METABOLIC ALTERATIONS TO SUDDEN INTRODUCTION OF HIGH CARBOHYDRATE DIETS IN RUMINATING DAIRY BULL CALVES

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(ABSTRACT)

The objective of this study was to investigate whether it is possible to create acute laminitis in young ruminating calves by feeding high carbohydrate diets. Three experiments were performed. In the first, 16 calves, 17 wk of age, were fed one of four diets (4 replications) that contained either 71 or 81% of TDN and either 15 or 20% CP. Jugular blood and rumen fluid were sampled and hoof temperature measured at frequent intervals over a subsequent 2-d period. Calves responded acutely to the 81% TDN diets by anorexia, stiffness and diarrhea. Ruminal pH was lower and L- and D-lactates greater in the rumen of 81% TDN treatments. Total ruminal VFA decreased as pH declined. Proportion of acetate increased while propionate decreased in 81% TDN treatments. Butyrate differed but was not dietary related. Whole blood L-lactate did not differ by treatments. Blood D-lactate increased significantly in the calves fed 81% TDN, peaking at 32 h (65 mg/dL). Hoof temperature was significantly lower in 81% TDN treatments at 28-32 h.

In the second experiment dietary sodium bicarbonate (.9% of DM) attenuated lactic acidosis in animals which consumed high quantities of concentrate. Although some animals in the buffer group suffered from acidosis, sickness was potentiated in nonbuffer group. Buffer inhibited the decline of ruminal fluid pH, and the increase of lactate in the rumen. Total VFA in the rumen declined with pH. Proportions of major VFA remained unchanged. Blood L-lactate increased at 28 h in animals which did not receive buffer. Blood D-lactate increased in both treatments and was greater in nonbuffer treatment.

In the third experiment, 24 calves, 17 wk of age, were fed diets containing either 68

or 80% TDN. The latter diet was supplemented with either ionophore, buffer or contained no supplementation. Sudden introduction of the diets resulted in transient lactic acidosis. Buffer was more efficient in the prevention of acidosis than ionophore. The acetate : propionate (Ac:Pr) ratio tended to be lower in ionophore treatment. Growth performance did not differ between the treatments. Acute laminitis was not detected but the reduction of ruminal pH and a many fold increase in blood D-lactate observed in this study, may contribute to occurrence of laminitis.

DEDICATION

I dedicate this dissertation to my family.

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CHAPTER 1

INTRODUCTION

Laminitis is an aseptic inflammation of highly vasculated corium in the hoof. It is a major cause of lameness in dairy cattle which has been regarded as the third most common problem in modern animal production. The disease shortens animal productive life. The precise etiology of the laminitis is not known. Although the multifactorial character of laminitis has been recognized, nutrition, management and genetics are considered especially important to the etiology of the disease.

Factors associated with nutrition such as lactic acid, endotoxin, and histamine, have been considered to be the most important. It has been learned from either clinical observations or experimentally induced cases that a sudden introduction of high carbohydrate diets can cause accumulation of lactic acid in the rumen and subsequent acidosis. Absorbed lactic acid into the blood may result in acidemia and depression of normal blood pH (7.4). When this occurs acidotic conditions in the rumen may promote a massive death of Gram negative bacteria which, when absorbed into the blood, cause endotoxemia. Simultaneously, proteolysis in the rumen may lead to accumulation of histamine.

Pathogenesis of acute bovine and equine laminitis is characterized with thrombosis and disturbances of the hemodynamics in the digital circulation, which results in ischemia of the hoof corium. An apparent paradox is that ischemia of the corium coincides with an increase of blood flow to the hoof. Therefore, arterial-venous shunting of blood has been proposed. Clinical findings in the affected animal may coincide with signs of systemic illness. Nevertheless, intense pain in the digits, stiffness, arched backs and lameness are indicative of laminitis, especially in the equine. Depending on severity of the disease the animal will be reluctant to move, rise and it even may eat while resting on carpal joints. Hoof temperature can increase and pedal bone in the hoof may rotate.

Pathological changes in chronic laminitis are reflected through the quality of the hoof horn. Health of the bovine digit depends on proper keratinization and adequacy of the blood supply that supports it. Anything that reduces normal blood supply to bovine hoof corium will disrupt normal keratinization and cause malgrowth of the claw. Hoof horn may be softer and overgrown. Massive hemorrhage may be seen on the sole surface.

Many attempts have been made to create a reproducible model for studying laminitis in dairy cattle. Some of these are based on either histamine or endotoxin injection, while others are based on sudden introduction of high levels of carbohydrates into the rumen.

CHAPTER 2

REVIEW OF LITERATURE

Anatomy and Histology of the Bovine Hoof

Bone and soft tissue

The digits are composed of proximal (first), medial (second) and distal (third) phalanges and related structures (Figure 2.1). The hoofs in cattle consist of central stabilizing parts and the surrounding modified skin which is hairless and extremely cornified (Geyer, 1994). These parts include the horny epidermis and the contents which include corium (dermis), digital cushion (hypodermis), distal part of the second phalanx, third phalanx (pedal bone), distal sesamoid bone, bursa, ligaments, tendons, blood vessels and nerves (Ashdown, 1984).

The pedal bone sits on the digital cushion, an elastic layer which is very important in relieving pressure inside the claw. This layer is very thin at the top of the claw and toward the toe it almost disappears. At that point the pedal bone is fixed with corium and linked to the other parts of the foot.

Below the digital cushion is the dermis or corium, a highly vascular layer that consists of the fine blood vessels and elastic tissue sandwiched between the horny hoof and toe bone (Greenough, 1992). Anatomically, dermis is divided into stratum periostale, stratum vasculosum and stratum lamelattum (laminar layer) or stratum papillare (other areas) (Maclean, 1971a). Macroscopically, it is possible to see the long leaflets of primary dermal laminae, and microscopically, on each of them, numerous secondary dermal laminae. They interdigitate with primary and secondary epidermal laminae forming an elaborate dermoepidermal complex (Leach and Oliphant, 1983). The primary function of the dermis

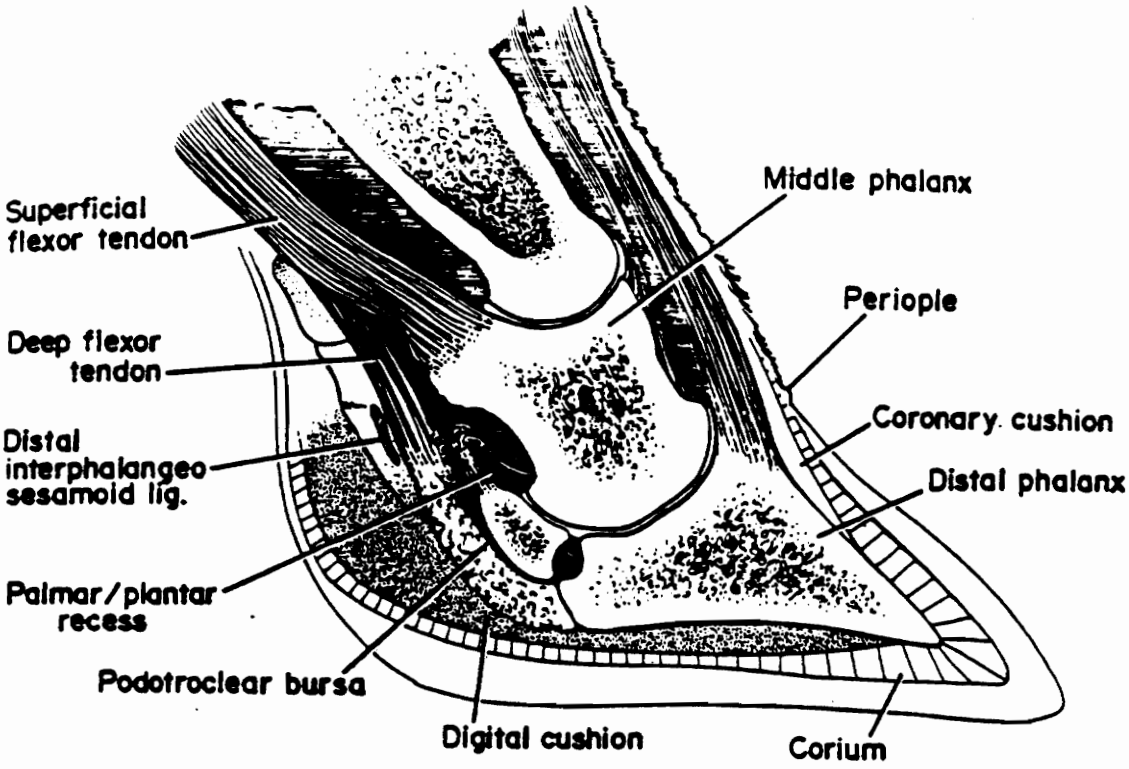

Figure 2. 1. Schematic drawing of a longitudinal section of the bovine hoof (Greenough, 1980).

is nourishment of the epidermis where the germinal layer has produced the claw or the hoof. Between the corium and epidermis is the basement membrane. Since the epidermal part is not vascular, all nutrients have to pass through the basement membrane.

The epidermis consists of the two layers, stratum germinativum and stratum corneum. Stratum germinativum consists of the stratum basale and the stratum spinosum, only living part of the epidermis. Cell multiplication occurs there. Stratum spinosum is the section in which cell differentiation, as well as cell death, occurs. Since no vascularization of the epidermis takes place, it was traditionally thought that epidermis did not receive innervation. For that reason epidermal lamina were called 'insensitive' lamina, while dermal lamina (which receive blood and nerve supply) were known as 'sensitive' lamina. More recently it was found that the germinative layer does receive some innervation, and for that reason the classification on sensitive and insensitive lamina has been abandoned. Each of the mentioned structures is a potential site of disorder and malfunction.

Blood supply to the bovine digit

New insights into a macroscopic and microscopic organization of the vascular system of the bovine hind limbs has recently been reported by Vermunt and Leach (1992a, 1992b). Using a plastic corrosion cast technique, they found that the arterial blood supply to the bovine hind digit is primarily provided by the dorsal common digital artery (*Arteria [A.] digitalis dorsalis communis III*) which is the continuation of the third dorsal metatarsal artery. Other arteries that provide blood to the digits are the common plantar digital artery (*A. digitalis plantaris communis III*) and abaxial proper plantar digital arteries (*Arteriae [Aa.] digitalis plantares propriae II et IV abaxialis*). The dorsal digital artery bifurcates within the interdigital space into two proper digital arteries (*Aa. digitales plantares propriae III and IV axiales*). All digital arteries communicate at the caudal border of the pedal bone, where they form an anastomotic network (Vermunt and Leach, 1992a).

The same authors observed that all venous blood from the claw is drained by the dorsal proper digital vein (*Vena [V] digitalis dorsalis propria III and IV axialis*) and axial and abaxial proper plantar digital veins (*Venae [Vv.] digitale plantares propriae III or IV axialis et abaxialis*). All the veins are well anastomosed within the digit (Vermunt and Leach, 1992a). Although vasculature of the hind limb was investigated, the authors pointed out that it is assumed that the vascular organization is 'almost similar' between hind and front digits. An adequate blood supply is essential to digital health. Since the vast majority of nutrient exchange occurs across the capillary bed and basal membrane, it is important to understand the microcirculation in this region. To explain bovine hoof circulation in the bovine it was proposed long ago that arterio - venous anastomoses (AVAs) exist. They were first demonstrated in horses (Pollitt and Molyneux, 1990) and then in bovine by Vermunt and Leach (1992b). The AVAs are vessels that vary in length from microns to several millimeters. They are widely present in all regions of the hoof (Vermunt and Leach, 1992b). In general, AVAs connect arterioles and venuoles, and allow blood to pass from the arterial to the venous system, thereby bypassing the capillary bed. If they are open, blood flow through the digit intensifies. Therefore AVAs are considered to be important to control of hoof temperature (Vermunt and Leach, 1992b). They are sensitive to different substances, such as histamine (Greenough, 1992) and to decreased blood pH (Walder, 1952).

The AVAs are one of the features that help to relieve the pressure inside the claw. According to Greenough (1992), there are three of these features: a) glomus bodies which are expandable vessels forming bridges between arterioles and venules into which blood can be directed if the inside claw pressure increases, b) arterio-venous shunts, simple bridges between arteries and veins which have musculature in their walls, and c) the coronary cushion which lies directly beneath the coronet and which comprises of a spongy network of veins. Talukdar et al. (1972) indicated that arterio-venous anastomoses had been observed in forty-three body areas. They were present as a direct junction of an artery and

vein, arterioles with epithelioid cells in the wall; arterioles with intimal cushions; and occasionally a glomus in a convoluted connecting vessel. These mechanisms are extremely important in maintaining normal function of the claw. If damaged, laminitis may occur (Greenough, 1992). Boosman et al. (1989) noticed that the number of 'neo-capillaries' (AVAs) increased with age. These authors proposed that the 'neo-capillary' is indeed a pathologically changed AVA shunt. The organism regulates blood flow in the hoof by providing sympathetic vasomotor tone (Eyre et al., 1979).

Growth of the hoof

As claw tissue is continually lost through surface wear, it must be continually replaced. This process of a continuous claw replacement is actualized by keratinization which occurs in the hoof epidermis. Keratins are small proteins which can be subdivided into two groups based on their sequence relatedness (Fuchs, 1988). Type I keratins are small and relatively acidic (pKi = 4.5-5.5) while type II keratin are larger and more basic (pKi = 5.5=7.5) (Fuchs, 1988). Type I and type II keratins are equally represented in keratin intermediate filaments (Miller et al., 1993). They are immunologically detected in all segments of the epidermis (Green, 1979). However, their concentration is the highest in the stratum corneum where they may account for more than 80% of the total protein (Green, 1979).

Keratinization is one of the events of cell differentiation, and keratins are important markers of differentiation (Viaene and Baert, 1994). In general, synthesis of keratin in the stratum corneum occurs in stages. Cells, which multiply in stratum basale, lose the ability of further proliferation (Green, 1979). They are pushed distally toward the surface of the claw (Leach and Oliphant, 1983). In the stratum spinosum, biosynthesis and cell mass accumulation still occur, but this is arrested when the cells reach the stratum corneum (Green, 1979). In this phase the cells are the subject of destructive processes which are

characterized by nuclear destruction and intensive keratin synthesis. Keratinization does not appear to start until the cells reached the stratum granulosum where keratohyalin appears in the cytoplasm (Maclean, 1971a). The cells, at this point keratinocytes, are essentially keratin-filled sacs with no functional organelles.

It is thought that the primary reason that this process precedes as described, is that distally-pushed cells are more distanced from the source of nutrients. An observation by Green (1979), however, does not support this proposed explanation for the cell differentiation. The author pointed out that in cultured keratinocytes, destructive processes take place in the upper layers of the epidermis. Since these layers are closest to the nutrients, they should be the best nourished and not differentiated.

From a histological point of view there are two types of keratinization: 'soft' and 'hard' (Maclean, 1971a). Soft keratinization is characterized by formation of basophilic granules (keratohyalin) in the cytoplasm of the cells in the stratum granulosum. Hard keratinization occurs in the area between the stratum spinosum and the stratum corneum, which is recognized by the appearance of acidophilic fibrils - 'keratin precursors' (onychogenic substance) (Ekfalck et al., 1985). Although keratins can be very variable in their chemical composition, the common characteristic is that their stability largely depends on the number of disulfide bonds that they contain (Clark and Rakes, 1982; Hahn et al., 1986; Manson and Leaver, 1988b; Ekfalck et al., 1990a; Singh et al., 1992).

Epidermal growth factor (EGF) stimulated cell division in cultured keratinocytes and extended their life, while inhibiting cell differentiation (Rheinwald and Green, 1977). Ekfalck et al. (1988) proposed the role of EGF as a basis for a possible new approach to study laminitis. They emphasized that EGF interferes with normal keratinization. The initial stages of laminitis may be due to a problem in keratin synthesis, therefore EGF may have an initial role. They estimated the EGF binding ability of bovine epidermis to be comparable to the capacity of the liver tissue for EGF binding. Singh et al. (1992) indicated that EGF can stimulate excessive horn production and reduce hardness of the sole.

Ekfalck et al. (1991) listed calcium, growth factors and hormones, vitamins and bacterial toxins as the factors which could interfere with keratinocyte differentiation. Green (1979) stated that all agents known to increase the level of cAMP by different means could promote a multiplication of keratinocytes, and delay in differentiation. Roberts et al. (1980) found an increase in keratinization in the animals affected with chronic laminitis. The authors pointed out that the hyperplasia resulted only when necrosis was not extensive. Funkquist (1992) concluded a short review by pointing out that laminitis can be induced by any agent that increases multiplication of hoof keratinocytes and/or subsequently suppresses their differentiation.

Hoof measurements

Very few studies related to the hoof measurements in domestic animals have been published. Since, hoof measurements on live animals involves a certain hazard, more data have been collected from slaughtered animals. More recently, a research group, lead by McDaniel, have set some standards for measuring growth and shape of the live bovine hoof (Hahn, 1979; Hahn et al., 1984a; Hahn et al., 1984b; Hahn et al., 1986; Choi and McDaniel, 1993).

Hoof measurements are important not only for comparison of animals, or expression of dysfunction, but also for calculation of heredity for certain hoof parameters. The most frequently measured parameters are the angle of the hoof, hoof length, heel depth and hoof growth and wear. Hoof angle is measured on the dorsal side of the abaxial wall with the respect to the standing surface (Hahn et al., 1984a). Hahn et al. (1984b) measured hoof angle in eight herds, and found that it ranged from 42 to 45° and that the angle decreased during the first lactation. Hoof length is the distance from the top of the wall to the lower edge (Hahn et al., 1984a). This parameter is very important because it may reflect nutritional status of the animal. Frequent overgrowth of the hoof is repeatable under certain conditions

(Glicken and Kendrick, 1977; Andersson and Lundstroem, 1981; Hale, 1985; Hahn et al., 1986; Ekfalck et al., 1990a). Also, it may be a symptom of chronic laminitis (Ekfalck et al., 1990b). Budras et al. (1989) proposed that during laminitis the hoof wall is not attached firmly to the dermis which allows proliferation of the cells in the epidermis. Heel depth is the distance from the hair line to the ground. It is not a very accurate method of measurement because it is subjective and additional training was necessary to increase repeatability between independent observers (Hahn et al., 1984a). Rates of hoof growth and wear in Holstein cattle were measured by Hahn et al. (1986). They measured monthly growth rate of 6.04 mm for front claws and 6.58 m for rear claws. At the same time rates of hoof wear were 5.78 and 6.11 mm, respectively.

Lameness - laminitis

According to definition lameness is the disability in movement of the limbs (Webster, 1987), and laminitis is a diffuse acute, subacute or chronic aseptic pododermatitis, usually affecting the tissues of several digits and with systemic signs in the acute and subacute stages (Greenough et al., 1981).

Lameness can be caused by many different diseases. Failure of nerves, bones and muscles are some of them. Most of the causes of lameness are associated with pain. Depending on the exact site of pain, the animal will express different kinds of posture. Lameness related postures are described by Greenough (1992). According to him, camping under is the posture of an animal that puts the hind legs well under the body due to pain in the toe region. If the pain is located in the heel region, an opposite type of posture, camping back, will be demonstrated. Knuckling over also indicates pain in the heel. Walking narrow points to pain in inside claws.

In more than 90% of lameness cases, the pathology is localized in the claws (Peterse, 1985). Weaver et al. (1981) observed lameness in 5.5% of 7528 cases and 88% of all lesions

were localized in the feet. Philipot et al. (1994b) wrote that 9 out of 10 cases of lameness originate from lesions on hind feet. Lameness is usually detected through locomotion scoring which is a reliable tool in spite of its subjective nature (Wells et al., 1993).

Lameness is often confused with conformational defects. Greenough and Gacek (1987) pointed out that many animals that have been culled for conformational defects were in fact affected with laminitis. Indeed, lameness is the third most common cause of culling amongst dairy cattle (Greenough, 1985).

According to many, laminitis is an important cause of lameness (Peterse, 1985; Greenough, 1990; Vermunt and Greenough, 1992). Some believe that laminitis is the most important cause of lameness (Weaver, 1979; Peterse, 1985; Mgasa, 1987; Whittier, 1993). Weaver (1979) suggested that 50% of foot lameness in the UK is caused by laminitis. Laminitis can be acute, subacute, chronic and subclinical.

