

Nutrition: Building the Bovine Claw

Dana Tomlinson Ph.D., and Michael Socha Ph.D.
Zinpro Corporation, Eden Prairie, MN, USA

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, off-feed, metabolic disturbances, toxins/mycotoxins, age). A considerable body of literature 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 claw horn lesions. More recently, emphasis is being placed on the metabolic disturbances and mechanical changes of the claw which occur during the transition period. These changes may be impacted by hormones, vitamins, minerals, and trace elements and the roles they play in development of quality claw horn tissue.

The objective of this paper is to summarize some of the factors 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 (3 wk prior to through 3 wk after parturition) remains a problematic area on many commercial dairy farms, and metabolic disorders continue to occur at economically important rates (Burhans et al., 2003). Toussant Raven (1989) considered parturition as a “healthy” disease that could significantly impact claw health of the dairy cow through the interaction of the cow, parturition and nutritional shifts that occur during this period (Figure 1). Drackley (1999) reported that success during the transition period effectively determines the profitability of the cow during the subsequent lactation. He indicated that most infectious diseases and metabolic disorders occur during this time. Milk fever, ketosis, udder edema, retained fetal membranes, metritis and displaced abomasum primarily impact cows during the periparturient period (Table 1).

Figure 1. Diagrammatic representation of the interrelationship between the cow, transition period and nutrition on lameness (E. Toussant Raven, 1989).

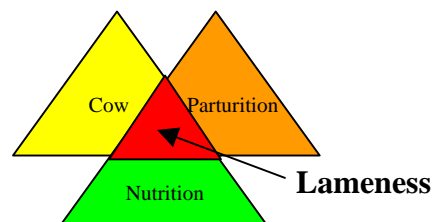


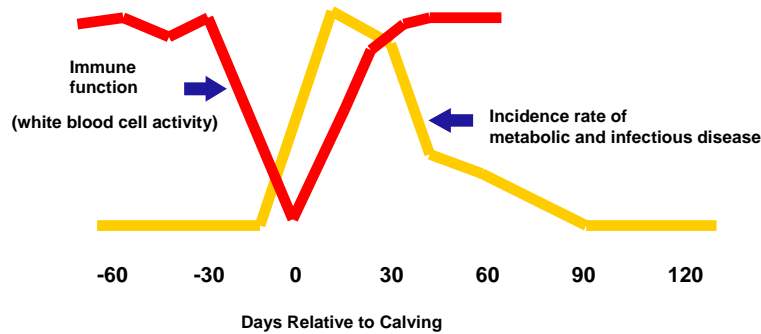
Table 1. Mean and range for incidence of selected periparturient health disorders in 61 herds of high producing dairy cows^a.

Disorder	Mean (%)	Range (%)
Milk fever	7.2	0 to 44.1
Displaced abomasum	3.3	0 to 14
Ketosis	3.7	0 to 20
Retained fetal membranes	9.0	0 to 22.6
Metritis	12.8	0 to 66

^a Adapted from Drackley (1999)

In addition, periparturient stress, hormonal shifts, and decreased dry matter intake lead to immune suppression and increased potential for metabolic and infectious diseases, during this most critical phase in the lifecycle of a cow (Figure 2).

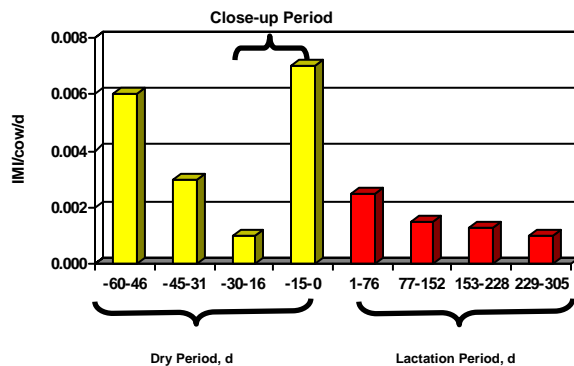
Figure 2. Immune suppression and incidence of metabolic and infectious disease during the periparturient period.



Adapted from Kehrl et al., 1999.

Immune suppression, coupled with depressed trace mineral status, would suggest a period of increased risk for new intra-mammary infections (IMI) may exist. Research at The Ohio State University indicates that incidence of IMI are highest immediately following dry-off and just prior to or at calving (Figure 3). Endotoxins from IMI result in the production of pro-inflammatory mediators which may affect blood flow to the developing claw horn and result in production of inferior quality tissue (Toussant Raven, 1989).

Figure 3. Incidence of intramammary infections (Smith et al., 1985).



Goff and Horst (1997) stated “The transition from the pregnant, non-lactating state to the non-pregnant, lactating state is too often a disastrous experience for the cow”. This is no more apparent than in the research by Godden et al. (2003) reporting 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 cull cows were leaving dairy herds by 60 DIM (Overton, 2003). Many of these cows suffer from claw abnormalities which occur in early lactation (Figure 5, Green et al., 2002) and may be partly due to the result of nutritional deficiencies or hormonal changes occurring during the periparturient period.

