramide content of the sole horn samples was 1346.4±221.3 (µg/g) and was significantly (p<0.01) higher than that of wall horn samples, which was 638.6±82.6 (µg/g) (Table 1). This is the first report on quantity and types of ceramide from the bovine hoof horn. It has been reported that seven types of ceramide were detected in the stratum corneum of human skin. In this study, the patterns of chromatograms of hoof horn ceramides from sole horn samples were similar to those of human skin reported by Imokawa et al (1991). The reason for the differences in quantity of ceramide from sole and wall horn was not clarified in our study. The contents of total ceramide and ceramide type IIa, IIb, III and VII of hoof horn from cows with subclinical laminitis were significantly (p<0.05) lower than those in claw horn samples from normal cows. Among the 6 types of ceramide fraction, ceramide 1 was found to be most significantly reduced in atopic lesional skin of humans. It has been reported that keratinocytes in the basement membrane cell layer are important for the formation of claw horn, and that cell metabolism was markedly inhibited in cow with laminitis. From our findings, we speculated that the decreased quantity of ceramide of hoof horn in cows with laminitis was caused by the inhibition of ceramide synthesis by keratinocytes in the condition of subclinical laminitis. Determination of the types of ceramide in the bovine claw horn may be important for clarification of the mechanism of bovine claw disease.