Acute laminitis

Acute laminitis in cattle may occur either as a primary entity or as a complication of other diseases such as severe mastitis, metritis and acetonemia (Edwards, 1982). It is manifested by pain, muscular tremor, and in early stages, by sweating (Greenough et al., 1981). Often nothing but severe pain was found when the animal was examined (Bazeley and Pincent, 1984). The symptoms of acute laminitis observed in fattening steers were reluctance to rise, arched back and lowered head, the limb posture depended on which feet were the most painful (Edwards, 1982). Finally, difficulty in rising can cause an animal to eat while resting on carpal joints (Sonoda et al., 1977; Greenough et al., 1981). Similar symptoms were observed in ponies which, in addition were depressed and anorectic (Ekfalck et al., 1990a). Also, 'paddling' has been recognized as one of early symptoms (Greenough et al., 1981; Takahashi and Young, 1981a; Edwards, 1982). And, as Edwards (1982) writes: "Many 'paddling' cows dismissed as 'calving' or 'early milk fever' are in fact demonstrating

symptoms of laminitis".

Actual posture depends upon which feet are the most painful. It has been observed that acute laminitis primarily affects the lateral digit of hind legs in cattle (Maclean, 1971b; Greenough et al., 1981)) and medial digit in front legs (Boosman et al., 1991b). This has been confirmed indirectly by Bergsten (1994) who found more hemorrhage in the inner than in the outer front hooves, while the situation was reversed relative to hind hooves. Accordingly, it is possible to see animals having crossed legs (Edwards, 1982; Boosman et al., 1991b; Greenough, 1992). Bradley et al. (1989) found significant differences in the severity of laminitis between lateral and medial digits, while no difference was found between lateral and medial hooves from left and right legs. Similarly, Livesey and Fleming (1984) observed sole ulcers exclusively in the lateral hind claws. Peterse (1985) emphasized a great similarity in the pathological processes in left and right legs.

Increased heat in the feet during acute laminitis is not a consistent finding (Edwards, 1982), while swelling of the skin, and tenderness below the coronet and the bulbs often occur. Increased pulse rates (120 - 130 per minute) and increased respiratory rates are a common finding during the acute laminitis in cattle (Edwards, 1982). Deviation of the pedal bone (Maclean, 1971b; Sonoda et al., 1977; Andersson and Bergman, 1980; Roberts et al., 1980; Greenough et al., 1981) and its distal rotation may follow the acute phase of laminitis in cattle (Sonoda et al., 1977; Edwards, 1982; Livesey and Fleming, 1984) and in horses (Colles, 1977; Denoix et al., 1993; Hunt et al., 1993). Rotation of the distal phalanx is irreversible (Greenough et al., 1990).

Hunt (1993) summarized some data in an attempt to make a satisfactorily predictive model of the outcome of pedal bone rotation. He found that the degree of rotation correlated with the severity of lameness. In a more severely affected group of horses he measured rotation of 11.10±8.19°, while in a less affected group it was 5.89±6.48°. However, he did not find an appropriate model for good prediction.

Congestion of the dermis is present in the acute phase (Greenough et al., 1981).

Histopathology of acute laminitis shows gross hyperaemia in the stratum vasculosum, thromboses in large and small vessels, while hemorrhages are common in solar corium (Maclean, 1971a; Roberts et al., 1980; Weiss et al., 1994). Blood pressure increases (Garner et al., 1975; Clarke et al., 1982; Weiss et al., 1994).

Maclean (1971a) pointed out that thrombosis can be caused by any factor which causes adherence of platelets. Some of the most important factors are damage of the vessel wall and the presence of a foreign substance. Damage of the blood vessel wall can be caused by: a) toxins or endotoxins acting directly on the vessel wall or acting indirectly through an allergic reaction; b) edema between the unyielding horn and bone which may pressure the vessel wall; and c) an inflammatory lesion in the hoof (Maclean, 1971a).

Diagnostics of acute laminitis is based on typical symptoms of stiffness, abnormal posture, arched back, reluctance to move, heat and pain in the hooves. However, heat in the feet is an inconsistent finding (Edwards, 1982). It should be noted, however, that none of the above-mentioned symptoms are specific for acute laminitis, and clinical findings may often be outweighed by symptoms of other diseases (Boosman et al., 1991b).

Hood et al. (1978) demonstrated the hemodynamic changes in the horse foot by use of a nuclear medicine technique. They observed an apparent decrease in the regional blood flow and a decrease in capillary perfusion. A significant degree of arteriovenous shunting occurred which correlated with severity of lameness. The two capillary beds present in the hoof were not visible during the acute phase of the disease. A similar finding was reported by Weiss et al. (1994) who found a lack of blood supply to the hoof in the pony affected by laminitis.

Trout et al. (1990) employed a scintigraphic method to investigate distribution of the blood during the onset of acute laminitis. They found no decrease in lamellar blood supply during the acute phase as was observed by Hood et al. (1978), and concluded that their results do not support a concept of lamellar ischaemia as a primary cause of acute equine laminitis.

Another technique used in the study of laminitis is thermography. Turner (1991) suggested that thermography can detect slight changes in hoof temperature and blood flow. The coronary band is normally 1 to 2°C warmer than the rest of the hoof, therefore, if temperature in the rest of the hoof equals or exceeds the coronary band temperature, laminitis is present. Magnetic resonance imaging has provided another tool to study pathological changes that occur during the onset of laminitis (Denoix et al., 1993).

The normal keratinization pattern is disrupted in the epidermis, which is manifested as disorientation of the cells in stratum germinativum and complete disappearance of onychogenic substances (Maclean, 1971a; Singh et al., 1992). Epidermal lamellae are atrophic (Roberts et al., 1980). The same authors note that keratinization increased in the epidermis of the septicemic animals. Regardless of the exact mechanism, vascular leakage is a common factor in acute laminitis.

Subacute laminitis

This term relates only to cattle and depicts only animals which have suffered from acute laminitis for more than 10 days (Boosman et al., 1991b). Toussaint Raven (1989) describes subacute laminitis as a phase which takes place between acute and chronic laminitis. He pointed out extensive yellow and red discolorations in the horn of the sole, resulting from a disturbed blood supply in the corium. Again, lateral hind claws are affected (Toussaint Raven, 1989). As soon as hoof circulation is recovered, the discolorations grow off with the horn.

Chronic laminitis

By definition, chronic laminitis that lasts for more than 6 weeks (Boosman, et al. 1991b). The initial phase of laminitis is often unrecognized or does not occur. Clinical signs

of chronic laminitis depend on severity of the disease. Arched back, stiffness and pain occurs in severe cases. Stanchioned animal's standing at the edge of the gutter may indicate pain in the heel region (Greenough et al., 1981).

One of the most prominent features of chronic laminitis is distal rotation of the pedal bone (Greenough et al., 1981). In the normal hoof there is no angle between distal surface of the pedal bone and the sole surface, and likewise for the dorsal border of the pedal bone and dorsal hoof wall (Greenough et al., 1981). In laminitis, however, rotation of the pedal bone may occur and an angle between pedal bone and appropriate surface can be detected. The same authors indicate the rotated bone may be only 5 mm from the sole in the toe region, while 9-12 mm in the plantar region. Maclean (1971b) reported that the overall thickness of the sole can be decreased in affected animals. He measured 9.7 mm thickness in control animals while in affected ones it was only 5.8 mm.

Detection of pedal bone rotation by lateral radiography is the most important clinical sign of chronic laminitis (Greenough et al., 1981). O'Grady (1993) pointed out that radiographic evaluation should be an integral part of the management of a laminitis case. Rotation of the pedal bone in the horse can be relatively slow in the subacute and chronic cases (Colles and Jeffcott, 1977). Andersson and Bergman (1980) reported that no macroscopic changes could be detected in the reference material, while pedal bone deviation was found in 35% of hooves examined and hemorrhages were frequently found in the horn of the sole.

Microscopic examination reveals a pathological process which was marked with hyperaemia, edema, local fibrinous thrombi and hemorrhages. Hyperkeratosis and parakeratosis were frequently observed in chronic laminitis. The authors emphasized the arteriosclerotic changes that were primarily located in blood vessels in the corium. They concluded that hypertension which may occur during the onset of acute laminitis was responsible for the arteriosclerosis and arteriolosclerosis, and that the endothelium is the site of primary insult. In other words, even in this phase, pathological processes are still marked

by vascular changes (Maclean, 1971a; 1971b). Hypertension in the horse may last more than three weeks after the cessation of clinical signs (Colles and Jeffcott, 1977).

Dilation of venules and arterioles were accompanied with hypertrophy of tunica media, proliferation of the tunica intima and fibrosis of the tunica adventitia (Maclean, 1971a). Hyalinized thrombi were found in small blood vessels (Maclean, 1971a; Andersson and Bergman, 1980) which caused recanalization of the blood and neocapillary formation (Maclean, 1971a). Dermal laminae were swollen, broader than normal, while epidermal laminae were compressed in appearance (Maclean, 1971a; Andersson and Bergman, 1980) and in some cases onychogenic substance had disappeared completely (Maclean, 1971a; Greenough et al., 1981).

Boosman et al. (1989) did find sclerotic and proliferative processes in the bovine claws, however, there was no difference in the intensity of degenerative changes between animals that had severe laminitis and normal ones. Based on this, the authors suggested that it is possible to induce chronic laminitis in the bovine solely by altered hemodynamics (ischemia) in the digit. Ischemia can also be a cause of downer cow syndrome (Cox, 1982).

By angiographic studies, chronic laminitis was characterized by the existence of completely avascular areas. Ackerman et al. (1975) proposed that alteration in hoof vascularity could be a major factor in the development of abnormal feet because it would interfere with normal keratinization.

Changes caused by chronic laminitis are primarily located in the hooves and could be seen as concavity of the dorsal hoof wall, irregular hoof shape and hoof overgrowth. Some of these were confirmed by Andersson and Lundstroem (1981). Flattening of the hooves relative to the walking surface, which is usually associated with hoof overgrowth, is one of the indicators for chronic laminitis (Greenough et al., 1981). Another clinical sign that is often associated with laminitis, is formation of ridges on the dorsal wall (Greenough et al., 1981; Boosman et al., 1991b; Greenough, 1992). These ridges are parallel to the coronary margin. By measuring their distance from the coronary margin it is possible to

estimate the time of the primary insult (Toussaint Raven, 1989; Boosman et al., 1991b; Greenough, 1992). The "hardship groove" is another term used by cattlemen to describe a shallow groove that may be found running around the hoof wall parallel to the coronary band (Greenough, 1992). This records events that might interfere with normal horn synthesis.

Subclinical laminitis

When cattle are not affected by clinical laminitis, yet the sole shows signs characteristic of laminitis 80-100 days postpartum (hemorrhages, yellow discoloration, sole lesions) then the term subclinical laminitis applies (Boosman et al., 1991b). It is difficult to distinguish subclinical laminitis from the chronic form. Peterse (1985) writes that the process remains subclinical as long as there is no sole ulcer which is a part of laminitis. No lameness is apparent in animals affected by subclinical laminitis, and only hoof trimming would reveal existence of it (Boosman et al., 1991b). According to some (Andersson and Lundrstroem, 1981; Bradley et al., 1989) subclinical laminitis is a major predisposing cause of other forms of lameness. Thus, Greenough (1985; 1992) proposed that if the annual incidence of lameness exceeds 5%, the presence of subacute laminitis should be considered. In addition, when more than 50% of a herd is lame within the fifty days post partum, subclinical laminitis is present (1985). Weaver (1985) declared that if the incidence of lameness exceeds 15%, it was a problem herd.

Significant changes may take place in quality of the claw. Greenough (1985) writes that the claw from an affected animal is softer and contains more moisture. This is in agreement with Maclean (1971b) who found great differences in moisture content in hooves from laminitic and normal animals. He measured 26.8% moisture in the abaxial wall vs 15% , 28.7% vs 18.2% in axial wall, 34.7 vs 14.1% in sole and heel, and 66.2% vs 16.2% in horny laminae, in laminitic versus normal animals, respectively. Overall, moisture level of normal horn varied from 14 to 18%, but affected horn showed variation from 27 to 66%.

To determine at what age symptoms of subclinical laminitis appeared, Bradley et al. (1989) investigated hooves from 136 Holstein-Fresian cattle, which varied from 4 mo to 2 yr of age. They found horn hemorrhages in calves as young as 5 mo. In general, sole hemorrhages were more severe in animals older than at 10 mo. They concluded that laminitis of varying degrees is a common condition in young dairy heifers. Similarly, Bergsten (1994) found a negative correlation between the total hemorrhage and the age of the cows, while Andersson and Lundstroem (1981) reported an opposite trend.

Frankena et al. (1992) investigated sole hemorrhages in young female dairy animals in a large number of Dutch farms. In total, 1141 calves were investigated. No clinical lameness was observed but sole hemorrhage affected 37.9% (433) of the calves. Greenough (1985) indicated 'toe ulcers' were associated with subclinical laminitis. Bradley et al. (1989) wrote that subclinical laminitis is considered to be a major predisposing cause of other hoof problems, particularly sole ulcers in newly calved heifers. Sole ulcers nearly always occurred in the sole of the outside claw of the hind feet (Greenough, 1992).

Etiology of laminitis

Despite the large body of data that has been collected either by experimental approach or by clinical observations, the precise cause of laminitis is not known. This has led researchers to believe that the disease is multifactorial (Weaver, 1979; Edwards, 1982; Mortensen and Hesselholt, 1986; Mgasa, 1987). Among the most important factors are lactic acidosis, histamine and gram negative endotoxemia (Boosman et al., 1991a). Other factors such as metabolic, season, housing, genetics and management are also considered as important to the etiology of the disease (Edwards, 1982).

There is no agreement on the site of primary insult. There are two schools of thought (Robinson et al., 1975): a) acute laminitis is defective keratin synthesis, which leads to damage of the laminar corium and subsequent changes in the blood flow, or b) laminitis

results from the decreased blood flow to the hoof with increased flow through the tissues at the coronary band. Authors generally favor the second option (Maclean, 1971a; Ackerman et al., 1975; Robinson et al., 1975; Robinson et al., 1976; Hood et al., 1978; Roberts et al., 1980; Peterse, 1985; Singh et al., 1992).

There is an apparent contradictory phenomenon: in the acute phase of the disease there is an increase in blood flow to the hooves which is simultaneously accompanied with an ischaemia in the digit. An explanation for this is presence of anastomoses which shunt the blood away of the dermis. This was clearly demonstrated on scintillographic angiography images of the hoof from animals that had experimentally induced laminitis (Ackerman et al., 1975). It clearly demonstrated that hoof perfusion was poor during the onset of laminitis, while it improved after the insult was gone.

Based on these results Ackerman et al. (1975) concluded that changes in circulation caused by endotoxin, in this case, could alter elasticity of the arterial wall, and predispose the artery to spasm. Ultimately, this leads to irregular keratinization and abnormal hoof development which was confirmed by results of histological studies in dairy cows by Maclean (1971a). He found that the regular arrangement of the cells disappeared during the acute laminitis onset, that was accompanied with simultaneous disappearance of onychogenic substance. This finding was confirmed by other authors (Ekfalck et al., 1985; Ekfalck et al., 1991; Singh et al., 1992). Also, Maclean (1971a) emphasized that the vascular changes are primary during the acute as well as the chronic expression of laminitis.

Considering the structure of the foot, blood flow is very important to the normal replacement of the claw. Mechanisms for relief of the claw pressure have already been mentioned. When these mechanisms fail to function properly, problems are created. The first phase of laminitis is characterized by increased blood volume that comes into the foot. It is assumed that pressure-relief mechanisms are paralyzed by vasoactive substances causing increased pressure in the claw. In this phase blood leakage under the horn claw occurs which is visible from the outside as the places of pink coloration of the sole (Greenough,

1992). Gradually, horn of poor quality is produced, which is the result of the decreased blood flow through capillary network, and decreased supply to the germinative layer. Similar results were reported by Ekfalck et al. (1988). In this study with horses it was found that inhibited differentiation of keratinocytes is the most prominent feature during the earliest stages of laminitis. Blood flow could be influenced by either changes in systemic circulation or locally.

An elaborate network of blood vessels, which consists of a large number of capillaries that have very small volume, could be blocked by clot or collagen (Andersson and Bergman, 1980). In a case of incomplete blocking, nourishment will be reduced, leading to poor hoof quality. However, in a case of complete blockage of the capillaries, a part of the germinative layer which receives nutrients from the blocked circulation, will suffer from ischemia and subsequent necrosis (Ackerman et al., 1975; Andersson and Bergman, 1980; Toussaint, 1989; Greenough, 1992). Longer existence of such conditions may lead to the occurrence of a sole ulcer, one of the symptoms that laminitis is the underlaying cause of lameness (Livesey and Fleming, 1984; Peterse et al., 1984; Lucey et al., 1986; Bradley et al., 1989).

Apparently the ischaemia that occurs in the acute phase is accompanied with lamellar hypoxia (Hood et al., 1993). When circulation is reestablished a reactive hyperaemia occurs, causing a tingling or pain. This happens in the human as well and is known as a Raynaud's phenomenon.

Nutrition

Nutritional factors have been recognized as among the most important in the etiology of laminitis (Weaver, 1979; Mgasa, 1987; Greenough, 1992). Describing the relationship between laminitis and nutrition, Greenough (1992) writes that there are very few common facts that apply in every circumstance. Nutrition is cited as the reason for differences in the

manifestation of chronic laminitis between dairy and beef cattle (Singh et al, 1992). So far, many findings have been reported which illustrate a relationship between laminitis expression and a particular feed component. The most frequently referred are energy, protein and crude fiber level, barley, frequency of feeding, histamine and appearance of toxic components originated by lysis of gram-negative bacteria.

Energy

"A diet rich in carbohydrate is the most important contributing factor in laminitis" (Greenough, 1990). The feeding of diets containing over 70% TDN (Greenough, 1990; Greenough et al., 1990) as rapidly digestible carbohydrates, has been recognized as one of the most important factors leading to onset of laminitis (Suber et al., 1979; Takahashi and Young, 1981b; Peterse et al., 1984; Hale, 1985; Hahn et al., 1986; Sprouse et al., 1987; Manson and Leaver, 1988a; Manson and Leaver, 1988b; Whittier, 1993). Edwards (1982) emphasized carbohydrate overload in the peripartum period and the period of the peak milk yield as a likely cause of laminitis. Using a high starch diet, based on flaked corn, Suber et al. (1979) caused laminitis in three of six steers. The animals were engorged through permanent ruminal fistulas, and acute laminitis occurred 14-16 h after the engorgement.

Mortensen and Hesselholt (1986) observed reduced feed consumption and feed efficiency in dairy cows fed 12 kg of concentrate per day when compared to those fed 6 kg of concentrate. More hoof lesions occurred in the high concentrate group and tended not to regress at the end of lactation. Peterse et al. (1984) observed an increase in the occurrence of sole ulcers in cows fed concentrate diets. They suggested that high levels of concentrates influenced the metabolism in such a way that sole ulcers developed. The authors added that it is possible that even small increases in some substances (lactate, endotoxin, histamine) which may be produced during a period of decreased ruminal pH would result in sole ulcers. In a similar approach Manson and Leaver (1988b) fed cows two

levels of concentrate, 7 and 11 kg. They observed more lameness in the group fed 11 kg of concentrate. Although numbers of affected animals did not differ statistically between the treatments (6 of 24 animals for 7 kg of concentrate vs 9 of 24 animals for 11 kg concentrate), severity and duration of the lameness were higher in high concentrate fed group. Weekly rates of lameness were .077 for high group vs .021 for low concentrate group.

Occurrence of laminitis in Holstein steers in feedlot was described for the first time in Japan by Sonoda et al. (1977). Some animals were seen resting on their carpal joints. The authors theorize that the presence of breakdown products of concentrates in the rumen of the steers was a major cause of observed pathology. Yearlings and calves fed 73.5 and 78.5% TDN diets had an increased prevalence of toe and heel hemorrhages in calves and heel hemorrhages in yearlings when the high energy diet was fed (Greenough et al., 1990).

One of the most convincing evidences of the importance of high carbohydrate is a reproducible model for creating laminitis in horses introduced by Garner et al. (1975). The diet is based on high starch and wood flour gruel which is administered via stomach tube at the dosage rate of 17.6 g/kg. Eleven of twelve horses developed laminitis within 32-48 h after the dose. Hale (1985) considered high carbohydrate diets as a common nutritional background for both liver abscesses and founder in feed lot cattle.

Feeding barley has been mentioned by several authors as a specific factor in etiology of laminitis. Weaver (1971) reported a high incidence of laminitis after barley was introduced into the ration of heifers and dairy cows at one farm. Bargai et al. (1992) emphasized the role of barley in the outbreak of laminitis. Maclean (1971a) suggested that there is a toxic factor in barley leading to laminitis.