Figure 4. Summary of percent of cows culled by 21-day periods from 2800 Midwest dairy herds (Godden et al., 2003).

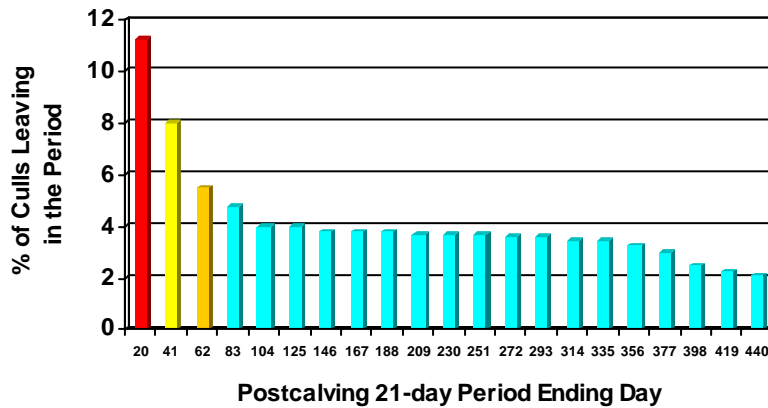
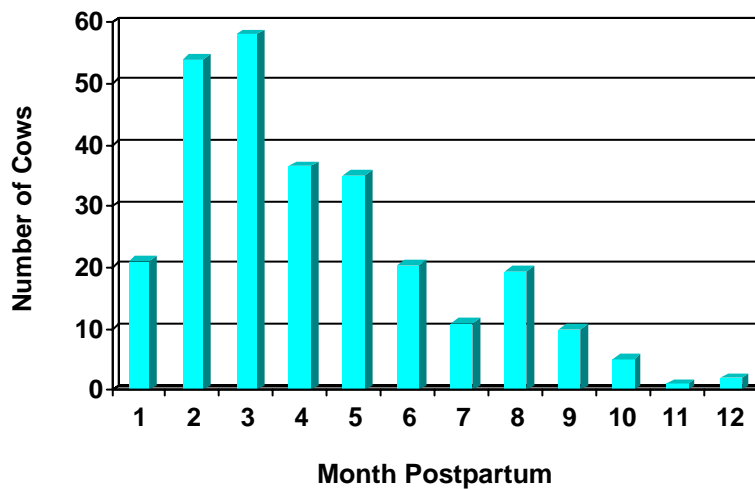


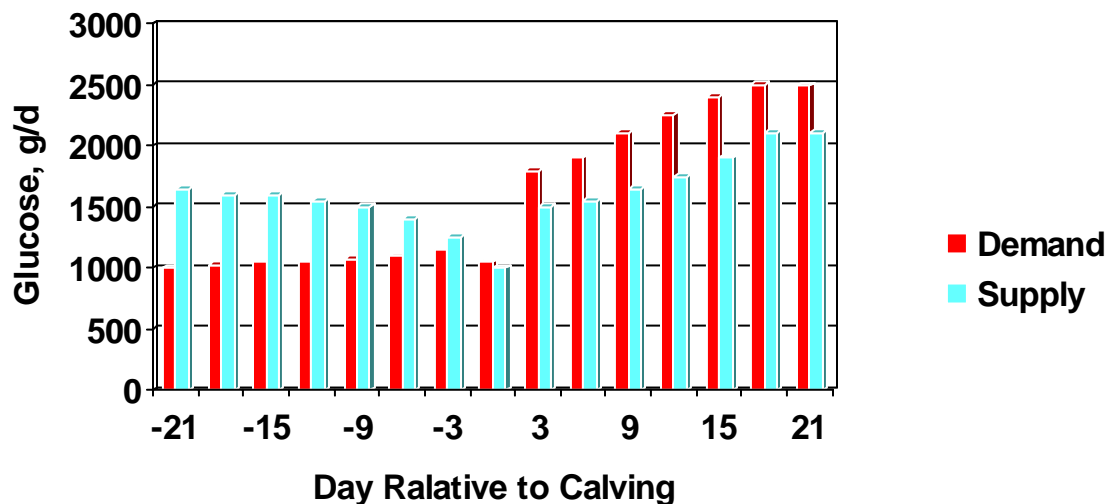
Figure 5. Incidence of lameness by month of lactation (Green et al., 2002).



Parturition and 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). Overton et al. (2000) indicated that in late gestation glucose supply exceeds demand, while post-parturition the significant demand by the mammary gland for glucose exceeds that supplied by dietary intake and gluconeogenesis. 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 (Figure 5).

Figure 6. Glucose supply and demand during the transition period of the dairy cow (Overton et al., 2000).



In research to investigate production of new claw horn, 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) dermis in the claw shares the post-receptor insulin resistance shown by other tissues during the periparturient period (Vernon, 1988).

Other hormonal changes occurring at or around parturition that may affect claw integrity:

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 (Mülling et al., 1999).

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-dependent 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).