Fiber

Crude fiber is also potentially important in laminitis onset (Bazeley and Pinsent, 1984; Livesey and Fleming, 1984; Peterse et al., 1984; Greenough, 1992). A typical diet in early lactation is high in starch and low in fiber (Livesey and Fleming, 1984). The diet that contained low fiber - high starch levels (16.5% crude fiber), caused an incidence of 68% laminitis and 64% sole ulcers, in contrast to 8% for each in cows fed 19% crude fiber diets (Livesey and Fleming, 1984).

Similar results were found in a study that was based on the feeding of 12 kg concentrate (low roughage) which caused more digital lesions in the cows than the feeding of 6 kg concentrate (high roughage) (Mortensen and Hesselholt, 1986). Sole ulcers which have been considered as a symptom as well as a cause of laminitis, mostly occurred in animals fed low fiber diets (Bazeley and Pinsent, 1984; Livesey and Fleming, 1984; Peterse et al., 1984). In an epidemiologic study done on 23 Danish herds, Enevoldsen et al. (1991a) found that the roughage ration is more likely to be low in fiber and high in protein content during certain periods of the year.

Kaufman (1976) recommends feeding 20% crude fiber in the diet of high producing cows as a minimum to maintain a ratio of Ac:Pr of 3:1 in the rumen. Forage needs to comprise at least 30% DM of the diet in order to maintain stable rumen conditions (Greenough, 1992). Greenough stressed the importance of the crude fiber form (less chopping - longer fiber) for increased amounts of the 'effective fibre'. Whittier (1993) described an outbreak of lameness at the Virginia Tech Dairy which was apparently due to a low level of effective fiber. Physical length of the fiber should be 2.5 cm or greater Vermunt and Greenough (1994). Greenough (1990) considered feeding silage as a predisposing factor of laminitis. Greenough and Gacek (1987) observed that good quality silage contains a high amount of lactic acid. Laminitis decreased when cattle returned from concrete floor to straw bedded housing, probably due to eating straw.

Presence of effective fiber in the diet is important to chewing and salivation. It is well documented that saliva helps in maintaining favorable rumen pH. The most important consideration in relating saliva secretion to buffering capacity is the amount of saliva secreted per unit of energy intake (Kromann, 1975). Saliva contributes more than 70% of the water and most of the salts to the rumen (Bartley, 1975). In a 700 kg cow consuming hay and grain, 24-h saliva production equals 190 kg yielding 1100 g of $NaHCO_3$, 350 g of Na_2HPO_4, 100 g of NaCl, 30 to 80 g of nitrogen, protein equivalent 200 to 500 g, and 50 to 130 g of urea (Kromann, 1975).

Changes in the rumen

There is wide agreement that rumen lactic acidosis is probably the most important contributing factor to laminitis which is a result of rapid fermentation of sugars in the rumen (Dunlop, 1972). This is characterized as an increase in the concentration of rumen lactic acid which could be followed by an increase of blood lactic acid. This may be associated with Gram negative endotoxemia, the result of shifting of rumen microbial population, and an increase in histamine concentration. Overall outcome and course of lactic acidosis are dependent upon the rate of entry of lactate into body fluids (Huber, 1975).

Lactic acidosis

Lactic acid is an organic acid present in two isomeric forms, L-(+)- and D-(-) lactate. The capital letters indicate absolute configuration of the molecule (Dunlop 1972). The pKa estimated to be about 3.87 (Dunlop and Hammond, 1965). Fermentation of rapidly digested carbohydrates increases the rate of lactic acid production in the rumen. When production rate exceeds the rate of its metabolism, lactic acid accumulation in the blood may occur (Dunlop and Hammond, 1965). They found an exponential decline of added lactate (11.4

mM/kg) with half removal time of 33 min. They refer to several factors which influence level of lactate in the rumen such as passage to the lower tract, dilution by saliva; absorption through rumen epithelium; and changes in the amount of water in rumen. Lactate anion was poorly absorbed from the rumen, while free acid was absorbed fairly rapidly (Dunlop and Hammond, 1965).

Rate of absorption of L- and D-isomers from the rumen into the blood appears to be equal (Dunlop, 1972; Huber, 1975). In a case of simple diffusion, D-lactate would be absorbed slightly faster because its concentration in the blood is normally near zero (Dunlop, 1972) . Differences in blood concentrations of the isomers after absorption is explained by a lower capacity of bovine liver to metabolize D-lactate. Dougherty et al. (1975b) confirmed that D-lactate is poorly metabolized. Huber (1975) added that the reduced rate of D-lactate metabolism is due to an absence or reduced activity of a D-specific lactic dehydrogenase. Jones (1993) offered a schematic explaining the reasons for observed stereospecificity of lactate dehydrogenases. Harmon et al. (1985) measured the rates of L- and D-lactate absorption and metabolism in steers with subacute and acute acidosis. L-lactate absorption rates were 97 and 164 mmol/h in subacute and acute acidosis, respectively; and D-lactate were 10.5 and 71.8 mmol/h. L-lactate absorption increased only 70% while D-lactate absorption increased by sixfold.

Dunlop and Hammond (1965) measured pH of ruminal fluid as low as 4.0 in a steer engorged with soluble carbohydrate. The ratio between the two lactate isomers, L:D, ranged from 2.5:1 to 1:1.2. Blood lactate increased and blood pH decreased from 7.4 to 7.0. D-isomer was the major isomer in the blood and was apparently absorbed in an undissociated form. Metabolic half-life for injected L-isomer was 22 min which compared to 108 min for D-form. Rate of metabolism of D-lactate was about .2 mM/kg BW/h (Dunlop, 1972), and 49 mmol/h (Harmon et al., 1985).

Harmon et al. (1984) compared the rates of D-lactate metabolism in bovine tissues at varying substrate concentrations to that of L-lactate utilization. They found bovine tissues

actively metabolize both isomers. Kidney cortex is the most active tissue. Rates of oxidation of isomers were similar at .1 mM, but, at 50 mM, D-lactate utilization decreased relative to L-lactate.

Rapid metabolism of L-lactate could be why Takahashi and Young (1981b) found no change in blood L-lactate in soluble carbohydrate engorged bulls, probably due to rapid metabolism of L-lactate. Dougherty et al. (1975b) engorged both sheep and cattle by feeding 70 g of grain per kg of BW. Blood D-lactate increased while blood L-lactate remained mostly unchanged. Huntington and Britton (1978) observed a significant shift from predominantly L-lactate to almost all D-lactate by 40 d in concentrate-fed sheep.

Since acidosis is associated with an increase in osmotic pressure of the rumen content a hemoconcentration is often present. This leads to a decrease of the plasma volume and subsequent reduction of tissue perfusion rate. Under these conditions any absorption of lactate would lead to accumulation (Dunlop and Hammond, 1965). A decrease in blood pH and an increase in lactic acid concentration (Dougherty et al., 1975b; Suber et al., 1979) causes change in the capillary wall and subsequent change in circulation. Low plasma L-lactate corresponded to low blood pH. So, Suber et al. (1979) concluded that these two parameters were not related in the typical acid - pH meaning.

Lactic acid has an negative effect on certain cells, which may lead to permanent damage and abnormal hoof growth (Hale, 1985). Some attention should be devoted to the interaction between rumen pH, buffering and protozoal activity, since protozoa have a central role in lactate metabolism (Newbold et al., 1986).

More frequent feeding of concentrate is thought to reduce the incidence of acidosis (Kaufmann, 1976; Manson and Leaver, 1988a), which may reduce the expression of laminitis. Bergsten (1994) found a negative correlation between hemorrhage in the hoof and the number of concentrate feedings per day. Kaufmann (1976) found that higher feeding frequency resulted in less decline in rumen pH. Slyter and Rumsey (1991) suggested that concentrate should always be available in order to prevent engorgement and acidosis.

Nagaraja et al. (1978) engorged two cows with 1:1 mixture of ground corn and wheat through a rumen fistula at the rate of 55 g/kg BW. Ruminal pH dropped from 7.5 to 4.1. Simultaneously, blood L-lactate increased from 17 mg/dL to 47.0 mg/dL. Severe diarrhea, dehydration and laminitis occurred.

Clinically, laminitis is quite similar in cattle and sheep (Dougherty, 1975). D- lactate increased in carbohydrate engorged sheep (70 g of grain/kg BW), from near zero to 79.5 mg/dL in 72 h post-feeding. At the same time L-lactate varied but in a different manner and magnitude. In a different experiment, laminitis was associated with symptoms of diarrhea, anorexia, hemoconcentration and drop of ruminal pH as low as 4.0. Laminitis was primarily observed in front legs. Garner et al. (1977) engorged 31 horses with a diet to induce laminitis and got a positive response in twenty-one animals. L-lactate increased in the blood from .76 mM/L to 2.35 mM/L.

Hoof overgrowth in cattle usually has been associated with high energy (Sonoda et al., 1977; Hale, 1985; Greenough, 1992) or high protein diets (Manson and Leaver, 1988a). Hoof growth was fastest in animals fed a high carbohydrate diet (Manson and Leaver, 1988a). However, Greenough et al. (1990) did not find a significant correlation between excessive horn growth and energy or protein intake. Mackey (1975) proposed several measures to prevent or treat acidosis in cattle. He believed that addition of 30 g of sodium bicarbonate per 100 kg of BW is good husbandry practice.

Endotoxin

Since it has not been possible to explain all adverse effects of systemic acidosis by lactic acid only, attention of workers has focused on endotoxin. Endotoxins are a group of thermostable lipopolysaccharides that result from degradation of gram negative bacteria. During rumen acidosis gram negative bacteria die and the resulting endotoxins cause pathological effects (Dougherty et al., 1975a; Vermunt and Greenough, 1994). Dougherty

et al. (1975a) observed an increase of endotoxin in three engorged sheep and one steer. Nagaraja et al. (1978) observed a 15 to 18 fold increase of endotoxin in engorged animals which was attributed to lysis of gram negative bacteria. However, *in vitro,* they found endotoxin increased without a proportional decrease in number of gram negative bacteria. They proposed that low pH favored release of endotoxin from intact gram negative bacteria.

Since it is possible to cause laminitis by single injection of endotoxin in cows (Mortensen and Hesselholt, 1986) it became clear that it plays a role in etiology of laminitis. Boosman et al. (1991a) did not cause acute laminitis by endotoxin injection. Endotoxins trigger other pathologic events such as thrombosis in the hoof vasculature and changes in hemodynamic aspect of the corium (Boosman et al., 1991b). Although they did not confirm laminitis in horses given endotoxin, Duncan et al. (1985) did observe some signs of hoof discomfort, like shifting weight from one front hoof to the other. Simultaneously, hoof temperature decreased 3°C which may be explained by blood shunting. No difference occurred in endotoxin concentration in the rumen between high and low concentrate fed groups, and no animals suffered from endotoxemia (Mortensen and Hesselholt, 1986).

Garner et al. (1978) studied the changes in the caecal flora in the horse during laminitis. They found that *Lactobacillus spp.* and *Clostridia spp.* increased, while *Streptococci spp.* and Enterobacteriaceae spp. decreased by 8 h after carbohydrate overload. The authors supposed that death and lysis of gram-negative bacteria could cause the release of large amounts of endotoxin into the caecum.

Chaplin and Jones (1973) observed a rapid increase in rumen *Lactobacilli* peaking 48 h after the engorgement of sheep with ground barley. *Streptococci* increased, peaking 18 h after the engorgement, and were accompanied with increased ruminal lactate from 1 to 100 mM. Rumen pH fell rapidly to 4.2. Moore et al. (1979) found an increase in Streptococcal and Lactobacillus populations which coincided with a decrease in intraluminal (cecal or ruminal) pH.

Endotoxin alone might not cause the described pathology so a mechanism of activation, enhanced absorption or interaction with other nutrients, is necessary to promote the activity of endotoxin (Dunlop, 1972). Burrows (1979) observed in endotoxin-treated ponies that blood lactate of 70 mg/dL was associated with lethality while those with a concentration with 40 mg/dL recovered. A significant increase in blood lactate was a reflection of the anaerobic metabolism in tissue which was accompanied by a peripheral perfusion deficiency (Burrows, 1979). Sprouse et al. (1987) measured the increase of endotoxin from 0.1 ng/l to 2.4 - 81.5 ng/l in laminitis-induced horses. Endotoxemia was present in 11 of 13 lame animals. However, the authors warned that gram-negative endotoxins may not be involved in all cases of acute laminitis. Hunt (1993) noted that laminitis was more frequent in horses which suffered from gastrointestinal disorders, indirect support for the role of endotoxin in the etiology of laminitis.

Histamine

Early recognized as a mediator of inflammatory reactions, histamine has been the subject of considerable research. It increases permeability of blood vessels, which may be an important factor in pathogenesis of laminitis. A permeability concept, however, was questioned by Robinson et al. (1976).

Role of histamine on circulatory mechanisms has been searched extensively. Although it is known as a vasodilator, it appears that histamine has primarily a vasoconstrictive role of importance in laminitis. Elmes and Eyre (1977), found that histamine caused vasoconstriction in both arteries and veins but digital vein strips were 100X more sensitive than arterial. Since histamine activity was inhibited by mepyramine, which is H_1 blocker, they concluded that H_1 type of receptor predominates in bovine hoof vasculature. Relative to the *in vivo* limb perfusion, Elmes and Eyre (1977) found dose-dependent increases in perfusion pressure following histamine injection. They postulated

that the venous portion of the perfused bed was responsible for the greater proportion of the resistance changes. Takahashi and Young (1981b) found, however, a reduction in digital and systemic arterial blood pressure upon histamine injection, while digital vein pressure remained unchanged.

Rautschka et al. (1991) tried to relate histamine level, laminitis and corticosteroid interactions. Using radioenzymic methodology, higher levels of histamine were found in the circulation of laminitic horses (12.46 nmol/l) than in non-laminitic animals (4.46 nmol/l). Histamine release from basophils and histamine absorbed from the gut, were possible sources for histamine increase. Corticosteroids did not increase plasma histamine.

Eyre et al. (1979) investigated the effect of histamine, norepinephrine, epinephrine and serotonin on arterial and venous strips with or without addition of corticosteroids. They found that all preparations contracted in a dose-dependent manner, but contractility in venous strips was 4X greater than in arterial strips. While corticosteroids did potentiate the effects of norepinephrine, epinephrine and serotonin, they only slightly potentiated the histamine effect. Robinson et al. (1975) measured the histamine effect on the blood flow in ponies. They concluded "that histamine in large doses actively decreases vein caliber." In a later study with horses, the role of histamine was not confirmed leading the authors to conclude that a combination of factors may affect hoof circulation (Robinson et al., 1976). Suber et al. (1979) found 3 µg/mL of acetylhistamine in the blood of carbohydrate-foundered cattle that had become laminitic. Ruminal fluid histamine peaked at 12 h after the engorgement, but there was no correlation between ruminal and blood histamine. They did find a significant increase in blood acetylhistamine.

Dain et al. (1955) observed a direct negative correlation between the level of histamine in the ingesta and the well-being of sheep. Sjaastad and Stormorken (1963) measured histamine in ruminal fluid of sheep and found a moderate capability of rumen wall for metabolism of histamine. When this capacity is exceeded liver tissue detoxified the remaining histamine.

Wicki and Schatzmann (1977) found that ruminal fluid is able to synthesize histamine by decarboxylating histidine. Under normal feeding conditions it appears that histidine is limiting to histamine production. They found 20-fold increase in histamine production upon addition of 4.5 mM of histidine to the ruminal fluid. If protein breakdown is not accompanied with protein synthesis, such as in acidosis, free histidine can contribute to histamine increase.

Wicki and Schatzmann (1977) found that net histamine production decreased with decreases in pH. They theorized that low pH may select bacteria rich in histidine decarboxylase and/or impair transport of histamine across the rumen wall. The former concept is supported by Dain et al. (1955) who found that eight species of *Lactobacilli* have histidine decarboxylase activity. Histamine dissociates on pH as low as 5 and therefore it is poorly absorbed (Edwards 1982). Ruminal smooth muscle is remarkably insensitive for histamine. An increase of histamine by 100-fold is required to observe any effect of histamine in that regard (Wicki and Schatzmann, 1977).

Takahashi and Young (1981a) applied 45 to 59 mg of histamine diphosphate into the neck of cattle. Shortly, they observed a rise in heart rate, paddling action and increase in hoof temperature. In the second experiment they injected the same histamine levels in a repeated manner to two groups of bulls; one group was exposed to low environmental temperature (-10 to -15 °C) and the other to room temperature. Laminitis, accompanied with lameness, occurred in both groups but was more severe in the low temperature group. Recovery from lameness was also prolonged in this group.

Takahashi and Young (1981b) combined high energy diets with and without addition of 30 mg of histamine into the hoof. Histamine itself did not cause laminitis, but did intensify symptoms when in combination with a high grain diet. Laminitis lasted for 29 to 101 days. Higher temperature was observed in the histamine injected hoof. They concluded that prolonged action of histamine in the hoof caused severe tissue damage leading to the development of laminitis.

Others (Andersson and Liberg, 1980) think that histamine plays just a subordinate role in hypersensitivity in cattle. Others doubt the possible role of histamine since only 1% of the total histamine in the blood is in the free form, which is the active form (Rautschka et al., 1991). Also, thrombosis is an regular finding in laminitis, however, histamine is not implicated in thrombus formation (Maclean, 1971a).

Histamine acts by binding to its receptors; H_1, H_2 and H_3. There is a lot of data about H_1 receptor; much less about H_2 while H_3 receptor is currently heavily studied. A functional H_1 receptor cDNA clone was isolated for the first time from bovine adrenal medulla by Yamashita et al. (1991). Based on this it was determined that bovine H_1 receptor is a protein which consists of 491 amino acids with seven putative transmembrane domains. Histamine H_2 receptor in bovine remains to be cloned, but based on results of cloning in canine by Gantz et al. (1991), H_2 gene encodes 358 amino acids. H3 receptor has not been cloned as yet.

Histamine H_1 receptor protein prevails in peripheral vasculature in normal conditions (Elmes and Eyre, 1977; Alexander, 1990), while H_2 predominates in gastric mucosae where it is associated with secretion of hydrochloric acid. Presence of H_3 receptor, originally described in central nervous system (CNS) (Arrang et al., 1983), has been demonstrated in guinea pig ileum too (Poli et al., 1991). There is an shortage of data relative to its distribution in other tissues. Guanidine and adenylate cyclase-coupled systems mediate the signal of binding histamine to H_1 and H_2 receptors, respectively (Buschauer et al., 1989).

Histamine may act on endothelium of blood vessels through stimulation of nitric oxide (NO) synthesis. Nitric oxide is cleaved from its precursor, the amino acid arginine, by specific enzymes (Yang et al., 1991), and it is primarily a vasodilator. Endothelium is particularly sensitive to histamine stimulation. More NO was released in artery compared to vein and it was partially due to a presence of more endothelium in internal mammary artery than in saphenous vein (Yang et al., 1991). This would make histamine a dilator in the artery constrictor in the vein. However, histamine was constrictor in both vessels when

endothelium was removed. Szabo et al. (1993) found evidence suggesting that endotoxin injection caused hypotension in the rat. The primary mechanism for that was a stimulation of NO synthase by endotoxin. Simultaneously, there was hyporeactivity to noradrenaline.

Protein

It has been thought that protein is important to etiology of laminitis. However, there is little information that would suggest what levels of protein and what nitrogen forms (ammonia, amino acids) night be important in the development of the disease (Vermunt, 1992; Vermunt and Greenough, 1994). Some data indicates a role of protein in outbreak of laminitis (Bazeley and Pinsent, 1984; Manson and Leaver, 1988b; Bargai et al., 1992). Laminitis could be caused by an allergic reaction to protein from either grain feeds or from retained placenta (Blood and Radostits, 1984).

Since laminitis may arise from the feeding of animals concentrate diets that contain more than 16% of protein, such diets should not be used in nutrition of calves for the first three months of life (Bargai et al., 1992). Animals fed 18% digestible protein concentrate were affected by laminitis, while those fed 15.3% digestible protein concentrate, were not. The authors did not provide information that would indicate the possible role of other factors which might be responsible for the described situation (Whitaker, 1992).

Manson and Leaver (1988b) found that feeding dairy cattle concentrates containing 19% protein significantly increased lameness, while feeding 16% concentrates did not. Other authors failed to demonstrate laminitis when a 19% protein diet was fed (Greenough, 1990; Greenough et al., 1992). Greenough et al. (1990) found no significant correlation between excessive horn growth and protein intake. Other authors (Bazeley and Pinsent, 1984) consider protein level as a possible factor contributing to occurrence of laminitis. They suggested that free ammonia from silage could be a contributor, particularly since it can indicate presence of other toxic products of fermentation.

Hardness of claw is related to the number of disulfide bonds formed in keratin. Degree of keratinization seems to be associated with the cystine disulfide bonding (Vermunt and Leach, 1992). Black horn is considered by many to have qualities superior to that of light colored horn (Greenough and Gacek, 1987). Clark and Rakes (1982) however, found no relation between hoof color and hardness.

Lack of sulphur amino acids caused softening of hoof (Manson and Leaver, 1988b) that predisposed the animal to sole ulcers. Maclean (1971b) measured a remarkable reduction of methionine and cysteine concentrations in hooves from laminitic animals when compared to non-laminitic ones. Clark and Rakes (1982) found that feeding methionine hydroxy analog stimulated hoof growth but decreased hoof hardness. They measured a lower level of cysteine which suggested a reduced number of disulfide bonds. Moore et al. (1989) found a positive trend in hoof hardness upon addition of 200mg/d of zinc-methionine (Zinpro 40). The visual hoof score (texture, heel cracks and interdigital dermatitis) was significantly improved.

Others have stressed the importance of sulphur-bearing amino acids at the growth site for normal hoof formation (Hahn et al., 1986). Ekfalck et al. (1990a), found that labelled methionine was mainly incorporated into the stratum basale and spinosum of the matrix, while labelled cysteine was specifically taken by keratogenous zone of the hoof. Methionine incorporation was much more intensive than cysteine (Ekfalck et al., 1985).

Colles and Jeffcott (1990) mentioned that failure of keratinization is mostly the result of deficiency in methionine and cystine supply, resulting from impaired blood flow. Administration of methionine may be beneficial for both long and short term treatment of laminitis. They recommended a rate of 10 g daily for 4 d followed by 5 g daily for a further 10 d. Edwards (1982) recommended feeding of 10 g of methionine for 1 week in order to provide a disulfide bound substrate for maintenance and repair of the hoof pedal bone bond.

Other nutritional factors

Vermunt (1992) theorized that 1-2% nitrate levels in the ration can produce blood levels of nitrite capable of vasodilation. According to him, the vasodilation may contribute to the development of tissue anoxia by causing circulatory failure or may lead into endothelium damage and subsequent leakage of blood components into the dermis. This author considered mycotoxins in fungi-damaged feedstuffs such as corn silage to be of importance in the etiology of subclinical laminitis and sole hemorrhages.

Although biotin was significantly lower in high producing cows with history of lameness, clear deficiency was not observed (Roberts and Bagotti, 1982). Cooke and Brumby (1982) reduced lameness in cows by supplementing their diet with 20 mg of biotin per cow per day.

Management

The structural and functional organization of anatomical and histological parts and activities has been developed over the very long period of time, and it serves the animal well. However, modern styles of management, housing, and nutrition creates conditions for potential hoof diseases and malfunctions. Factors from management considered to be important to the etiology of laminitis are housing, bedding and exercise. Narrow concrete cubicles may exacerbate severity of laminitis (Bazeley and Pincent, 1984). Symptoms of laminitis were always more severe when cattle were on concrete. Philipot et al. (1994b) found that subacute laminitis, detected in 160 French dairy herds, was often associated with variations in the floor level in the building for livestock. Risk factors for heelhorn erosion were associated with poor hygiene.

Inadequate exercise that could be a result of modern housing may be a predisposing factor for lameness (Weaver, 1979). Lack of exercise decreases blood flow through claw

corium making it more susceptible to insult (Greenough, 1990). Toussaint Raven (1989) calculated from a biomechanical point of view that small changes (5 cm) in a cow's standing position would create large differences (40 kg) in load between outer and inner claw.

Higher environmental temperature could cause vasodilation of foot blood vessels and stimulate of hoof growth. Furthermore, if a soft floor, hoof overgrowth may occur. This agrees with data of Hahn et al. (1986) who found that hooves on concrete wore 35% more than those on dirt. Takahashi and Young (1981b) measured increase of hoof length in laminitic animals to be 11.5% and in nonlaminitic animals 1.6%. Seasonal outbreak of laminitis (winter or early spring) probably more relates to the type of housing and/or calving which is accompanied with sudden changes in nutrition (Vermunt and Greenough, 1994). An outbreak of laminitis, investigated at one dairy farm was probably due to increased consumption of the feed as a result of increased metabolic demands at low environmental temperature (Bargai et al., 1992). In a large study, Weaver et al. (1981) found peak lameness months were January, February and March, followed by November. In contrast, Enevoldsen et al. (1991b) suggested that in warmer months the bacterial and chemical (ammonia) degradation of the heel horn may be promoted.

Walking a long distance can cause sole bruising and subsequent lameness (Gicken and Kendrick, 1977). Vermunt (1992) depicted a typical situation in New Zealand where cows have to walk for couple of miles to the milking shed to be milked twice daily for about 300 d per year. It can be a predisposing factor to laminitis. Laminitis may have a cumulative effect (Greenough, 1990), repeated insult to the animal increases susceptibility.

Species and breed differences

The horse appears to be more susceptible to acute laminitis than ruminants. Ponies appear to be even more susceptible to laminitis than horses (Colles and Jeffcott, 1977; Clarke et al., 1982). Apparently, there are two reproducible models to study laminitis in horses

(Garner, 1975; Minnick et al., 1987), but none in other species. Nothing suggests major differences in laminitis susceptibility between sheep and cow. Furthermore, there are no major differences in pathological processes in the hoof between horse and bovine (Ekfalck et al., 1985). However, it becomes clear that horses are primarily affected in front feet while cattle and sheep suffer in rear feet. Different distribution of body weight may be a possible cause for this. Also laminitis is associated with an increase in body weight (Cohen et al., 1994). The latter authors found that horses weighing more than 550 kg were about twice as likely to develop laminitis than lighter horses.

Because some cows develop laminitis while others do not, there is an widely held notion that genetics might play a role in laminitis. Some thought a particular breed more susceptible to laminitis (Greenough, 1990; Whitaker, 1992). So far, however, an inherited tendency for laminitis has only been demonstrated in Jersey cattle (Merrit and Riser, 1968). Greenough and Gacek (1987) stated that Aberdeen-Angus bulls were much less troubled by hoof problems than were five other breeds examined. Hale (1985) described Brahman type cattle as very susceptible to founder perhaps because they eat feed selectively. At the same time Hale (1985) credited Holstein steers as resistant to founder. Data reported by Maclean (1971b) indicated that Guernsey breed was more susceptible to sole ulcer. Channel Island cattle were more severely affected in the medial hind claws, while Friesians were affected in both the lateral and medial claws (Maclean, 1971b)

Age is a factor as well (Weaver, 1971; Vermunt and Greenough, 1994). Primiparous cows were much more sensitive to laminitis than older cows (Enevoldsen et al., 1991b). Bazeley and Pincent (1984) noticed that heifers were always more severely affected than cows. Furthermore, in three herds the disease was exclusively confined to heifers, and on four farms all the heifers were affected (Bazeley and Pincent, 1984). Greenough and Vermunt (1991) observed that the heifers generally had more severe hemorrhages than older cows 4 mo before calving. In addition, the animal with the highest daily body weight gain (950 g/d) had the highest score for sole hemorrhage. Andersson and Lundstroem (1981) and

Bergsten (1994) reported that Swedish Friesian cows were more prone to hemorrhage in the sole horn than the Swedish Red and White cows.

Heritability of some hoof parameters

Some hoof abnormalities may be heritable (Hahn, 1979). The author found that angles of the hoof are less heritable for medial than for lateral claws. Also, heritability of heel depths and hoof areas were greater for front hooves. Glicken and Kendrick (1977) proposed that hoof overgrowth may be under different sets of genes and it is possible that one set of genes regulates the age of expression and another controls the extent of hoof overgrowth. Although all four feet were affected, hoof overgrowth has a greater degree in hind legs, and it reaches greater proportions in older cows. Other authors believe that heredity is involved in some hoof problems (Livesey and Fleming, 1984).

Choi and McDaniel (1993) estimated heredity for hoof angle to be 0.18 (range from 0.03 to 0.39); for hoof length to be 0.25 (range from 0.08 to 0.53); and for heel depth to be 0.07 (range from 0.02 to 0.16). Hoof length had the highest positive phenotypic relationships with milk or fat yields and days open, and deeper heels were always associated genetically with more days open. Hoof angles were positively correlated with survival rate for the first lactation, but negatively with milk and fat yields. Genetic correlations of fat and milk yield were generally negative with hoof angles, but positive with hoof length.

Some experimental attempts to create laminitis in ruminants

Since the importance of laminitis in the pathology of modern animal production has been recognized, there has been a need for a reproducible model that would serve as a tool to investigate it. There have been many attempts to find a valid model in cattle (Dougherty et al., 1975b; Svendesen, 1979; Andersson, 1981) and in sheep (Morrow et al., 1973).

Morrow et al. (1973) injected DL-lactate into the rumen of 16 lambs. A classical picture of laminitis occurred in all animals within 12 to 24 h upon the injection. To do the same on cows, Svendesen (1979) induced acidemia by intravenous injection of racemic lactate in three cows. Although some effects such as bradycardia and depressed blood pressure were observed, laminitis did not occur. Andersson (1981) performed intraruminal infusion of lactic acid in dairy cows. Despite high blood lactate concentrations, laminitis was not observed. Suber et al. (1979) induced laminitis in steers after engorging them with a concentrate ration of 90% flaked corn grain. Clinical signs of laminitis and lethargy were observed 14 to 24 h after engorgement. Nagaraja et al. (1978) caused laminitis in the two experimentally overfed cows.

Frequency of occurrence of laminitis and economic importance

Assuming 10 to 50% reduction in milk production for a lame cow and a 10% incidence rate of laminitis, Whittier (1993) estimated that a herd with 110 cows and a rolling average of 18,000 lb of milk would lose $3,000 to $15,000 in milk production alone per year due to lameness. Philipot et al. (1994a; 1994b) examined cows from 160 dairy farms. The observed lameness rate was 8% while 89% of cows were affected by at least one pedal lesion (Philipot et al., 1994a). More specifically, of the 4,896 cows examined, 77% suffered from chronic and subacute laminitis or heelhorn erosion. Similar findings were reported by Andersson and Lundstroem (1981) who examined digital diseases in 594 cows and found that only 25.9% of the cows were free from lesions.

The study of lameness has largely been neglected. This is surprising because lameness affects dairy cows, young beef bulls and horses. Greenough (1992): "When one cow in ten becomes lame in any period of twelve months for reasons other than foot rot, claw deformity or accident, it can be said that a lameness problem exists". A severely lame

cow in the peak of lactation could lose 20% of its production for that lactation (Greenough, 1992). Weaver (1979) reported 8% of the national dairy herd becomes lame each year in the UK. Weaver (1985) pointed out that the average herd in UK has 5 to 25% of the cows lame at some time each year. Whitaker et al. (1983) stated that in England the incidence rate can range from negligible to over 50%.

Wells et al. (1993), reported the prevalence of lameness in 17 Minnesota and Wisconsin herds was 13.7% in summer and 16.7% in spring in lactating dairy cows. In this study, independent trained examiners found 2.5 times more lameness than that estimated by the herd managers. This would suggest that the lameness rate in USA is much higher than 4.4 to 9.5 cases per 100 cows/yr (as usually reported).

Prevention

Since rumen acidosis has been recognized as important in the etiology of laminitis, an effort has been made to prevent acute acidosis by controlling lactic acid synthesis. Huber (1975) pointed out three possibilities to feed safely high carbohydrate rations without having an adaptation period first: a) dietary buffers; b) antibiotics specific for lactic acid bacteria; and c) providing lactic acid utilizing bacteria to the rumen of unadapted animals. Increased feeding frequency is considered to be beneficial to maintaining high ruminal pH (Kaufmann, 1976).

Weaver (1979) recommended avoiding sudden feeding changes around parturition and a gradual introduction of concentrates over a longer period of time. Feeding of total mixed rations should minimize the risk of acidosis. Edwards (1982) noted that the system of complete diets is accepted in Israel, where, in spite of having the world's highest national average yield, laminitis is almost unknown.

Other approaches have been undertaken with the purpose of reducing the possibility of acidosis (Nagaraja et al., 1981; Nagaraja et al., 1982; Nagaraja et al., 1985) and eventually

subsequent laminitis (Rowe et al., 1994). Rowe et al. (1994) found that Virginiamycin reduced lameness score in horses. They believe this to be a dual effect from Virginiamycin: it reduces the intake of pellets during the first 3 d of feeding and it specifically inhibits lactic acid producing microbes.

Ionophore antibiotics have been investigated for their ability to prevent lactic acidosis (Nagaraja et al., 1981; Nagaraja et al., 1982; Nagaraja et al., 1985). Nagaraja et al. (1981) prevented corn or glucose induced lactic acidosis in cattle with lasalocid or monensin. It should be noted, however, that corn-induced acidosis was prevented only when ionophores were administered for 7 d prior to acidosis, while the administration for 2 d prior to treatment prevented glucose induced acidosis only.

Dennis et al. (1981) investigated antimicrobial effects of lasalocid and monensin. They found that both antibiotics selectively inhibit production of L-lactate but not D-lactate. Lasalocid and monensin inhibited major lactate-producing bacteria like *S.bovis, Lactobacillus, Butyvibrio,* and *Lachnospira,* while having no effect on lactate-utilizing bacteria.

Nagaraja et al. (1982) found that lasalocid prevented experimentally induced lactic acidosis in lower concentrations than monensin and thiopeptin. Lasalocid was effective when fed at rate of .33 mg/kg body weight, while monensin and thiopeptin were active at .65 and 1.3 mg/kg of body weight, respectively. Nagaraja et al. (1985) also found that salinomycin had an inhibitory effect on lactic acid production. Quigley et al. (1992) and Eicher-Pruiett et al. (1992) successfully supplemented calves with 1 mg/kg body weight of lasalocid for the prevention of lactic acidosis. The latter group believed that an even higher dose of lasalocid would be more effective.

Galyean et al. (1992) observed a negative effect with the addition of 6 mg of laidlomycin propionate on ruminal D-lactate in beef steers while L-lactate remained unchanged. Rumsey (1978) investigated the effects of dietary sulfur supplementation on lactic acid concentrations. He found that supplementation lowered peaks of both lactate

isomers in engorged animals. Hale (1985) observed that introduction of ionophores reduced occurrence of bloating and acidosis.

Another approach which will probably gain more attention in the future is a modification of the microbial population in the rumen. Martin and Nisbet (1990) investigated effects of the addition of *Aspergillus oryzae* to *in vitro* ruminal fermentation of some feedstuffs. The idea behind this is that *Aspergillus* could increase availability of nutrients and therefore the level of dietary energy could be lower. The presence of *Aspergillus oryzae* in the diet of dairy cows stimulated degradation of forage which may have increased forage intake by the cows (Williams et al., 1991). In addition, they measured higher ruminal pH in the group given *Aspergillus oryzae*.

Selenomonas ruminantium is a major ruminal bacterium which can account for about 50% of the total viable count (Nisbet and Martin, 1993). *S. ruminantium* is the focus of interest of some researchers as a potential tool for utilization of D-lactate in the rumen. Nisbet and Martin (1991) observed that L-malate could stimulate growth of *S. ruminantium* on lactate. Nisbet and Martin (1993) found that the addition of either L-malate or Amaferm (*A.oryzae* product) stimulated D-lactate utilization by *S. ruminantium*. Melville et al. (1987) proposed the presence of lactate racemase in *S. ruminantium* as a tool for utilization of D-lactate by the microorganism.

Megasphaera elsdenii is a lactate utilizer and when cocultured with Streptococcus bovis (a major ruminal lactate producer), it kept the pH of the culture high. When the pH was artificially decreased, *S. bovis* increased and *M. elsdenii* disappeared at pH 5.2 (Russell et al., 1981). Counotte et al. (1981) estimated the contribution of *M. elsdenii* to lactate fermentation in the rumen to be about 74%, and this contribution was even more important when a cow was fed readily fermentable carbohydrates. An extensive work on the purification and characterization of D-lactate dehydrogenase from *M. elsdenii* was done by Olson and Massey (1979). Hino and Kuroda (1993) proposed that *M. elsdenii* possesses lactate racemase and that D-lactate can be converted to acetate, butyrate or propionate.

Objectives

The overall objective of this study was to investigate metabolic alterations caused by sudden introduction of diets that varied in composition, on parameters which may be associated with the onset of laminitis.

Specific objectives were:

1. To study the acute changes in ruminal fluid pH and blood/rumen lactic acids in bull calves fed the diets that differed in energy and protein concentration.
2. To investigate the acute effects of different energy and protein levels on incidence of hoof abnormalities in dairy bull calves .
3. To study the modulating effects of ionophore and buffer on the blood/rumen parameters.
4. To investigate the effects of long term feeding of high concentrate diets on incidence of lameness in young dairy bull calves.
5. To learn more about the pathogenesis of laminitis, and thus extend knowledge about the mechanisms of the disease expression in domestic animals.

CHAPTER 3

EXPERIMENT 1

The objective of this study was to investigate metabolic alterations caused by sudden introduction of diets that differ in energy and protein on some parameters which can be associated with the onset of laminitis.

Materials and Methods

Sixteen Holstein bull calves, 17 wk old and 130 kg BW, were fed for two wk an adjustment diet that contained 71% TDN and 15% CP (Table 3.1). Feed was delivered twice daily at 8 AM and 4 PM. To train animals to meal feeding, the calves were allowed 2 h to eat after each feeding. Water was always available. The calves were held in individual pens (1.2 x 2.0 m) at the Virginia Tech Dairy Center. Since all the animals were not at the same age they were introduced into the experiment in groups of four according to their age order.

Experimental Diets and Design

Experimental diets contained two levels of protein (15 or 20%) and two levels of energy (71 or 81% TDN). Ingredients and chemical composition of the diets are shown in Table 3.1.

On the last day that the adjustment diet was fed (14^{th} d), the animals were given a normal amount of feed in the morning only, then deprived of feed until the following AM. They were then offered one of the four experimental diets. The adjustment diet was one of the experimental diets. The animals were randomly assigned to each treatment. The

Table 3.1. Dietary ingredients and chemical composition[a]

Item	Treatments[b]			
	LELP	LEHP	HELP	HEHP
Dietary ingredients	---------- % of DM ----------			
Alfalfa silage	30.4	30.6	6.4	6.4
Corn silage	25.7	25.9	5.7	5.7
Corn meal	16.3	9.1	38.6	31.7
Barley, ground	16.3	9.1	38.6	31.7
Soybean meal	8.2	22.2	8.7	21.5
Molasses, cane, dehy	2.9	2.9	1.8	2.7
Salt	.2	.2	.2	.2
Chemical composition				
DM%	61.5	61.5	80.6	80.8
CP	15.0	20.0	15.0	20.0
TDN	71.3	71.2	81.3	81.2
ADF	22.0	22.8	9.4	10.0
NDF	33.7	33.6	19.6	19.2
Calcium	.53	.57	.18	.22
Phosphorus	.31	.35	.37	.41

[a]Estimated by Dair4 except for silages which were analysed.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

experimental diets were offered initially for two days. If severe acidosis occurred the animals were fed a mixture of the experimental diet and hay for up to one week. Beyond that, only the experimental diets were fed for 2 mo. Feed not consumed was taken away at 8 PM of each day.

Samplings and Measurements

Blood and rumen samples were collected and hoof temperature measured just before refeeding in the AM of the first day (0 h) and at 4, 8, 12, 16, 24, 28, 32, 36 and 48 h. Blood was sampled by use of vacutainer tubes (Becton Dickinson, Rutherford, NJ). Two types of tubes were used: one contained 100 USP units of sodium heparin, and the other contained 8.0 mg of potassium oxalate and 10 mg of sodium fluoride. Upon sampling the tubes were submerged in ice, and taken to the laboratory. As preparation for lactate measurement, 1 mL of whole blood was mixed with 2 mL of cold 8% perchloric acid. After centrifugation (IEC Centra 8R centrifuge, International Equipment Company, Needham Heights, MA) at 3000 x *g* for 15 min at 4°C, supernatants were stored in duplicate at -20°C until analyzed.

Ruminal fluid was sampled via esophageal tube fitted with a suction strainer with vacuum applied (GAST Manufacturing Corp., Benton Harbor, MI). Of 100 mL collected, approximately 10 mL were taken for analysis. The sample tubes were iced immediately for transport to the laboratory. There pH was measured using an Accumet pH Meter (Fisher Scientific, Raleigh, NC) equipped with Ross® Sure-Flow combined pH electrode (Orion Research Incorporated, Boston, MA). One mL of ruminal fluid was deproteinized with 2 mL of 8% perchloric acid (PCA). After centrifugation, as described for blood, the supernatant was stored at -20°C until analyzed for lactate. The remaining rumen fluid was split in two subsamples, 2.5 mL each. To each was added .5 mL of 25% H_3PO_4. To one, .5 mL of 30 mM isocaproic acid (internal standard) was added for VFA analysis; both were stored at -20°C.