Relaxin. Lischer and Ossent (2002) have suggested that action by the hormone relaxin may be partially responsible for development of claw horn lesions during the periparturient period. Relaxin is a hormone produced by the corpus luteum in both pregnant and non-pregnant females. This hormone has a broad range of biologic activities which include the induction of collagen remodeling and consequent softening of the tissues of the birth canal (Bani, 1997). Fibrous tissue may also be affected by this hormone (Holah et al., 2000). Lischer and Ossent (2002) have proposed that increased production of relaxin before and at parturition result in weakening of the suspensory apparatus of the third phalanx allowing it to sink and cause the development of lesions. Impact by this hormone would be similar to those proposed to result in separation of the dermal-epidermal junction as a result of nutritionally induced laminitis (Ossent, 1999). Therefore, development of claw horn lesions at or shortly after parturition may not be a result of nutritional stressors but the result of normal hormonal shifts as a result of the culmination of pregnancy and the induction of lactation.

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 (horn producing cells) 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 cystine (Cys), histidine (His) and methionine (Met) play key roles in establishing the structural integrity of the keratinocyte (Ekfalck, 1990; Ekfalck 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.

Amino acid requirements of dairy cattle are not known with much certainty (NRC, 2001). However, the NRC (2001) does suggest that high producing dairy cows may not be able to produce adequate quantities of metabolizable protein to meet the demands of milk production, especially in early lactation when dry matter intake is depressed (Marquardt et al., 1977). This lack of metabolizable protein in early lactation could contribute to insufficient protein synthesis by developing keratinocytes and thus result in production of inferior horn.

Minerals. The onset of lactation places such a large demand on mechanisms of calcium (Ca) homeostasis that most cows develop some degree of hypocalcemia at calving (Goff and Horst, 1997). This is important in that calcium plays an integral role in the keratinization and cornification process. Calcium is needed for activation of epidermal transglutaminase (TG), which is active in cross-linkage of the cell envelope keratin fibers and in addition is involved in initiation and regulation of the terminal differentiation of the epidermal cells. This enzyme helps activate the final step in the production of the mature squame (i.e., fully cornified keratinocyte) by linking of cell envelope proteins on the cytoplasmic side of the cell wall via glutamyl-lysine bonds to form a cellular envelope of high proteolytic resistancy (Mülling et al., 1999).

Insufficient Ca provided to maturing keratinocytes due to inadequate vascular supply (Nocek, 1997) or Ca availability due to hypocalcemia may lead to depressed TG activity and formation of dyskeratotic horn. Mülling et al. (1999) reported that differentiating

epidermal cells were very sensitive to changes in plasma Ca levels. They suggested inconsistent levels of Ca around parturition, in particular with the onset of lactation, will certainly influence the metabolism in differentiating epidermal cells. This may provide an explanation for the horn rings consistently observed associated with pregnancy in cows. Horst (1986) reported that between 5 and 10% of all milking cows suffer with hypocalcemia during or shortly after calving. Therefore, it may be probable that some of the laminitic insults seen in high producing dairy cows (typically moderately hypocalcemic) and those that have suffered from hypocalcemia may be in part related to impaired TG activity and its impact on terminal differentiation control and formation of the cellular envelope.

Zinc. Zinc (Zn) 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). These catalytic enzymes are Zn metalloenzymes and as such are dependent upon Zn as an activator and thus an integral component in the differentiation of keratinocytes.

Zinc also plays a key role in formation 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 Ca^{2+} and carrying Ca^{2+} 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 action of calmodulin and protein kinase C. Inositol phosphate acts to increase Ca^{2+} by mobilizing the ion from intracellular stores, primarily from the endoplasmic reticulum.