The left rear leg from each animal was examined by x-ray twice from a lateral position by use of a portable device (MINIXRAY, Japan). This occurred during the period of depressed intake and within 24 h after the last sampling. Hoof temperature was measured at the time of each sampling. Dorsal hoof wall on the lateral hoof of the rear legs was cleaned and surface temperature was measured by use of the Digital Infrared Scanner, Model D501 (Exergen Corp., Newton, MA). Animal hooves were examined on two occasions, at approximately 3 and 7 mo after the first feeding. Hoof x-rays were performed at that time as well.

Analyses

Whole blood was analyzed for L-(+)- and D-(-)-lactate; plasma for total protein, albumin, urea nitrogen and glucose; and ruminal fluid was assayed for lactates, ammonia and VFA. All samples were analyzed in duplicate. Chemicals were purchased from Sigma (Sigma, St. Louis, MO). Absorbencies were measured using a Titertek Multiskan MCC/340 microplate reader (EFLAB, Finland) equipped with Skan Soft program, except for albumin which was measured on a Spectronic 1001 spectrophotometer (Bausch & Lomb, Rochester, NY)

Deproteinized supernatants from the whole blood were analyzed for L-(+)- and D-(-) -lactate by the enzymatic procedure outlined in the Sigma Technical Bulletin (No. 826-UV, Sigma, St. Louis, MO). L-lactate was analyzed with L-lactate dehydrogenase from beef heart, and D-lactate with D-lactate dehydrogenase from *Lactobacillus leichmanii*. The original procedure was modified by reducing the total reacting volume by 10 fold, which accommodated a 96-well microplate. Prior to analysis, a 6.1 mL of a reactant solution was prepared that contained 2.0 mL of glycine buffer, 4.0 mL of water, .10 mL of appropriate lactate dehydrogenase, and 10.0 mg of NAD. To each microplate well was pipetted .29 mL of this mixture. Then 10 μL of the sample or TCA (blank) was added to each well with

gentle mixing with pipette tip. The unknowns were determined from standard curves prepared on each plate for each lactate. The reactant mixture was incubated for about 15 min at 37°C and absorbance was measured at a wavelength of 340 nm. All lactate samples were analyzed within 48 h of storage at -23 °C.

Frozen plasma was thawed and analyzed for total protein by a biuret method outlined in Sigma Technical Bulletin (No. 541, Sigma, St. Louis, MO). A total protein reagent was used. To .3 mL of the reagent was added 6 µL of sample in microplate well. After reaction at ambient temperature for 10 min, absorbance was read at 540 nm.

Albumin in plasma was analyzed by the Sigma's procedure (No. 631, Sigma, St. Louis, MO). Albumin reagent which contained bromcresol green was used. To 1 mL of the reagent were added 10 µL of sample. Reading of absorbance was done on a spectrophotometer at a wavelength of 625 nm, after exactly 1 min of incubation at room temperature.

Plasma urea nitrogen was determined by the urease method (Weatherburn, 1967). Five µL of plasma sample were placed into a glass tube and incubated for 20 min with 100 µL of urease solution. A half of a mL of reagent one (50 g phenol and 250 mg sodium nitroferricyanide/L) and reagent two (25 g sodium hydroxide and 42 mL 8% sodium hypochlorite/L) were added. Samples were vortexed and kept at ambient temperature overnight. Absorbance were measured at 630 nm.

Plasma glucose was assayed according to Sigma's procedure (No. 16-UV, Sigma, St. Louis, MO) by use of Glucose (HK) reagent. The reagent was prepared according to instructions, and .3 mL of the reagent placed into microplate well when 3 µL of sample was added. Incubation was at 37°C for 5 min, followed by measuring absorbance at 340 nm. Ruminal fluid lactate was measured similarly as described for whole blood (see above).

Ruminal samples for ammonia and VFA measurements were thawed and filtered through a 25 mm .45 µm filter (Gelman Sciences, Ann Arbor, MI). Ammonia was determined similarly as described for plasma urea nitrogen (Weatherburn, 1967) except

urease incubation was omitted. Ruminal VFA was analyzed by gas chromatography by injecting .5 μL of sample into Varian Vista 6000 chromatograph (Varian Instruments, Palo Alto, CA) equipped with a flame ionization detector, a Varian Vista 4270 integrator (Varian Instruments, Palo Alto, CA), and a 6' x 1/4" o.d. and 2 mm i.d. glass column (Supelco Inc., Bellfonte, PA) packed with GP 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb WAW. The analysis was isothermal with temperatures of 115°C for the column, 170°C for the injector and 180°C for the detector. Nitrogen, used as a carrier, had a flow of 80mL/min and hydrogen and air as detector gasses with flows of 40 and 60 mL/min, respectively.

Statistical Analyses

Data were analyzed by ANOVA for a two-factor experiment (diet and time) with repeated measurements on time using the general linear models (GLM) procedure of PC-SAS (1990). Effects of diet and animal with repeated measurements on time were included. Overall effects from diet over time were tested by using Type IV MS for animal (diet) as an error term, according to a model by Ott (1988):

$$y_{ijk} = u + \alpha_i + \pi_{j(i)} + \beta_k + \alpha\beta_{ik} + \epsilon_{ijk}$$

where α_i, $\pi_{j(i)}$, β_k, $\alpha\beta_{ik}$ are fixed effects corresponding to main effects for diet, animal(diet), time and their interaction, respectively.

All results are shown as least-squares means (LSM) and pooled standard error of mean (SEM). Orthogonal contrasts for treatment means included effects due to energy (71 vs 81% TDN) LE vs HE, protein (15 vs 20%) LP vs HP, and the interaction between energy and protein INT.

Results and Discussion

Because feed had been withhold for 22 h, eager consumption of food was observed in all animals at 8 AM of d 1, resulting in a high dry matter (DM) intake. Animals fed 81% TDN, 15% CP (HELP), 81% TDN, 20% CP (HEHP), 71% TDN, 15% CP (LELP), and 71% TDN, 20% CP (LEHP), consumed 6.61, 5.49, 4.73 and 4.24 kg of DM, respectively. When expressed as a percentage of body weight (BW) and compared to their average daily DM intake in the adjustment period, animals in HELP increased consumption 61% (5.33 vs 3.32), HEHP, 49% (4.30 vs 2.88), LELP, 20% (3.93 vs 3.27), and LEHP, 8% (3.25 vs 3.02). On d 2 those animals offered 81% TDN refused to eat (Table 3.2).

Symptoms of anorexia and depression in HELP and HEHP treatments occurred as early as 12 h after the feed was offered, indicating digestive problems. Discomfort, lethargy, stiffness, muscular tremor and diarrhea were observed. Affected animals stood motionless, apparently unaware of their environment for a period of time and finally became unable to rise, usually between 16 to 24 h. Six of eight animals in HELP and HEHP groups were down by 24 h. Two animals, one each from HELP and HEHP group did not eat enough to engorge themselves. These animals demonstrated symptoms of mild diarrhea and stiffness. One animal from LEHP showed similar behavior after having consumed a large amount of diet. At 28 h, affected animals in HELP and HEHP treatments were medicated with a balanced electrolyte solution (Entrolyte®, SmithKline Beecham Animal Health, West Chester, PA). In general, this therapy proved beneficial for speeding recovery of affected animals.

Ruminal fluid changed color, consistency, and odor in animals fed 81% TDN diets beginning at 8 h. Ruminal fluid became a yellow, watery liquid with a progressively sour odor. This coincided with changes in rumen pH which declined significantly in HELP and HEHP animals by 8 h and remained lower in these two treatments for 36 h (Table 3.3). Although dietary energy differences explained most of the observed treatment differences,

Table 3.2. Dry matter consumption

	Treatments[a]				
	LELP	LEHP	HELP	HEHP	SEM
	---------- % of BW ----------				
Day 1	3.93[cd]	3.25[d]	5.33[b]	4.30[c]	.24
Day 2	2.95[b]	2.38[b]	.00[c]	.00[c]	.29

[a]LELP = 71% TDN,15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN,15% CP; HEHP = 81% TDN, 20% CP.

[bcd]Means within a row lacking a common superscript are significantly different ($P \leq .05$).

Table 3.3. Response of ruminal pH to overload of diets varying in TDN and CP[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
0	7.44	7.38	7.39	7.44	.99	.98	.83
4	6.59	6.35	6.53	6.31	.80	.63	.68
8	6.68	6.23	5.94	5.51	.01	.06	.86
12	6.47	6.14	4.99	4.98	.01	.47	.56
16	6.67	5.82	4.85	4.85	.01	.09	.10
24	7.05	6.84	4.70	4.72	.01	.71	.63
28	6.30	6.70	5.01	5.09	.01	.40	.52
32	6.61	6.41	5.03	5.36	.01	.80	.30
36	6.20	6.59	5.52	6.09	.02	.10	.84
48	7.18	7.22	6.40	7.02	.04	.21	.26

[a]LSM ± SEM; ± .26.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

protein also lowered pH at 8 and 16 h after feeding and interaction approached significance at 16 h. The pattern and degree of changes in ruminal fluid pH were similar to those obtained by Harmon et al. (1985) who infused a glucose solution into the rumen of steers. In that case pH declined below 5.0 at 12 h and remained less than 5.0 for 30 h. In the present study, pH was less than 5.0 at 12 h and remained less than 5.0 for 12 h. Chaplin and Jones (1973) and Nagaraja et al. (1978) measured pH of 4.4 at 12 h in engorged sheep and cattle, respectively. In an earlier study rumen pH fell below 5.0 by 6 h (Dunlop and Hammond, 1965).

Ruminal fluid L-lactate was low in all animals at 0 h prior to feeding (Table 3.4). It rose sharply in all treatments by 4 h, and continued to rise in all groups except LELP by 16 h. Further increases occurred only in HELP and HEHP treated animals. Ruminal L-lactate peaked at 24 h in HELP and HEHP groups (526 and 451 mg/dL, respectively). L-lactate increased in LEHP for 16 h, but protein differences were significant only at 8 h except for the interactions. Interaction was significant at 16 h after feeding which reflected a peak of rumen L-lactate in LEHP treatment and a decline in LELP treatment. The initial (4 h) L-lactate increase was numerically greater in lower energy treatments.

The maximal levels of rumen L-lactate, obtained in this study, were somewhat lower than those measured at a corresponding time by Harmon et al. (1985), 550 mg/dL and Wilson et al. (1975), 570 mg/dL, but higher than measured by Rumsey (1978), 126 mg/dL. Peak of L-lactate (1,450 mg/dL) measured by Dunlop and Hammond (1965) occurred at 6 h in an engorged steer. Slyter and Rumsey (1991) measured the increase of L-lactate in an engorged steer at 6 and 24 h (450 and 810 mg/dL, respectively). Suber et al. (1979) measured peak of L-lactate at 8 h.

Changes in ruminal fluid D-lactate followed a pattern similar to L-lactate, but peaked at lower levels (Table 3.5). It multiplied several fold within the first 4 h in all treatments. D-lactate increased faster in HEHP than in HELP group for the first 12 h. This resulted in different times of reaching maximum concentrations (295 mg/dL at 12 h vs 366 mg/dL at 24

Table 3.4. Response of ruminal L-lactate concentration to overload of diets varying in TDN and CP[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- mg/dL ----------						
0	8.0	10.8	8.1	3.9	.96	.99	.96
4	90.5	107.4	40.5	66.0	.49	.75	.95
8	59.0	155.9	105.1	235.2	.35	.09	.82
12	48.9	173.2	352.1	328.9	.01	.44	.27
16	23.5	213.3	441.4	361.2	.01	.41	.04
24	15.2	17.2	525.9	451.0	.01	.58	.56
28	62.7	43.0	426.2	420.8	.01	.85	.91
32	27.2	25.8	378.3	316.4	.01	.63	.65
36	26.8	22.2	283.1	62.2	.04	.10	.11
48	22.2	13.5	39.0	35.6	.61	.79	.79

[a]LSM ± SEM; ± 68.8.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

Table 3.5. Response of ruminal D-lactate concentration to overload of diets varying in TDN and CP[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- mg/dL ----------						
0	3.9	10.0	5.0	5.2	.97	.95	.95
4	78.9	89.8	29.0	58.4	.38	.70	.87
8	44.2	78.0	86.4	216.1	.13	.18	.51
12	34.6	93.0	211.7	294.5	.01	.16	.84
16	16.7	102.9	327.0	251.8	.01	.91	.10
24	10.2	13.4	365.6	236.3	.01	.19	.17
28	55.7	31.7	325.9	188.3	.01	.10	.24
32	23.8	23.3	273.2	151.1	.01	.20	.21
36	22.4	17.4	217.9	3.3	.07	.03	.04
48	17.8	11.2	48.6	1.3	.93	.55	.64

[a]LSM ± SEM; ± 50.7.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

h for HEHP and HELP, respectively). Effects from energy were significant from 12 through 36 h. Protein and the interaction were significant at 36 h, largely due to the high concentration in HELP group. Dunlop and Hammond (1965) determined D-lactate at 30 h to be as high as 810 mg/dL. Harmon et al. (1985) measured peak of D-lactate to be 423 mg/dL at 30 h, while Wilson et al. (1975) estimated D-lactate to be 100 mg/dL at 30 h. There is a difference in D-lactate pattern across the studies once the peak was reached. In the present study, a sharp decrease of D-lactate occurred at 36 and 48 h in HEHP and HELP groups, respectively. A similar pattern was observed by Rumsey (1978). However, Dunlop and Hammond (1965) and Harmon et al. (1985) observed that D-lactate remained high for this period of time.

The ratio of rumen L-lactate:D-lactate (L:D) varied across time (Table 3.6). It was significantly different between two protein levels (81% TDN) at 0, 36 and 48 h. However, at those times both lactates were low in both treatments (Table 3.4 and 3. 5). The greatest concentrations of both isomers were between 16 and 32 h. At this time interval HELP group yielded numerically greater proportions of L:D than HEHP group. The ratio observed in HELP group was greater than those reported by Harmon et al. (1985) (64:36), similar to those observed by Rumsey (1978) (70:30), while lower than those reported by Wilson et al. (1975) (80:20). Dunlop and Hammond (1965) reported a decrease in the ratio from 70:30 at 6 h to 45:55 at 28 h. Huntington and Britton (1978) observed an significant shift in proportion between the two isomers in the rumen at Day 35, from predominantly L- to almost all D-lactate.

Ruminal VFA concentration doubled in all treatments within 4 h (Table 3.7). This was followed by a steady decline in VFA in HELP and HEHP treatments between 12 and 32 h. The decline was particularly expressed at 28 h in HEHP treatment when total VFA concentration fell to 16, 19, and 38% of that measured in LELP, LEHP, and HELP, respectively. Effects from dietary TDN dominated observed changes in total VFA concentration between 12 and 36 h. Protein only tended to be associated with lower total

Table 3.6. Effect of diets varying in CP on ruminal L- to D-lactate ratio[a]

	Treatments[b]		Contrast (P ≤)
Time (h)[c]	HELP	HEHP	LP : HP[d]
0	66 : 34	43 : 57	.01
4	59 : 41	56 : 44	.59
8	53 : 47	60 : 40	.24
12	60 : 40	56 : 44	.59
16	61 : 39	57 : 43	.56
24	73 : 27	66 : 34	.28
28	72 : 28	70 : 30	.73
32	74 : 26	66 : 34	.21
36	75 : 25	89 : 11	.05
48	56 : 44	80 : 20	.01

[a]LSM ± SEM; ± 4.
[b]HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.
[c]Feed was offered after samplings at 0 and 24 h.
[d]Difference in L- to D- ratio between two protein levels.

Table 3.7. Total VFA concentration in the rumen as a response to overload with diets varying in TDN and CP[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- mM ----------						
0	48.59	46.09	43.98	42.19	.82	.83	.97
4	100.77	105.68	105.79	117.10	.57	.57	.82
8	103.14	92.74	100.51	97.92	.93	.65	.79
12	109.47	92.49	66.95	72.37	.03	.69	.44
16	101.62	97.81	53.14	74.02	.01	.56	.39
24	76.63	93.81	48.54	33.25	.01	.95	.26
28	113.33	96.21	48.18	18.11	.01	.10	.65
32	108.52	114.60	37.86	29.65	.01	.94	.62
36	125.70	106.64	44.36	72.10	.01	.76	.12
48	67.78	67.13	71.77	54.60	.69	.68	.71

[a]LSM ± SEM; ± 14.88.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

VFA at 28 h ($P < .10$). No significant interaction occurred. Wilson et al. (1975), Harmon et al. (1985) and Chaplin and Jones (1973) reported an overall decline of rumen total VFA in animals with metabolic disturbances. Moreover, Slyter and Rumsey (1991) showed dramatic reduction of total VFA in engorged animals compared to normally fed ones (19 vs 101.3 mmole, respectively). Rumsey (1978), however, measured a steady increase of total VFA in restricted steers, from a baseline created by 96-h fasting.

Acetic acid closely followed the pattern of total VFA when it was expressed as absolute concentration. However, when it was expressed as a percentage of total VFA, molar proportion was greater in HELP and HEHP animals at 24, 28, 32 and 48 h (Table 3.8). Protein was associated with a decrease of acetate proportion in 20% CP treatments at 36 h. The observed increase in proportion of acetate agrees with Slyter and Rumsey (1991). Wilson et al. (1975), however, measured a decrease in relative acetate concentration as acidosis progressed. Rumsey (1978) found a high molar proportion in fasted animals which lowered after refeeding.

Propionate molar proportion decreased at 16 h in high TDN groups and remained lower for the next 16 h (Table 3.9). The greatest decrease of relative propionate occurred at 24 h when it dropped one-third to one-half in HELP and HEHP treatments, respectively, relative to 16 h. At 24 h propionate proportion in 81% TDN treatments was about one-fourth of that in 71% TDN treatments. This agrees with data reported by Slyter and Rumsey (1991) who found a tendency for a decrease of molar proportion of propionate in the engorged animal. Wilson et al. (1975) and Rumsey (1978), however, observed an opposite trend.

Acetate to propionate ratio was high in all treatments at 0 h (Table 3.10). It declined in all groups through 12 h. A dramatic increase in the ratio occurred in HEHP group at 16 h due to both a decline in propionate and an increase in acetate. Both energy and protein effects were significant. Only TDN was significant at 24 h when 81% TDN treatments had maximal ratio observed in the study. Dietary TDN was still significant at 28 h, but beyond

Table 3.8. Rumen acetate as a molar proportion of total VFA[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- mol/100 mol ----------						
0	76.17	71.76	75.24	72.98	.98	.51	.83
4	66.12	65.80	64.63	64.17	.76	.94	.99
8	63.50	65.27	60.98	62.62	.61	.73	.99
12	59.68	64.17	62.21	64.71	.76	.49	.84
16	58.88	69.24	74.20	69.77	.12	.56	.14
24	67.49	62.51	81.13	80.62	.01	.59	.66
28	62.83	63.23	75.68	79.74	.01	.66	.72
32	59.68	61.99	72.98	67.44	.06	.75	.44
36	58.80	61.81	67.47	43.19	.33	.04	.10
48	72.08	70.45	51.53	50.11	.01	.54	.75

[a]LSM ± SEM; ± 5.10.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

Table 3.9. Rumen propionate as a molar proportion of total VFA[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- mol/100 mol ----------						
0	14.99	16.22	15.94	17.98	.63	.56	.89
4	21.81	21.25	22.54	23.84	.56	.90	.74
8	21.22	21.46	23.41	24.67	.34	.79	.86
12	23.23	22.20	25.31	22.52	.67	.50	.75
16	24.24	20.38	18.45	11.36	.01	.05	.57
24	21.01	26.08	6.51	6.19	.01	.40	.34
28	21.72	23.90	13.97	8.81	.01	.60	.20
32	24.29	23.45	16.56	21.73	.10	.44	.29
36	25.68	22.56	19.83	31.26	.57	.14	.01
48	19.12	18.64	21.65	23.70	.29	.56	.45

[a]LSM ± SEM; ± 2.86.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

Table 3.10. Effect of diets on acetate : propionate ratio[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
0	5.3	4.4	4.8	4.2	.90	.80	.95
4	3.1	3.1	2.9	2.7	.92	.98	.97
8	3.1	3.1	2.6	3.1	.94	.94	.92
12	2.6	2.9	2.5	6.4	.56	.48	.54
16	2.5	3.7	5.3	17.2	.01	.03	.07
24	3.3	2.8	21.4	23.6	.01	.78	.66
28	2.9	3.2	8.3	9.5	.05	.80	.87
32	2.5	2.9	8.2	4.5	.22	.57	.49
36	2.3	2.9	7.4	1.4	.69	.28	.21
48	3.8	3.9	3.8	2.1	.63	.67	.64

[a]LSM ± SEM; ± 2.9.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

that time there was no difference across the treatments.

Molar proportion of rumen butyrate doubled numerically by 4 h in all treatments (Table 3.11). Its change across time was not dietary related except at 48 h when HELP and HEHP treatments had a significantly higher proportion of butyrate. Numerically, however, butyrate tended to be lower at 28 and 32 h in 81% TDN groups. Interaction was significant at 16 h. In the lower energy diets, butyrate seemed to follow a repeatable diurnal pattern. Wilson et al. (1975) observed the similar trend while others, Rumsey (1978) and Slyter and Rumsey (1991) reported an overall increase in butyrate proportion in engorged animal. Molar proportions of minor rumen VFA including isobutyrate, isovalerate and valerate were occasionally significantly different between the treatments, however, they had little metabolic importance in this study (Appendix 1.1., 1.2., 1.3).

Concentrations of L- and D-lactate were pooled and expressed as total lactate in mmol/L, and then compared to total VFA (Figure 3.1). Total VFA concentration in 71% TDN animals was always greater than lactate concentration. However, in those animals fed 81% TDN diets, lactate concentration surpassed VFA concentrations from 16 to 32 h with differences significant at 24 and 28 h. The concentration reversed again at 48 h indicating ruminal recovery from acidosis.

Greater dietary protein resulted in lower blood L-lactate at 32 h with 20% CP (Table 3.12). Interaction was significant at 28 h. Dietary TDN apparently did not influence blood L-lactate. L-lactate in the blood remained fairly insensitive to L-lactate changes in the rumen. Harmon et al. (1985) observed a slight increase in blood L-lactate which coincided with the lowest rumen pH. Nagaraja et al. (1978) measured an rapid accumulation of blood L-lactate beginning at 15 h, and transitory increase occurred at 28 h (Dunlop and Hammond, 1965). In contrast, Suber et al. (1979) observed an decrease in plasma L-lactate in engorged steers when compared to normal animals.

Blood D-lactate, followed an entirely different pattern than blood L-lactate (Table 3.13). From negligible amounts, measured at 0 and 4 h, D-lactate increased ($P < .01$) in

Table 3.11. Rumen butyrate as a molar proportion of total VFA[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- mol/100 mol ----------						
0	4.92	6.71	5.15	5.05	.82	.79	.76
4	9.13	9.56	10.09	9.66	.86	.99	.89
8	12.15	10.45	13.19	10.58	.85	.49	.88
12	13.90	10.88	10.54	10.03	.50	.57	.69
16	13.44	8.10	5.62	12.72	.61	.78	.05
24	7.50	8.13	8.84	8.74	.75	.93	.91
28	11.94	9.66	7.14	7.19	.25	.72	.71
32	12.59	11.30	6.28	6.85	.09	.91	.77
36	11.80	12.13	7.43	15.22	.86	.21	.25
48	5.71	7.13	18.90	16.21	.01	.98	.69

[a]LSM ± SEM; ± 3.22.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

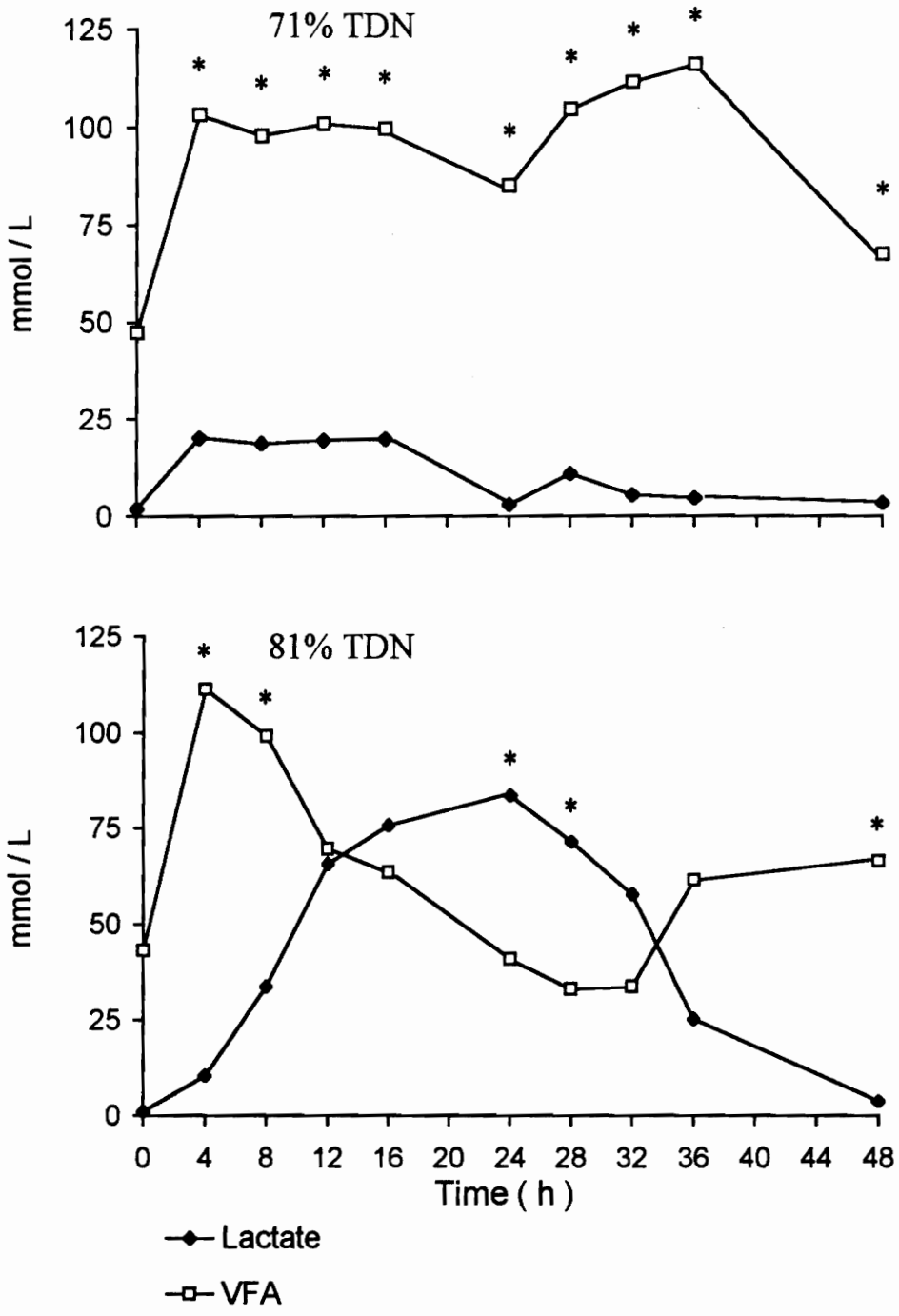

Figure 3.1. Ruminal lactate and VFA in animals fed 71 and 81% TDN, (* = P < .05).

Table 3.12. Response of blood L-lactate to overload of diets varying in TDN and CP[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- mg/dL ----------						
0	5.4	3.0	7.0	6.1	.10	.25	.57
4	11.8	10.2	9.8	10.2	.47	.70	.48
8	12.6	9.5	9.6	9.5	.30	.26	.29
12	11.1	8.8	7.7	8.2	.17	.52	.35
16	5.9	5.2	6.2	6.4	.61	.87	.74
24	7.5	4.3	6.8	7.2	.44	.33	.21
28	11.7	6.8	6.8	6.9	.11	.10	.09
32	9.5	5.0	8.3	7.2	.73	.05	.24
36	4.1	5.0	5.3	2.3	.76	.59	.26
48	5.5	5.1	4.9	2.4	.37	.45	.60

[a]LSM ± SEM; ± 1.4.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

Table 3.13. Response of blood D-lactate to overload of diets varying in TDN and CP[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- mg/dL ----------						
0	.4	.4	.0	.2	.97	.99	.99
4	.4	.7	.0	.3	.97	.97	.99
8	.3	1.3	3.5	9.9	.48	.66	.75
12	.1	5.6	17.1	29.9	.01	.27	.66
16	.0	7.5	33.8	45.2	.01	.26	.81
24	.4	5.7	56.8	58.6	.01	.67	.84
28	.2	2.3	61.3	64.8	.01	.74	.93
32	.0	.3	59.8	69.1	.01	.56	.59
36	.1	.2	59.6	45.4	.01	.40	.39
48	.4	.7	43.9	16.1	.01	.11	.10

[a]LSM ± SEM; ± 8.5.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

HELP and HEHP groups by 12 h, and remained higher in these two treatments. It peaked at 28 and 32 h, reaching 61 and 69 mg/dL in HELP and HEHP treatments, respectively. Blood D-lactate increased in the LEHP relative to LELP group, which persisted at very low levels. TDN dominated changes even though greater dietary protein seem to increase D-lactate. Harmon et al. (1984) measured 43 mg/dL of blood D-lactate at 30 h in engorged animals. Dunlop and Hammond (1965) reported 76 mg/dL at 30 h. Nagaraja et al. (1978) measured 27 mg/dL of D-lactate in an engorged cow at 15 h and saw no further increase after that time. Dougherty (1975) observed a delay in D-lactate accumulation in the blood of engorged sheep. The peak (44 mg/dL) appeared at 48 h, while ruminal fluid pH was around 4.0 for more than 36 h. There is no data which report measurement of D-lactate in the blood during onset of laminitis.

Rumen ammonia was numerically greater in animals fed 20% CP compared to 16% CP diets most of the time (Table 3.14). However, at 4 h, 71% TDN treatments yielded greater values for ammonia, probably because rates of fermentation were slower relative to feed ammonia release immediately after feeding. Amount of dietary protein was also a factor at 4 h ($P < .06$). Overall, dietary TDN was not significant at 8, 28, 32 and 36 h, but was significant at 4, 32, and 36 h. The interaction was significant at 12, 16, 24 and 48 h, primarily because of such low values in the LELP fed animals. At 48 h, reverse occurred which began at 36 h. Greater acid concentration probably formed ammonia nitrogen in the 81% TDN groups.

Plasma urea nitrogen (PUN) varied across treatment and time in a manner similar to the pattern of rumen ammonia. LEHP treatment had the greatest PUN throughout the experiment (Table 3.15). It was higher also in the pretreatment sampling. TDN effects were significant at most sampling intervals. Protein was significant all of the time, except at 0 h. Interaction was significant at most sampling intervals also, largely due to greater values in LEHP diets. Greater PUN was reported by Bargai et al. (1992) who found higher PUN in calves on a farm affected by laminitis. These calves were fed higher protein level, compared

Table 3.14. Response of ruminal ammonia to overload of diets varying in TDN and CP[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- mg/dL ----------						
0	1.92	6.68	3.44	3.10	.63	.30	.24
4	15.61	22.02	8.82	10.54	.01	.06	.27
8	6.63	15.95	7.43	11.27	.37	.01	.20
12	3.59	16.54	10.57	15.00	.21	.01	.05
16	.90	14.95	13.30	15.54	.01	.01	.01
24	1.26	13.04	13.54	16.62	.01	.01	.04
28	10.40	14.90	9.68	16.83	.78	.01	.54
32	5.50	10.26	8.76	16.93	.02	.01	.43
36	4.57	9.41	8.78	19.18	.01	.01	.23
48	3.90	4.98	4.30	17.33	.01	.01	.04

[a]LSM ± SEM; ± 2.21.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

Table 3.15. Response of plasma urea nitrogen to overload of diets varying in TDN and CP[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- mg/dL ----------						
0	5.44	8.64	5.78	5.39	.10	.11	.04
4	7.60	11.11	6.02	6.79	.01	.02	.12
8	5.62	11.41	5.14	5.16	.01	.01	.01
12	4.54	10.66	4.54	5.70	.01	.01	.01
16	3.99	9.33	4.72	4.82	.03	.01	.01
24	5.45	11.31	6.27	6.96	.05	.01	.01
28	6.08	14.52	6.07	8.63	.01	.01	.01
32	4.40	12.35	6.88	10.58	.69	.01	.02
36	4.56	10.83	6.71	10.87	.10	.01	.46
48	5.36	12.04	8.10	11.99	.06	.01	.28

[a]LSM ± SEM; ± .91.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

to unaffected calves at a different farm. Hilwig (1975) pointed out a relationship between rumen ammonia and PUN, stating that feeding high protein results in high rumen ammonia and PUN.

Plasma total protein was greater at 16 h in HELP and HEHP treatments but mean differences were modest (Appendix 1.4). Plasma albumin was slightly more responsive to dietary treatments than plasma total protein (Appendix 1.5). Significant responses were due to TDN, protein or the interaction, depending on the time interval. This makes any reasonable interpretation very difficult. In general, plasma glucose was high in all treatments. Except for samples at 8 and 28 h after feeding no differences were observed (Table 3.16). A moderate increase in plasma glucose in laminitic animals were observed by Sonoda et al. (1977) and Nagaraja et al. (1978).

Hoof temperature was quite variable across the treatments and sampling times. Temperature was significantly lower at 28, 32 and 36 h for both feet in animals fed 81% TDN diets (Table 3.17). Hoof temperature was inversely related to dietary protein at 8 and 28 h, but this was not consistent. Duncan et al. (1985) found a decrease in hoof temperature in animals during acute laminitis while Takahashi and Young (1980b) measured an increase of hoof temperature in affected animals. X-rays did not reveal any significant changes in hoof angle which could be associated with dietary treatments (Appendix 1.6).

Hooves were inspected at 3 and 7 mo after engorgement for any deviant morphology. At 3 mo the hoof inspection indicated possible chronic laminitis (Table 3.18). Separation of the hoof wall, erosion of the sole and the heel as well as double sole were found. A hardship groove was present at the dorsal hoof wall which resulted in a concavity of the dorsal wall, the similar to that described by Greenough (1992). The groove was positioned between 2 to 3 cm from the coronary band, which indicated its relationship with time of engorgement.

At the second inspection, an improvement in hoof condition was observed. Hoof hemorrhage was present in only one case. The hardship groove was pushed further distally,

Table 3.16. Response of plasma glucose to overload of diets varying in TDN and CP[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- mg/dL ----------						
0	79.1	82.4	81.4	76.2	.97	.86	.41
4	81.4	85.4	79.0	76.9	.29	.85	.55
8	91.6	86.4	93.5	83.7	.10	.01	.21
12	90.5	96.0	101.9	96.0	.26	.97	.27
16	100.3	95.8	96.8	99.1	.98	.82	.50
24	97.2	90.4	97.5	97.2	.49	.49	.52
28	77.6	80.7	99.0	101.0	.01	.62	.91
32	93.2	84.2	92.9	92.1	.46	.34	.42
36	83.9	80.1	86.9	79.5	.74	.36	.82
48	85.4	72.3	77.3	86.8	.48	.82	.03

[a]LSM ± SEM; ± 5.2.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

Table 3.17. Temperature in the left hoof[a]

Time (h)[c]	Treatments[b]				Contrasts (P ≤)		
	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- °C ----------						
0	14.3	11.7	13.8	12.9	.88	.45	.71
4	19.6	17.2	19.3	18.3	.86	.47	.75
8	26.1	23.7	25.1	19.5	.26	.09	.50
12	25.6	22.1	22.1	19.7	.20	.21	.80
16	26.2	26.2	25.4	18.3	.06	.14	.13
24	17.0	15.9	17.0	14.6	.77	.45	.77
28	27.2	16.0	18.3	12.7	.01	.01	.23
32	25.6	21.7	14.8	15.6	.01	.51	.31
36	19.6	22.9	11.6	12.1	.01	.88	.13
48	15.0	13.7	11.3	10.3	.15	.64	.93

[a]LSM ± SEM; ± 2.4.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

Table 3.18. Hoof inspection in the calves 3 and 7 mo after being suddenly introduced to diets that contained various amounts of CP and TDN[a]

Trtm.[c]	Observed morphology[b]											
	Hardship groove		Hoof wall separation		Sole hemorrhage		Erosion of the sole		Erosion of the hill		Double sole	
	3 mo	7 mo	3mo	7 mo	3 mo	7 mo	3 mo	7 mo	3 mo	7 mo	3 mo	7 mo
LELP	4	4	1	--	--	--	1	--	1	--	1	--
LEHP	3	3	--	--	--	1	1	--	1	--	--	--
HELP	4	2	3	--	--	--	1	--	1	--	--	--
HEHP	4	2	1	--	--	--	2	1	2	1	--	--

[a]Four animals per treatment.
[b]Number of animals with the observed morphology.
[c]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

and was positioned between 4 and 6 cm from the coronary band. In some calves the groove had disappeared due to a wear. Beyond that point the dorsal hoof wall appeared to be free of any irregularities. Apparently, any earlier abnormalities were completely overcome.

General Discussion

Sudden alteration in ruminal fermentation can result in lactic acidosis which may create a sequel of events, including laminitis. Many attempts to induce laminitis were based on engorgement by using high amounts of readily digestible carbohydrates.

A rapid consumption of high amounts of fermentable components in the diet is a basic precondition to creation of lactic acidosis and potentially laminitis. For that purpose fasting for different lengths of time to create hunger has been employed by many authors. Nagaraja et al. (1992) fasted steers for 24 h, Takahashi and Young (1980a) for 36 h, Suber et al. (1979) for 40 h, Slyter and Rumsey (1991) for 72 h and Rumsey (1978) for 96 h. Since an extended fast could affect microbial population in the rumen we limited fasting only to create an eager desire for feed.

In several studies, the animals were forcefully engorged by placing an allotment of feed into the rumen via permanent fistula. However, voluntary intake is a natural way of engorgement under practical circumstances. Dunlop and Hammond (1965) believed it is difficult to induce animals to ingest a damaging amount of feed by voluntary intake and that environmental factors, such as cold weather, must be involved in a stimulation of appetite.

The 80% TDN animals in this study consumed quite high amounts of concentrate. When expressed as g/kg of BW, this equaled 17 for LELP, 15 for LEHP, 50 for HELP and 42 for HEHP treatment. HELP and HEHP consumption rates are comparable with the engorgement rates used in other studies. Thus, Suber et al. (1979) used flaked corn at the rate of 32 g/kg BW; Nagaraja et al. (1978) used a 1:1 mixture of corn and wheat at the rate of 55 g/kg BW while Dunlop and Hammond used corn at the rate of 62 g/kg BW.

Early symptoms of illness were behavioral differences observed during sampling. Non-affected animals resisted sampling while ill ones become lethargic. Observed lethargy was probably due to approaching acidotic coma and neural depression. Increased respiration rate found in affected animals was probably an effort to rid the body of CO_2 (Huber, 1976).

Diarrhea, lack of appetite, and stiffness observed in this study are frequently observed in lactic acidosis and in laminitis.

Low ruminal fluid pH was a prominent characteristic which dominated throughout the experiment. It fell significantly in engorged animals by 8 h after feeding. It further declined and at 16 h was below 5.0. Dunlop and Hammond (1965) measured pH below 5.0 at 6 h, Harmon et al. (1985) and Nagaraja et al. (1978) at 12 h after engorgement. This discrepancy probably reflects different approaches to engorgement. In the present study, animals ate feed over several hours, while in some studies feed was forced into the rumen in a short time.

Changes in concentrations of lactic acid isomers was inverse to ruminal fluid pH. While in other studies a sharp increase in L-lactate concentration was followed by either a further gradual increase (Harmon et al., 1985) or decrease (Dunlop and Hammond, 1965; Suber et al., 1979; Slyter and Rumsey, 1991), in the present study a several fold increase or decrease could occur between two samplings.

D-lactate in the rumen was similar in pattern, but not in quantity to L-lactate. Differences in quantity may be due to different rates of either synthesis or removal from the rumen. Slyter and Rumsey (1985) indicated that there may exist a strictly D-lactate producing bacterium. Therefore a lower level of D-lactate measured in this study may reflect less favorable conditions for such an organism. In general, it has been thought that rate of absorption for both isomers is the same (Dunlop and Hammond, 1965). However, Harmon et al. (1985) measured a several fold increase in D-lactate absorption and little increase for L-lactate. Racemization of D-lactate to L-lactate in the rumen is a less likely explanation since it had been observed that this process is not very extensive in the rumen (Slyter and Rumsey, 1985).