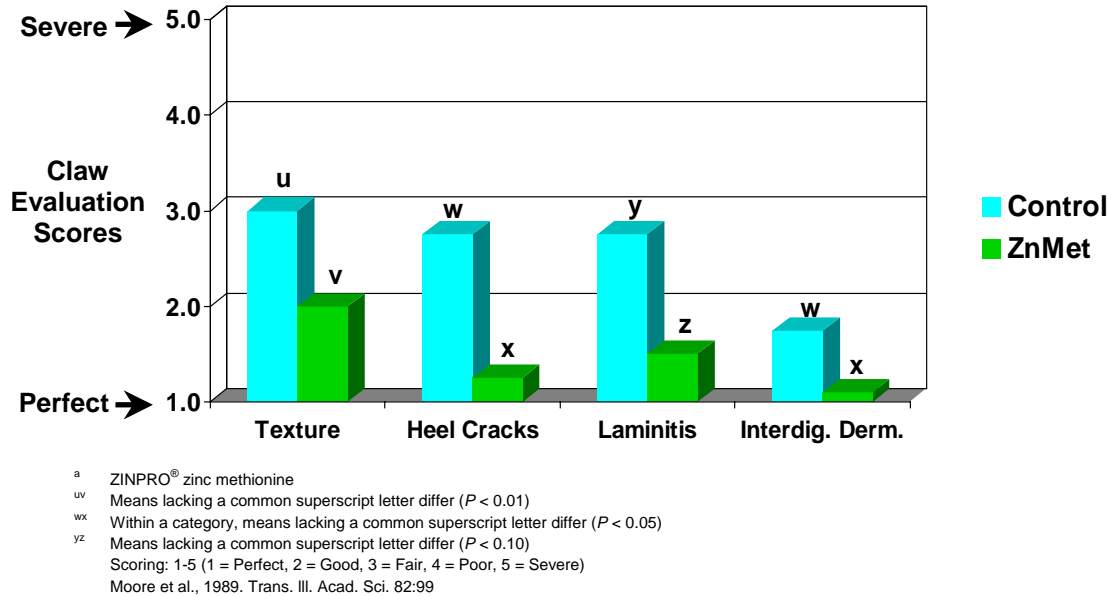
Zinc is also required for activation of the cytosolic enzyme Cu/Zn superoxide dismutase (Cu/Zn SOD). Cu/Zn SOD is responsible for prevention of lipid peroxidation. Protection of the intercellular cementing substance is critical in maintenance of the structural integrity and biological function of the 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 keratinization 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 (ZINPRO[®], Zinpro Corporation, Eden Prairie, MN) had fewer cases of foot rot, heel cracks, interdigital dermatitis and laminitis than cows not fed Zn Met (Moore et al., 1989).

Figure 7. Effects of zinc methionine^a on claw evaluation scores of dairy cattle (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 (ZINPRO) 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 (Se) is a constituent of the enzyme glutathione peroxidase. Glutathione peroxidase is responsible for reduction of H_2O_2 and free O_2 to H_2O (NRC 2001). By acting much like Cu/Zn SOD, glutathione peroxidase plays a role in protecting both the intra and extra-cellular lipid membranes against oxidative damage. This way Se may contribute to protection and maintenance of physiological function of the lipid rich intercellular cementing substance.

Excessive supplementation of Se may be damaging to developing keratinocytes. Selenium in the form of selenomethionine (SeMet) is readily absorbed by the same mechanism as Met (Combs, 2000). Inorganic Se absorption does not appear to be regulated and is quite high (>50%, NRC, 2001). Bodily storage of inorganic Se from selenite or selenate occurs as seleno-amino acids SeCys and SeMet. Combs (2000) indicated that the most biologically efficacious of these is the SeMet form, yet SeCys is also very active. Combs (2000) reported that Se and/or seleno-amino acids may be

preferentially incorporated into sulfur requiring AA sites during protein production and thus change the integrity of the protein structure.

Larson et al. (1980) reported that dairy cows supplemented with 50 mg of injectable Se (over 6.6 X NRC requirement) during the dry period suffered severe claw problems in the postpartum period. They indicated that between 48 and 69% of cows receiving the supplemental Se injection had increased lameness, sore feet, deformed claws and loss of hair from the tail versus 28 to 30% claw problems in non-supplemented cows. It is very likely that the excessive Se supplement was incorporated into keratin fibers of the maturing keratinocytes with the key Cys and Met sites replaced by SeCys or SeMet. During this process critical disulfide bridge formation may have been reduced or inhibited during the cornification process creating inferior hoof horn lacking structural rigidity with poor integrity. The recommended level of Se in dairy diets is 0.3 mg/kg DM and should be closely monitored to ensure over-supplementation does not occur, especially during the dry and early lactating periods (NRC, 2001).

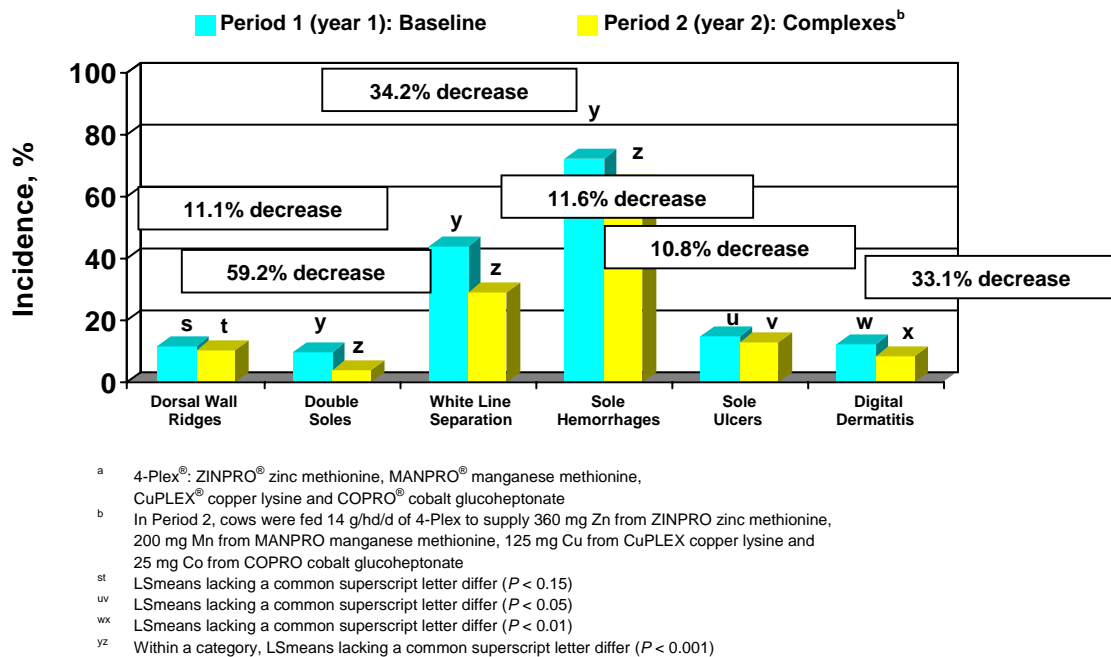
Manganese. Manganese plays an indirect role in the keratinization process. Manganese helps minimize feet problems by maintaining proper leg formation (Miller et al., 1988). Manganese is needed for activation of galactotransferase and glycosyltransferase enzymes, which are needed for the synthesis of 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 (4-Plex[®], Zinpro Corporation, Eden Prairie, MN), in combination with inorganic trace minerals, resulted in better claw integrity than cows fed only 360 mg of complexed Zn (ZINPRO) or no complexed trace minerals (Figure 8, Nocek et al., 2000).

Supplementation of the diet with a combination of complexed trace minerals reduced the incidents of double soles, white line separation, digital dermatitis, sole hemorrhages and ultimately, sole ulcerations (Figure 8). In addition, three hundred cows on a large commercial dairy in Florida were fed a combination of complexed Zn, Mn, Cu and Co (Availa[®]4, Zinpro Corporation, Eden Prairie, MN) 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 painfulness of the lesion, as compared to control cows that developed a claw lesion.

Figure 8. Effect of complexed trace minerals^a on incidence of claw disorders (Nocek et al., 2000).



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 dependent process, vitamin D is responsible for minute-by-minute calcium and mineral homeostasis. In its biologically 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 succumb to minor vitamin D deficiencies. Therefore, any lack of vitamin D will certainly impact calcium metabolism and thus affect the keratinization process.

Vitamin E. The best understood role of vitamin E is as a lipid-soluble cellular antioxidant (NRC, 2001). Via this function and possibly others, vitamin E is involved in maintenance of cellular membranes. This function may be important to the integrity of keratinized tissues as the intercellular cementing substance is composed of lipid rich material (Mülling et al., 1999). A deficiency of vitamin E at the cellular level is generally accompanied by an increase in lipid peroxidation of cellular membranes (Sokol, 1996). This may lead to decreased energy production by mitochondria, oxidation and mutation of DNA and alteration of normal transport processes of the plasma membrane (Sokol, 1996). Therefore, cells exposed to oxidative stress (i.e. a laminitic insult) will show more rapid injury and necrosis when rendered vitamin E deficient (Sokol, 1996). This may also help explain why transition dairy cows fed low levels of vitamin E and subjected to undue stress at parturition incur higher than normal levels of lameness and production of poor horn tissue (Nocek, 1997).

Biotin. A water-soluble “B” vitamin, biotin is possibly the vitamin of greatest importance to the keratinization process. Biotin is essential for the formation and integrity of the keratinized tissues (skin, hair, claws and footpads) in mammals and birds (Maynard et al., 1979). Biotin is a cofactor for enzymes used in a diverse array of metabolic pathways. Amino acid metabolism, cellular respiration, gluconeogenesis and lipogenesis involve enzymes that require biotin (Mock, 1996). Four biotin-containing enzymes are found in mammalian cells: acetyl-CoA carboxylase, B-methylcrotonyl-CoA carboxylase, propionyl-CoA carboxylase and pyruvate carboxylase. All four enzymes require biotin to become activated (Weiss and Zimmerly, 2000).

With regard to claw horn formation, biotin-dependent enzymes are directly involved in synthesis of lipids and glucose with particular importance placed on synthesis of long-chain fatty acids (Meyer et al., 1998; Weiss and Zimmerly, 2000). Mülling et al. (1999) demonstrated that biotin was essential for formation of the complex lipid molecules in the intercellular cementing substance. He also demonstrated, in biotin deficient calves, that biotin deficiency affected keratinizing epidermal cells as well as composition of the intercellular cement (Mülling et al., 1997). Research in pigs and horses has shown that biotin positively influenced the integrity of the hoof horn (Geyer, 1998).