Rumen total VFA decreased in the acidotic animals. Initially, an increase of VFA coincided with a fall in ruminal fluid pH. Later, as lactate accumulated, both VFA and pH declined. This is in agreement with the concept that an increase of VFA lowers ruminal fluid

pH normally, but drastic decline in pH is caused by increased lactate (Dirkson, 1970). Lactate is a much stronger acid than any of the VFA (Dunlop and Hammond, 1965). The observed changes in total VFA in this study agree with Chaplin and Jones (1973), Wilson et al. (1975), and Slyter and Rumsey (1991) and disagree with Rumsey (1978). Variation in total VFA undoubtedly reflected the substrate availability and the change in a type of rumen fermentation. Reduced VFA concentration can be partially explained by a dilution effect. Increased lactate in the rumen raises osmolality which draws fluid from other tissues causing dehydration of the animal (Dunlop, 1972).

However, not all VFA declined at the same rate. Acetate decreased less than propionate, which resulted in high acetate : propionate ratio. In a few cases almost no propionate was present at certain sampling times. In that situation acetate would contribute as much as 96% of total VFA. The pattern of rumen butyrate differed from any other VFA. Others found that butyrate had the highest negative correlation with ruminal fluid pH of all VFA (Harmon et al., 1985). Molar proportion of rumen valerate increased in engorged animals which is in agreement with observations done by Rumsey (1978) and Slyter and Rumsey (1991).

Differences of L-lactate in the blood was not dietary related and did not correspond directly to rumen L-lactate. In fact, L-lactate tended to be highest in blood of 71% TDN animals. It is possible that L-lactate was absorbed into the blood at a slower rate than D-lactate, or that it was metabolized more rapidly. According to Prins et al. (1974), the latter is probable because they measured faster metabolism for L- than for D-lactate in the rumen epithelium.

When pH declined to less than 5.0 (4.2 in some cases) a rapid accumulation of D-lactate in the blood occurred. At that low pH, lactate may be absorbed in undissociated form and its metabolism may be slower (Dunlop and Hammond, 1965; Prins et al., 1974). Former authors proposed that D-lactate absorption in the presence of hemoconcentration leads to accumulation of high amounts of lactate in the blood. Researchers have usually

measured L-lactate in the blood of animals affected by acute laminitis while neglecting D-lactate.

Rumen ammonia increased in all treatments at 4 h after feeding, and was greater in 71% TDN treatments. This difference could be a result of higher soluble protein in the diets of LELP and LEHP (37 and 33%, respectively) than in HELP and HEHP animals (27 and 25%, respectively). This supports a concept that when ruminal proteolysis is faster than starch digestion, an uncoupling effect between energy and protein may occur. Higher rumen ammonia in 20% CP treatments reflected higher concentration of dietary CP. These observations are in line with the finding by Chaplin and Jones (1973) who observed threefold increase in rumen ammonia over 72 h in engorged sheep. In general, plasma urea nitrogen could be high in three situations; when high degradable protein was fed, when insulin is low or when renal function is impaired.

Plasma total protein was fairly insensitive to diet composition. For acidosis purposes plasma protein is usually measured as a indicator of hemoconcentration. In this study it was higher in affected animals for a short time, pointing out transitory hemoconcentration probably caused by diarrhea and spleen contraction.

Change in hoof temperature may indicate the presence of laminitis. In this study hoof surface temperature was variable. The measurement took place in a semi-enclosed barn affected by great changes in ambient temperature. Other workers have drilled a hole in the hoof wall in order to improve accuracy of hoof temperature measurements (Duncan et al., 1985). Since animals could lay down and possibly change the circulation through the limbs, we measured temperature in both hind legs. Furthermore, some non-specific inflammations, unrelated to the experiment could occur. Svendesen (1979) explained the hypertensive effect of lactic acidemia as peripheral vasoconstriction elicited by stimulation from the sympathetic nervous system. A tendency for lowering the hoof temperature observed in this study may indicate less blood coming into the extremities because of shock created by acidosis. Another possible cause is less tissue circulation in the extremities which could be due to the

decline in plasma volume as fluid passed into the gastrointestinal tract (Dunlop and Hammond, 1965). We propose that the reason for lower hoof temperature, and eventually for the absence of laminitis, was the sympathetic nervous system stimulation which induced peripheral vasoconstriction which shifted blood to the central parts of the body. This could result in less hypothetical compounds (histamine, endotoxin) reaching the dermal lamina, and accordingly, there was not enough substance to cause the inflammation in the lamina.

Rotation of the pedal bone is an irreversible process associated with acute laminitis (Greenough, 1990), which can occur shortly after the insult (Edwards, 1982). The changes associated with rotation are generally small and therefore a high quality X-ray image is necessary for changes to be observed. We did not find changes in rotation of the pedal bone.

Since no unequivocal signs of laminitis were observed in the acute phases, it was decided to inspect hooves 3 and 7 mo later. According to Greenough (1992) this allowed sufficient time to observe hoof hemorrhages which happen at the time of the primary insult. Hardship groove, double sole, separation of the hoof wall, erosion of the sole and the hill and sole hemorrhage are symptoms of laminitis. The observed deviant morphology at 3 mo was not dietary related. Very few signs of chronic laminitis were observed on the second inspection, four mo after the first inspection, suggesting that hooves recovered. Based on these it is evident that lactic acidosis created 3 or 7 mo before the inspections, did not correspond with changes in the frequency of the hoof abnormalities.

Conclusions

Feeding 81% TDN diets resulted in severe acute acidosis in bull calves. This was manifested as anorexia, lethargy, diarrhea and stiffness. Ruminal fluid pH declined below 5.0 within 12 h of feeding as a result of a rapid fermentation. This promoted a many-fold increase in both isomers of lactic acid in the rumen, which peaked at 24 h. Simultaneously, a very moderate increase of lactate occurred in animals fed 71% TDN. Concentration of L-lactate was always greater than concentration of D-lactate in the rumen. Rumen VFA declined in acidotic animals which indicated a disruption of normal rumen activity. Blood L-lactate did not differ between the treatments while blood D-lactate accumulated in acidotic animals peaking between 28 and 32 h. This suggested slower metabolism of D-lactate. Acute laminitis was not observed perhaps due to the overwhelming symptoms of acidosis. Acidosis was associated in some instances with lower hoof temperature possible due to altered hoof circulation. Hooves, examined 3 mo after acute acidosis, indicated the presence of some aspects of chronic laminitis. On the second exam at 7 mo, any evidence of damage was reversed suggesting that recovery had occurred.

CHAPTER 4

EXPERIMENT 2

The objective of this study was to investigate if dietary buffer can prevent acute lactic acidosis caused by voluntary intake of high concentrate diets, and how this would effect some rumen and blood parameters.

Introduction

Feeding of buffers in high concentrate rations has been considered as important in normalyzing rumen pH and maintaining intake in lactating cows. Therefore, dietary supplementation of buffers is practiced.

Based on the previous experiment where high accumulations of both L and D lactic acids occurred in the rumen and accumulation of D-isomer in the blood, this study was designed to determine whether dietary buffer attenuates lactic acidosis when unadapted animals are allowed to consume high quantities of concentrate. Furthermore, it was important to determine if laminitis occurs and how this would differ between the dietary treatments.

Experimental Design and Diets

Twelve Holstein bull calves, 26 wk old and 190 kg BW, were used in this study. Eight of them were used in the previous study and had been fed a diet that contained 71% TDN and either 15 or 20% CP. Four of calves had not been used previously. The animals were taught to consume meals and after a 2-wk period were fasted and fed in the AM as described in Exp. 1.

At 8 AM the animals were given one of two experimental diets which differed relative to sodium bicarbonate; one contained sodium bicarbonate while another did not. Animals were randomly assigned to one of the two treatments.

Ingredients and chemical composition of the diets are shown in Table 4.1. Sodium bicarbonate was premixed with corn and then other concentrate ingredients were added to a final concentrate mixture. Concentrate ingredients were added to corn and alfalfa silages on a daily basis prior to feeding. The experimental diets were offered for 2 d provided the animals continued to eat.

Samplings and Measurements

All procedures and samplings as well as laboratory and statistical analyses were performed as for Exp. 1. Contrast was run for differences between non buffered vs buffered diet.

Table 4.1. Dietary ingredients and chemical composition[a]

Item	Treatments[b]	
	HE	HEB
Dietary ingredient	---------- % of DM ----------	
Alfalfa silage	7.7	6.3
Corn silage	8.4	6.9
Corn meal	36.3	37.2
Barley, ground	37.1	38.0
Soybean meal	8.6	8.8
Molasses, cane, dehy	1.7	1.7
Salt	.2	.2
Sodium bicarbonate		.9
Chemical composition		
DM%	79.8	81.3
CP	15.1	15.1
TDN	81.0	80.8
ADF	9.9	9.2
NDF	20.8	19.8
Calcium	.20	.18
Phosphorus	.36	.37
Sodium	.11	.35

[a]Estimated by Dair4 except for silages which were analyzed.
[b]HE = no buffer added; HEB = HE + sodium bicarbonate.

Results and Discussion

High intakes were observed in eleven of twelve animals in both treatments on d 1. When expressed in kg of DM/100 kg BW, intake averaged 4.79 for high energy (HE), and 4.84 for high energy + sodium bicarbonate treatment (HEB). One bull in HEB treatment ate less; 2.62 kg of DM/kg BW. Early symptoms of metabolic acidosis such as anorexia and distress, occurred within 12 h of feeding. Animals were standing with little movement, while diarrhea persisted. The lack of resistance to sampling indicated neural depression. Hyperventilation was observed in most animals on d 2. A 'glassy look' in their eye was noticeable.

Feed intake and sampling were planned for 48 h. However, in the morning of d 2 most animals were visibly ill and unable to stand. Feed was refused. Sampling continued until 28 h, then stopped and treatment with Entrolyte® began, but was not visibly effective.

In the afternoon of day 2, one animal from each treatment died within three hours of the noon sampling. Another eight were treated with intravenous infusion of a cocktail of minerals, vitamins, antibiotics and sodium bicarbonate in saline. Only two animals were not visibly ill. A third HE animal died on d 3 despite intense medical treatment.

Ruminal fluid changed from a dark-brown, dense suspension to yellow, watery liquid with an obnoxious odor. This coincided with a shift in pH which declined rapidly in both treatments. Numerical differences between treatments began at 8 h (Figure 4.1). Ruminal fluid in HE treatment had significantly lower pH at 12, 24 and 28 h. One animal in the HE treatment had a ruminal fluid pH of 3.96 at 28 h and died shortly after that. In this animal change in pH (7.49 at the beginning to 3.96) represents a 3,400-fold increase in hydrogen ion concentration. When average pH (7.54 vs 4.51) found in HE treatment were compared the same way, more than a 1,000-fold increase in hydrogen ion concentration had occurred.

Kezar and Church (1979) prevented acute acidosis with supplementation of sodium bicarbonate. Emery and Brown (1961) measured a significant increase in ruminal fluid pH

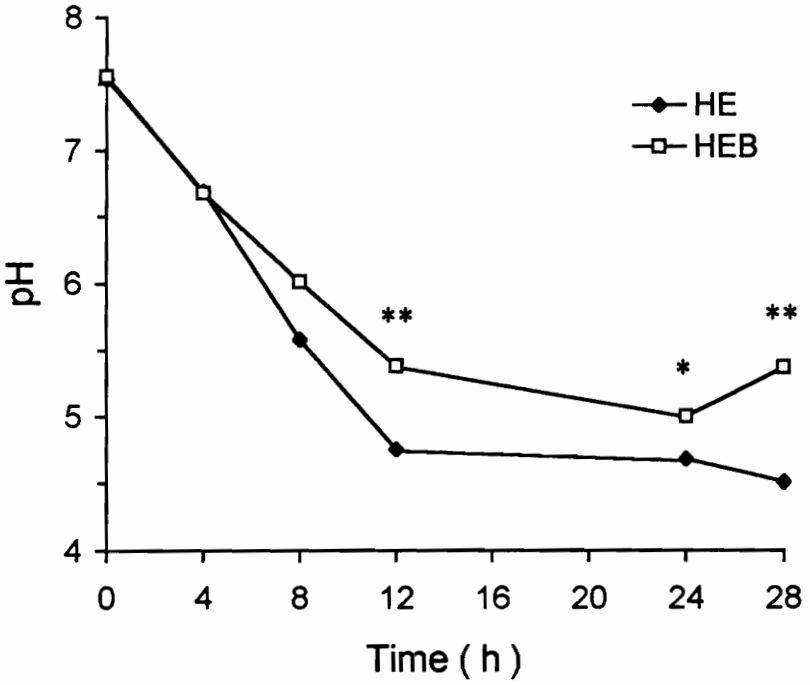

Figure 4.1. Response of ruminal pH in calves fed 81% TDN diets with or without sodium bicarbonate, (* = P < .10; ** = P < .05).

when sodium bicarbonate was added to a high grain diet, while Esdale and Satter (1972) increased ruminal fluid pH with continual infusion of sodium bicarbonate. Ralston and Patton (1976) measured greater ruminal fluid pH in animals fed sodium bicarbonate. Data from several studies (Trenkle et al., 1979) showed a consistently greater ruminal fluid pH with the addition of sodium bicarbonate.

L-lactate in the rumen was low at 0 h in both treatments (Figure 4.2) and increased many fold by 8 h. At 12 h the L-lactate concentration in the HE group was nearly double that in the buffered treatment. Beyond 12 h, L-lactate content of the HEB animals moved nearer to values in unbuffered animals, although they remained lower numerically. Ruminal D-lactate concentrations followed a progressive increase in a manner similar to L-lactate (Figure 4.3). It increased several fold within 8 h after feed was offered and fluid from unbuffered diets showed the greatest concentrations of D-lactate. Beyond 12 h the HE group yielded significantly greater D-lactate. The overall ratio L-lactate:D-lactate (L:D) was 55:45 for HE and 60:40 for HEB treatment. The literature reports that total rumen lactate was less in steers supplemented with sodium bicarbonate (Ralston and Patton, 1976), or was not present in ruminal fluid of engorged sheep supplemented with buffer (Kezar and Church, 1979).

Ratio of L- to D-lactate in the rumen at various times is shown in Table 4.2. In HE animals the ratio tended to decrease while in HEB it remained unchanged across time. This indicated that bicarbonate was effective in inhibition of D-lactate accumulation.

Rumen total VFA nearly tripled in both treatments by 4 h (Figure 4.4). VFA concentration declined sharply between 4 and 12 h in HE group. Further decrease occurred at a slower rate between 12 and 24 h. In HEB treatment, however, the decrease was more gradual and VFA concentration declined in a near linear manner from 4 to 28 h. As ruminal lactate increased, VFA concentrations decreased. This was especially true as lactic acid production reduced pH sufficiently to inhibit microbial fermentation. Similarly, Ralston and Patton (1976) found a negative correlation between total VFA and lactic acid in engorged

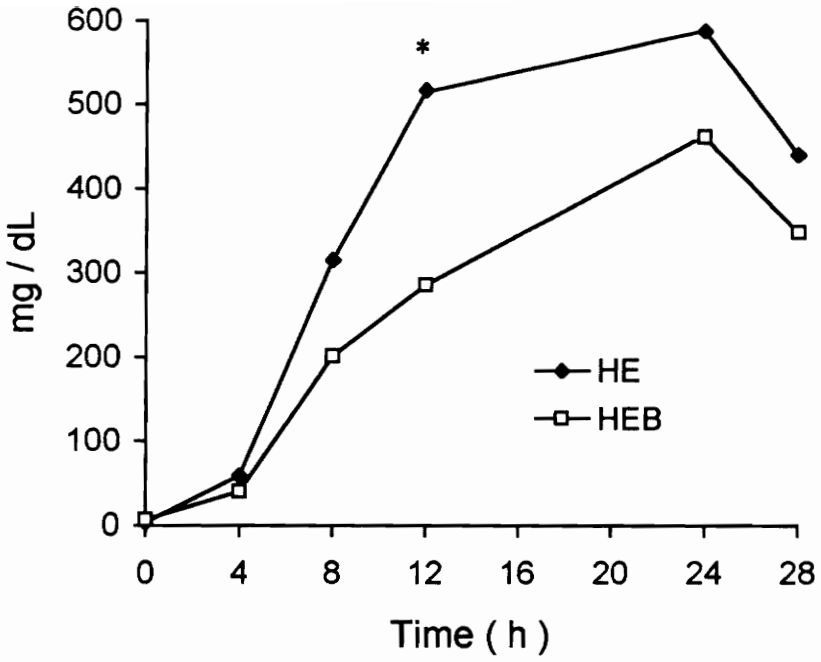

Figure 4.2. Response of ruminal L-lactate in calves fed 81% TDN diets with or without sodium bicarbonate, (* = P < .05).

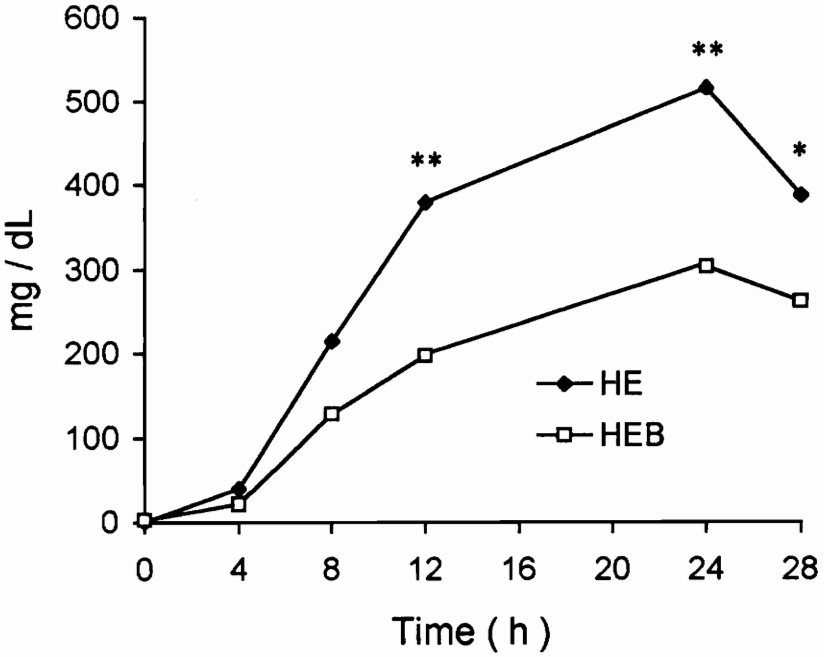

Figure 4.3. Response of ruminal D-lactate in calves fed 81% TDN diets with or without sodium bicarbonate, (* = P < .10; ** = P < .05).

Table 4.2. Ratio L-lactate to D-lactate in the rumen in calves fed 81% TDN with or without sodium bicarbonate[a]

	Treatments[b]		Contrast (P ≤)
Time (h)	HE	HEB	HE:HEB
0	90:10	81:19	.22
4	61:39	66:34	.52
8	61:39	67:33	.44
12	58:42	66:34	.30
24	56:54	67:33	.16
28	55:45	67:33	.14

[a]LSM ± SEM; ± 1.8.
[b]HE = no buffer added; HEB = HE + sodium bicarbonate.

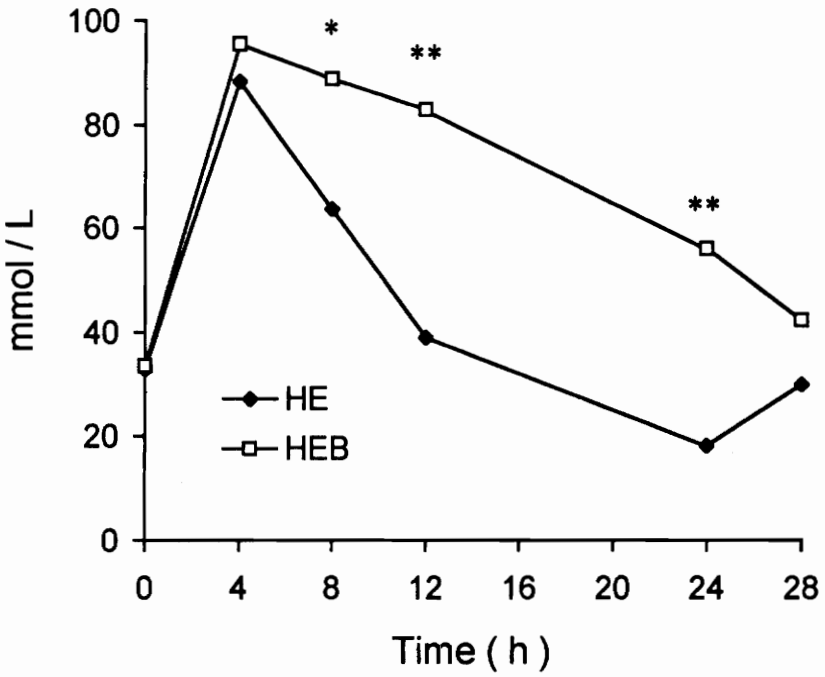

Figure 4.4. Response of ruminal VFA in calves fed 81% TDN diets with or without sodium bicarbonate, (* = P < .10; ** = P < .05).

steers. Emery and Brown (1961) found no difference in total VFA when sodium bicarbonate was added to high grain diet.