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, thus 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, Pöttsch 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 reduced 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 effect 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 horn 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 metabolically 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 nutritional, metabolic and hormonal changes that occur during the transition period. In addition, disorders such as milk fever, ketosis, udder edema, retained fetal membranes, metritis and displaced abomasum may impact nutrient flow to new horn cells. It is the process of nutrient flow 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

1. Baggott, D. G., K. J. Bunch and K. R. Gill. 1988. Variations in some inorganic components and physical properties of claw keratin associated with claw disease in the British Friesian cow. *Br. Vet. J.* 144:534-542.
2. Ballantine, H. T., M. T. Socha, D. J. Tomlinson, A. B. Johnson, A. S. Fielding, J. K. Shearer and S. R. van Amstel. 2002. Effects of feeding complexed zinc, manganese, copper and cobalt to late gestation and lactating dairy cows on claw integrity, reproduction and lactation performance. *Prof. Anim. Sci.* 18:211-218.
3. Bani, D. 1997. Relaxin: a pleiotropic hormone. *General Pharmacology* 28:3-22.
4. Bennick, M. R., R. W. Mellenberger, R. A. Frobish, and D. E. Bauman. 1972. Glucose oxidation and entry rate as affected by the initiation of lactation. *J. Dairy Sci.* 55:712 (Abstr.).
5. Brazle, F. K. 1993. The effect of zinc methionine in a mineral mixture on gain and incidences of footrot on steers grazing native grass pastures. *J. Anim. Sci.* 71(Suppl. 1):40 (Abstr.).
6. Burhans, W. S., A. W. Bell, R. Nadeau, and J. R. Knapp. 2003. Factors associated with transition cow ketosis incidence in selected New England herds. *J. Dairy Sci.* 86(Suppl. 1):247 Abstr.
7. Combs, G. F., Jr. 2000. Development of Anti-Carcinogenic Foods from Animals. Pages 40-45 in *Proc. 2000 Cornell Nutr. Conf. Feed Manuf.*, Rochester, NY. Cornell Univ., Ithaca, NY.
8. Cousins, R. J. 1996. Zinc. Pages 293-306 in *Present Knowledge in Nutrition*. 7th ed. E. E. Ziegler and L. J. Filer, Jr., eds. ILSI Press, Washington, DC.
9. Cowie, A. T., I. A. Forsyth and I. C. Hart. 1980. Hormonal control of lactation. Berlin; New York: Springer-Verlag (Monographs on endocrinology; vol. 15).
10. Cross, R. F. and C. F. Parker. 1981. Oral administration of zinc sulfate for control of ovine foot rot. *J. Am. Vet. Med. Assoc.* 178:704-705.
11. DaCosta-Gomez, C. M., A.L. Masri, W. Steinberg and H. Abel. 1998. Effect of varying hay/barley proportions on microbial biotin metabolism in the rumen simulating fermenter RUSITEC. *Proc. Soc. Nutr. Phys.* 7:30 (Abstr.)
12. Drackley, J. K. 1999. Biology of dairy cows during the transition period: the final frontier? *J. Dairy Sci.* 82:2259-2273.
13. Ekfalck, A. 1990. Amino acids in different layers of the matrix of the normal equine hoof. Possible importance of the amino acid pattern for research on laminitis. *J. Vet. Med.* 37:1-8.
14. Ekfalck, A., B. Funkquist, B. Jones and N. Obel. 1990. Distribution of labeled cystine and methionine in the matrix of the stratum medium of the wall and in the laminar layer of the equine hoof. *J. Vet. Med.* 37:481-491.
15. Elias, P. M. 1981. Lipids and the epidermal permeability barrier. *Arch. Dermatol. Res.* 270:95-117.
16. Fitzgerald, T., B. W. Norton, R. Elliott, H. Podlich and O. L. Svendsen. 2000. The influence of long-term supplementation with biotin on the prevention of lameness in pasture fed dairy cows. *J. Dairy Sci.* 83:338-344.
17. Fraser, R. D. B. and T. P. MacRae. 1980. Molecular structure and mechanical properties of keratins. Pages 211-246 in *The Mechanical Properties of Biological Materials*. J.F. Vincent and D. Currey, eds. Cambridge: Cambridge University Press.
18. Geyer, H. 1998. The influence of biotin on horn quality of hooves and claws. Pages 192-199 in 10th International Symposium on Lameness in Ruminants. Lucern, Switzerland.

19. Godden, S. M., S. C. Stewart, J. F. Fetrow, P. Rapnicki, R. Cady, W. Weiland, H. Spencer, and S. W. Eicker. 2003. The relationship between herd rbST-supplementation and other factors and risk for removal for cows in Minnesota Holstein dairy herds. Pages 55-64 *in* Proc. Four-State Nutr. Conf. LaCrosse, WI. Midwest Plan Service publication MWPS-4SD16.
20. Goff, J. P. and R. L. Horst. 1997. Physiological changes at parturition and their relationship to metabolic disorders. *J. Dairy Sci.* 80:1260-1268.
21. Green, L. E., V. J. Hedges, Y. H. Schukken, R. W. Blowey, and A. J. Packington. 2002. The impact of clinical lameness on the milk yield of dairy cows. *J. Dairy Sci.* 85:2250-2256.
22. Grosenbaugh, D. A., D. M. Hood, M. S. Amos and J. D. Williams. 1991. Characterization and distribution of epidermal growth factor receptors in equine hoof wall lamellar tissue: comparison of normal horses and horses affected with chronic laminitis. *Equine Vet. J.* 23:201-206.
23. Grosenbaugh, D. A. and D. M. Hood. 1993. Practical equine hoof wall biochemistry. *Equine Pract.* 15:8-14.
24. Hart, I. C., J. A. Bines, S. V. Morant and J. L. Ridley. 1978. Endocrine control of energy metabolism in the cow: comparison of the levels of hormones (prolactin, growth hormone, insulin and thyroxin) and metabolites in the plasma of high- and low-yielding cattle at various stages of lactation. *J. Endocr.* 77:333-345.
25. Hedges, J., R. W. Blowey, A. J. Packington, C. J. O'Callaghan and L. E. Green. 2001. A longitudinal field trial of the effect of Biotin on lameness in dairy cows. *J. Dairy Sci.* 84:1969-1975.
26. Hendry, K.A.K., A. J. MacCallum, C. H. Knight and C. J. Wilde. 1999. Effect of endocrine and paracrine factors on protein synthesis and cell proliferation in bovine hoof tissue culture. *J. Dairy Res.* 66:23-33.
27. Holah, D. E., K. M. Evans, G. R. Pearson, J. F. Tarlton and A. J. F. Weaver. 2000. The histology and histopathology of the support structures in the laminated region of the bovine hoof in maiden heifers and around the time of first calving. Pages 109-111 *in* III International Conference on Bovine Lameness. Parma, Italy.
28. Horst, R. L. 1986. Regulation of calcium and phosphorus homeostasis in dairy cows. *J. Dairy Sci.* 69:604-616.
29. Johnson, H. D. and W. J. Vanjonack. 1976. Effects of environmental and other stressors on blood hormone patterns in lactating animals. *J. Dairy Sci.* 59:1603-1617.
30. Keen, C. L. and S. Zidenberg-Cherr. 1996. Manganese. Pages 334-343 *in* Present knowledge in nutrition. 7th ed. E. E. Ziegler and L. J. Filer, Jr., eds. ILSI Press, Washington, DC.
31. Larson, L. L., F. G. Owen, P. H. Cole and E. D. Erickson. 1980. Relationship of periparturient administration of selenium and vitamins to health status in dairy cattle. *J. Anim. Sci.* 51(Suppl. 1):296.(Abstr.).
32. Linder, M. C. 1996. Copper. Pages 307-319 *in* Present knowledge in nutrition. 7th ed. E. E. Ziegler and L. J. Filer, Jr., eds. ILSI Press, Washington, DC.
33. Lischer, C. and P. Ossent. 2002. Pathogenesis of sole lesions attributed to laminitis in cattle. Pages 82-89 *in* 12th International symposia on lameness in ruminants. Orlando, FL. J.K. Shearer, ed.
34. Lischer, C. and P. Ossent. 1994. Laminitis in cattle: a literature review. *Tierarztl Prax* 22:424-432.
35. Kehrl, M.E.Jr.; Kimura, K.; Goff, J.P.; Stabel, J.R.; Nonnecke, B.J. 1999. Immunological dysfunction in periparturient cows - what role does it play in postpartum infectious diseases? *In* The Bovine Proceedings.

36. Marquardt, J. P., R. L. Horst, and N. A. Jorgensen. 1977. Effect of parity on dry matter intake at parturition in dairy cattle. *J. Dairy Sci.* 60:929.
37. Maynard, L. A., J. K. Loosli, H. F. Hintz and R. G. Warner. 1979. *Animal Nutrition*. 7th Ed. McGraw-Hill Book Co, NY.
38. Meyer, K., D. Hazlerigg, H. Galbraith and M. A. Lomax. 1998. Characterisation of keratin proteins in bovine sole horn. Pages 210-211 *in Proc. 10th Int. Symp. on Lameness in Ruminants*. Lucerne, Switzerland. C. J. Lischer, ed.
39. Milne, F. 1985. Steroid induced laminitis. *Equine Pract.* 7:32.
40. Mock, D. M. 1996. Biotin. Pages 220-235 *in Present knowledge in nutrition*. 7th ed. E. E. Ziegler and L. J. Filer, Jr., eds. ILSI Press, Washington, DC.
41. Moore, C. L., P. M. Walker, J. R. Winter, M. A. Jones and J. M. Webb. 1989. Zinc methionine supplementation for dairy cows. *Trans. Illinois Acad. Sci.* 82:99-108.
42. Mülling, Ch., H. Bragulla and K.-D. Budras. 1997. Nutritional factors and horn quality in cattle hooves. *In Proc. of IXth Int. Conf. On Prod. Diseases in Farm Anim.* H. Martens, ed.
43. Mülling, Ch. 1998. Anatomy and structure of hoof horn. Pages 176-188 *in Proc. 10th Int. Symp. on Lameness in Ruminants*. Lucerne, Switzerland. C. J. Lischer ed.
44. Mülling, Ch., and K.-D. Budras. 1998. Der Interzellularkitt (Membrane coating material, MCM der Epidermis der Rinderklaue. *Wien. Tierärztl. Mschr.* 85:216.
45. Mülling Ch., H. Bragulla, S. Reese, K. D. Budras and W. Steinberg. 1999. How structures in bovine hoof epidermis are influenced by nutritional factors. *Anat. Hist. Embryol.* 28:103-108.
46. Mülling, Ch. 2000. The use of nutritional factors in prevention of claw diseases – Biotin as an example for nutritional influences on formation and quality of hoof horn. *In XI International Symposium on Disorders of the Ruminant Digit*. Parma, Italy. C. M. Mortellaro, L. De Vecchis and A. Brizzi, eds.
47. National Research Council. 2001. *Nutrient requirements of dairy cattle*. 7th rev. ed. Natl. Acad. Sci. Washington, DC.
48. Nocek, J. E. 1997. Bovine Acidosis: Implications on Laminitis. *J. Dairy Sci.* 80:1005-1028.
49. Nocek, J. E., A. B. Johnson and M. T. Socha. 2000. Digital characteristics in commercial dairy herds fed metal-specific amino acid complexes. *J. Dairy Sci.* 83:1553-1572.
50. Norman, A. W. 1996. Vitamin D. Pages 120-129 *in Present knowledge in nutrition*. 7th ed. E. E. Ziegler and L. J. Filer, Jr., eds. ILSI Press, Washington, DC.
51. O'Dell, B. L. 1990. Copper. Pages 261-267 *in Present knowledge in nutrition*. 6th ed. M. L. Brown, ed. ILSI Press, Washington, DC.
52. Olson, J. A. 1996. Vitamin A. Pages 109-119 *in Present knowledge in nutrition*. 7th ed. E. E. Ziegler and L. J. Filer, Jr., eds. ILSI Press, Washington, DC.
53. Ossent, P. 1999. Subclinical bovine laminitis. *Cattle Practice* 7:193-195.
54. Overton, T. R., M. S. Piepenbrink and M. R. Waldron. 2000. Interactions of liver metabolism and health in transition dairy cows. Pages 251-261 *in Proc. of Cornell Nutrition Conf.* Cornell, Univ., Ithaca, NY.

55. Overton, T. R., K. L. Smith, and M. R. Waldron. 2003. Considerations for carbohydrate nutrition of transition dairy cows. Pages 89-97 *in* Proc. of Cornell Nutrition Conf. Cornell, Univ., Ithaca, NY.
56. Plaut, K. 1993. Role of epidermal growth factor and transforming growth factors in mammary development and lactation. *J. Dairy Sci.* 76:1526-1538.
57. Pöttsch, C. J., V. J. Collis, R. W. Blowey, A. J. Packington, and L. E. Green. 2003. The impact of parity and duration of biotin supplementation on white line disease lameness in dairy cattle. *J. Dairy Sci.* 86:2577-2582.
58. Puls, R. 1984. Mineral Levels in Animal Health. Diagnostic Data. 2nd. Edition. Sherpa International, Clearbrook, BC, Canada.
59. Raven, Toussaint E. 1989. Cattle Footcare and claw trimming. Farming Press, Miller Freeman UK Ltd.
60. Reynolds, C. K., P. C. Aikman, B. Lupoli, D. J. Humphries, and D. E. Beaver. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. *J. Dairy Sci.* 86:1201-1217.
61. Smart, M. and N. F. Cymbaluk. 1997. Role of nutritional supplements in bovine lameness *in* Lameness in Cattle. 3rd ed. P. R. Greenough, A. D. Weaver, eds. W. B. Sanders Co., Philadelphia, PA.
62. Smith, K. L. D. A. Todhunter and P. S. Schoenberger. 1985. Environmental mastitis: cause, prevalence, prevention. *J. Dairy Sci.* 68:1531-1553.
63. Sokol, R. J. 1996. Vitamin E. Pages 130-136 *in* Present knowledge in nutrition. 7th ed. E. E. Ziegler and L. J. Filer, Jr., eds. ILSI Press, Washington, DC.
64. Vermunt, J. J. and Greenough, P.R. 1994. Predisposing factors of laminitis in cattle. *Brit. Vet. Journal.* 150:151-164.
65. Vernon, R. G. 1988. The partition of nutrients during the lactation cycle. Pages 32-52 *in* Nutrition and Lactation in the Dairy Cow. P. C. Garnsworthy, ed. London: Butterworths.
66. Wedekind, K. J., A. E. Hortin, and D. H. Baker. 1992. Methodology for Assessing Zinc Bioavailability: Efficacy Estimates for Zinc-Methionine, Zinc Sulfate and Zinc Oxide. *J. Anim. Sci.* 70:178-187.
67. Weiss, W. P. and C. A. Zimmerly. 2000. Effects of biotin on metabolism and milk yield of dairy cows. Pages 22-30 *in* Proc. 62nd Cornell Nutr. Conf. For Feed Manuf. Cornell Univ., Ithaca, NY.