Acetic acid accounted for the greatest proportion of total VFA and accordingly, it varied similarly to total VFA across time. However, its molar proportion was not significantly different between the treatments (Table 4.3). Likewise, ruminal propionate, as a proportion of total VFA was not different for buffered and unbuffered animals (Table 4.4).

Rumen acetate to propionate ratio varied across the time but was not significantly different between the treatments at any time (Table 4.5). The same was observed by Emery and Brown (1961). Esdale and Satter (1972) found an increase in the Ac:Pr ratio when sodium bicarbonate was infused into the rumen. Trenkle et al. (1979) pointed out that sodium bicarbonate consistently increased the Ac:Pr ratio in cows while this was less consistent in beef cattle and sheep. Butyrate increased initially relative to other VFA in both treatments but returned to a starting value at the end of experiment (Table 4.6). At 12 h it was greater in the HEB treatment.

Rumen minor VFA including isobutyrate, isovalerate and valerate were not significantly different between HE and HEB treatments (Appendix 2.1., 2.2., 2.3). Buffering caused no changes in molar percentages of VFA in earlier studies (Emery and Brown, 1961; Ralston and Patton, 1976). However, Esdale and Satter (1972) measured an increase in molar proportion of acetate, butyrate and valerate, while propionate decreased, while Kezar and Church (1979) noted an increase in acetate proportion after buffer was offered.

The relation between total VFA and total lactate in the rumen is shown in Figure 4.5. In HE animals the pattern of changes resembled Exp. 1. Dietary sodium bicarbonate altered this by inhibiting the rate of increase and the maximal amount of total lactate in the rumen. At the same time, bicarbonate delayed decrease in total ruminal VFA.

Blood L-lactate did not differ between HE and HEB treatments for the first 24 h (Table 4.7). However, at 28 h HE treatment yielded a sharp increase of L-lactate relative to the previous sampling time, while HEB treatment remained unchanged. D-lactate began

Table 4.3. Rumen acetate as a molar proportion of total VFA in calves fed 81% TDN diets with or without sodium bicarbonate[a]

	Treatments[b]		Contrast (P ≤)
Time (h)	HE	HEB	HE:HEB
	---------- mol/100 mol ----------		
0	72.12	70.48	.79
4	61.52	62.44	.88
8	61.79	60.46	.83
12	73.24	68.17	.40
24	72.18	72.88	.91
28	65.87	71.90	.32

[a]LSM ± SEM; ± 4.48.
[b]HE = no buffer added; HEB = HE + sodium bicarbonate.

Table 4.4. Rumen propionate as a molar proportion of total VFA in calves fed 81% TDN diets with or without sodium bicarbonate[a]

	Treatments[b]		Contrast (P ≤)
Time (h)	HE	HEB	HE:HEB
	---------- mol/100 mol ----------		
0	14.85	14.70	.97
4	23.48	20.92	.51
8	22.12	21.66	.91
12	19.18	17.29	.63
24	16.02	17.89	.63
28	21.77	17.27	.25

[a]LSM ± SEM; ± 2.95.
[b]HE = no buffer added; HEB = HE + sodium bicarbonate.

Table 4.5. Rumen acetate to propionate ratio in calves fed 81% TDN diets with or without sodium bicarbonate[a]

	Treatments[b]		Contrast (P ≤)
Time (h)	HE	HEB	HE:HEB
0	4.9	4.8	.98
4	2.6	3.1	.83
8	3.1	3.0	.97
12	7.1	7.0	.96
24	7.7	6.3	.56
28	3.3	3.4	.93

[a]LSM ± SEM; ± 1.8.
[b]HE = no buffer added; HEB = HE + sodium bicarbonate.

Table 4.6. Rumen butyrate as a molar proportion of total VFA in calves fed 81% TDN diets with or without sodium bicarbonate[a]

	Treatments[b]		Contrast (P ≤)
Time (h)	HE	HEB	HE:HEB
	---------- mol/100 mol ----------		
0	7.38	8.29	.78
4	12.54	12.89	.92
8	13.54	15.05	.64
12	6.41	12.09	.09
24	7.42	5.81	.62
28	6.53	6.38	.96

[a]LSM ± SEM; ± 2.39.
[b]HE = no buffer added; HEB = HE + sodium bicarbonate.

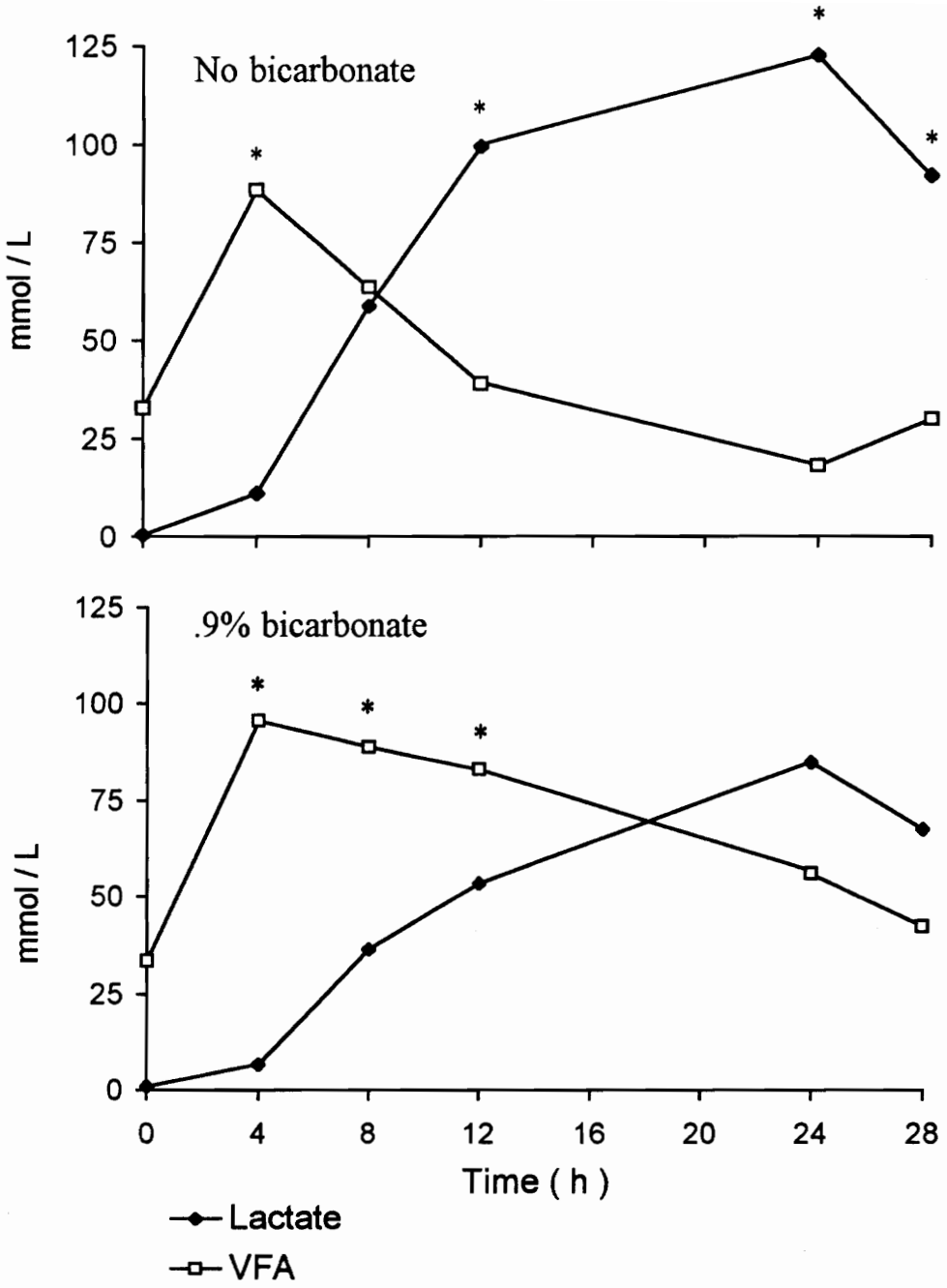

Figure 4.5. Ruminal lactate and VFA in animals fed 0 and .9% of sodium bicarbonate, (* = P < .05).

Table 4.7. Blood L-lactate in calves fed 81% TDN diets with or without sodium bicarbonate[a]

	Treatments[b]		Contrast (P ≤)
Time (h)	HE	HEB	HE:HEB
	---------- mg/dL ----------		
0	3.9	4.0	.98
4	9.9	7.4	.55
8	9.0	9.9	.83
12	7.2	9.5	.59
24	9.2	8.4	.84
28	17.9	7.5	.02

[a]LSM ± SEM; ± 3.2.
[b]HE = no buffer added; HEB = HE + sodium bicarbonate.

to appear in the blood at 8 h (Table 4.8). D-lactate increased several-fold in both treatments at 12 h and accumulation continued. Maximal values for HE and HEB treatments (90.9 and 49.6 mg/dL, respectively) were reached at 28 h. HE fed animals had significantly greater amounts of D-lactate in the blood at 12, 24 and 28 h. D-lactate increased about 90- and 50-fold for HE and HEB treatments, over the 28-h period.

Rumen ammonia increased over time in both treatments (Table 4.9). At 8 and 12 h it was significantly greater in HE group. After that time there was little difference between treatments. At 28 h it nearly doubled in both treatments relative to the baseline value. Emery and Brown (1961) found a tendency for increasing rumen ammonia when sodium bicarbonate was added to a high grain diet. Plasma urea nitrogen (PUN) gradually decreased in both treatments after the initial increase (Table 4.10). HE treatment had significantly greater PUN at 24 and 28 h. This could indicate a degradation of body protein for gluconeogenesis or poor kidney function. Overall, this likely indicates a general breakdown in energy metabolism since energy is required to clear urea from the body.

Table 4.8. Blood D-lactate in calves fed 81% TDN diets with or without sodium bicarbonate[a]

	Treatments[b]		Contrast (P ≤)
Time (h)	HE	HEB	HE:HEB
	---------- mg/dL ----------		
0	.0	.1	.99
4	.0	.0	.99
8	6.8	2.1	.64
12	36.5	14.5	.03
24	79.7	49.1	.01
28	90.9	49.6	.01

[a]LSM ± SEM; ± 7.2.
[b]HE = no buffer added; HEB = HE + sodium bicarbonate.

Table 4.9. Response of ruminal ammonia in calves fed 81% TDN diets with or without sodium bicarbonate[a]

	Treatments[b]		Contrast (P ≤)
Time (h)	HE	HEB	HE:HEB
	---------- mg/dL ----------		
0	6.12	7.38	.62
4	7.92	5.88	.43
8	12.59	7.57	.05
12	15.03	10.13	.06
24	13.65	14.30	.80
28	12.57	13.36	.76

[a]LSM ± SEM; ± 1.82.
[b]HE = no buffer added; HEB = HE + sodium bicarbonate.

Table 4.10. Plasma urea nitrogen in calvesfed 81% TDN diets with or without sodium bicarbonate[a]

	Treatments[b]		Contrast (P ≤)
Time (h)	HE	HEB	HE:HEB
	---------- mg/dL ----------		
0	8.52	9.96	.22
4	10.57	12.54	.10
8	7.58	7.62	.97
12	5.75	6.09	.77
24	8.71	5.81	.02
28	11.56	7.00	.01

[a]LSM ± SEM; ± .84.
[b]HE = no buffer added; HEB = HE + sodium bicarbonate.

General Discussion

According to Dunlop and Hammond (1965), laminitis is a sequel of acute lactic acidosis, which may be less evident in some cases (Dougherty, 1975), and it is often hard to detect at the time of its onset (Boosman et al., 1991b). This experiment was designed to determine if dietary sodium bicarbonate can prevent acute lactic acidosis and influence development of acute laminitis which appears associated with acidosis.

Animals engorged themselves by voluntary intake. Dietary supplementation of .75% of sodium bicarbonate as fed did not alter intake. Lactic acidosis occurred chronologically as in Exp. 1. A clinical picture, observed at 12 h, was characterized by diarrhea, lack of appetite, stiffness and clinical systemic illness; symptoms frequently described for the onset of acute laminitis. Suber et al. (1979) obtained a similar response from six engorged steers, and observed acute laminitis in three of them. The clinical picture of acute laminitis is well described (Greenough et al., 1981; Edwards, 1982). However, there are no signs which are pathognomonic for laminitis. Therefore, it is possible that signs of laminitis may be outweighed by other symptoms, especially those of acute systemic acidosis (Boosman et al., 1991b). In this study, clinical evidence of acute laminitis was not certain, the symptoms of acidosis were so overwhelming.

Acidosis quickly progressed between 24 and 28 h. At 24 h even the most affected animals did not appear in a life threatening situation. Four hours later animals were overcome and a decision of termination of the experiment was made. Just 2-3 h after that, two of the animals died while eight others were severely affected and required intense medical treatment to prevent death. Hyperventilation, which became dominant at that time, indicated an attempt of the organism to rid the system of CO_2.

Animals fed diets with or without buffer were affected with acidosis. Although one animal in HEB died and three more were ill, the symptoms of acidosis were even more pronounced in the HE animals of which two died and the remaining four struggled to

survive. Animals fed HEB treatment responded faster to medication and recovered fully in a shorter period of time.

Why did one animal from HEB treatment die? A possible explanation is that this animal ate in great excess. DM intake in this individual equaled 5.57 kg of DM/100 kg BW and was greater than any other in the group. Although the length of time that he spent eating was not measured it is very likely that he consumed much of the feed in a relatively short period of time. Therefore, the amount of bicarbonate was not adequate to prevent lethal lactic acidosis. Of the two animals which died, both also consumed a high amount of feed (5.48 and 5.16 kg of DM/100 kg BW). It should be noticed, however, that one other animal in HEB group also consumed a large amount of feed (5.30 kg DM/100 kg BW) and no visible symptoms of sickness were observed. This pointed out the individual differences among the animals.

In general, it is widely accepted that buffer increases ruminal fluid pH relative to unbuffered treatments. However, in this experiment sodium bicarbonate only slowed the decline in ruminal fluid pH. This is in agreement with other studies (Emery and Brown, 1961; Esdale and Satter, 1972; Ralston and Patton, 1976). There are few reports relative to the effect of bicarbonate in the prevention of acute lactic acidosis that permit a direct comparison with our data.

Sodium bicarbonate controlled an increase of L-lactate in the rumen for the first 12 h relative to unbuffered diet. However, it was even more effective in inhibition of accumulation of rumen D-lactate. A lower total lactate concentration when sodium bicarbonate was fed is reported by Ralston and Patton (1976).

Sodium bicarbonate either increased (Ralston and Patton, 1976) or had no effect on total VFA compared to controls (Emery and Brown, 1961). However, in the present study buffer slowed down a decline in total VFA concentration rather than increased it. This difference was clearly caused by the different experimental approach. Decline of total VFA concentration was rapid and profound in HE treatment after 4 h. Buffer did not change

molar proportion of any VFA. Accordingly, there was no effect on acetate to propionate ratio. This is in agreement with Emery and Brown (1961), while Esdale and Satter (1972) noted an increase in the ratio when buffer was added.

L-lactate in the blood remained fairly constant in both treatments until 24 h. It sharply increased in HE treatment at 28 h. At the same time blood D-lactate continually increased in both treatment groups but increased faster in HE. This might indicate either slower metabolism or higher absorption of D-lactate, or both. Buffer inhibited the increase of D-lactate in the blood. HE treatment yielded very high concentration of D-lactate in blood which peaked at 28 h. This coincided with peak of L-lactate in the blood. PUN was higher in HE group which indicated protein degradation in this treatment.

Acute laminitis was not evident in this study, possibly due to prevailing symptoms of severe acidosis. Sodium bicarbonate inhibited increase of rumen and blood lactate and slowed down the decline in ruminal fluid pH. This partially prevented or delayed acidosis. Higher quantity of dietary bicarbonate may have controlled symptoms even further.

Conclusions

Feeding 81% TDN resulted in acute lactic acidosis in young bulls. Buffer inhibited the increase in lactic acid concentration and the decrease in ruminal fluid pH. This resulted in less sick animals in the buffer-fed group. Blood L-lactate persisted at low levels for most of the sampling intervals until it increased in nonbuffered animals. Blood D-lactate, however, increased in both groups, but more in nonbuffered animals. Severe acidosis was fatal to some calves and caused sickness in most of the animals. Acute laminitis was not observed in this experiment. The data from this experiment provided further support to recommend feeding buffers in high concentrate diets.

CHAPTER 5

EXPERIMENT 3

Introduction

It has been well documented in the literature that sudden introduction of concentrate diets may lead to acute acidosis and laminitis. Furthermore, long-term feeding of concentrate diets may lead to chronic acidosis and subclinical or chronic laminitis. These have been observed particularly in young fattening steers.

Feeding of buffers is considered important in preventing acidosis. Buffers increase alkaline reserve and rumen pH. Although primarily used to improve the efficiency of feed use, lasalocid has been considered as a possible inhibitor of lactic acid accumulation in the rumen. Its mechanism of action is inhibition of lactic acid producing bacteria.

The objectives of this study were to investigate a) if dietary buffer or ionophore would prevent acute lactic acidosis in animals suddenly introduced to fermentable diets, and b) long term effect of feeding highly fermentable diets on sensitivity to modulation of blood pressure in hooves.

Experimental Design and Diets

Twenty-four Holstein bull calves, 17 wk old and 120 kg in BW, were fed for two wk an adjustment diet that contained 68% TDN and 16% CP. Feed was delivered twice daily at 8 AM and 4 PM. Animals were trained to meal feeding by permitting two h for feed consumption at each feeding. Water was available at all times. Calves were housed in individual pens (1.2 x 2.0 m) in an open-sided barn at the Virginia Tech Dairy Center.

Animals of similar age were randomly assigned to groups of four to each treatment.

On the last day of the adjustment period, animals were given feed in the AM only and deprived of the feed for the following 22 h. At 8 AM of the following day the animals were given one of four experimental diets. One group continued to receive the adjustment diet (CN). Three groups were fed diets that contained 80% TDN (Table 5.1). One group was supplemented with sodium bicarbonate (HB), another received ionophore (HI), while the third group contained neither (HE). Diets were isonitrogenous (16% CP). The animals in the ionophore group were given 1 mg/kg BW of lasalocid (Bovatec 68, Roche, Nutley, NJ) in gelatine capsule (Torpac Limited, India) at that time. In order to approximate a dose of 1 mg/kg BW, adjustments in ionophore amount were performed weekly after calves were weighed. Experimental diets were fed for three mo ad libitum in the AM with feed available until 8 PM of every day. Concentrate ingredients were premixed and stored until corn and alfalfa silages added.

Samplings and Measurements

All animal procedures, and samplings, as well as laboratory procedures were performed as described in Chapter 3 except that X-ray filming was not done and animals were weighed weekly. Hooves were examined at the end of three-month feeding period.

Statistical analyses were performed as previously described, except non-orthogonal contrasts were tested. Therefore, an adjustment of P-values was performed according to Games (1977), and the following formula was used for calculation of true P-value:

$$1 - (1 - \alpha)^g = FWI$$

where α = unadjusted P-value from the SAS output, g = number of contrasts, and FWI = Familywise Type I error. Contrasts included control vs three high energy diets, high energy vs high energy ionophore, high energy vs high energy buffer, and high energy ionophore vs high energy buffer.

Table 5.1. Dietary ingredients and chemical composition[a]

Item	Treatments[b]			
	CN	HE	HI	HB
Dietary ingredients	---------- % of DM ----------			
Alfalfa silage	26.3	4.1	4.1	4.1
Corn silage	46.6	7.4	7.4	7.3
Corn meal	9.8	36.8	36.8	36.3
Barley, ground	9.8	36.8	36.8	36.3
Soybean meal	4.7	10.7	10.7	10.7
Molasses, cane	2.0	2.7	2.7	2.6
2:1 mineral[c]	.8	1.4	1.4	1.4
Sodium bicarbonate				1.3
Chemical composition				
DM%	56.6	80.8	80.8	81.0
CP	16.0	16.0	16.0	15.8
TDN	68.0	80.0	80.0	79.0
ADF	24.3	8.7	8.7	8.6
NDF	39.4	18.9	18.9	18.7
Calcium	.87	.49	.49	.48
Phosphorus	.34	.51	.51	.50

[a]Estimated by Dair4 except for silages which were analysed.

[b]CN = according to NRC recommendations for 100 kg-large-breed growing males with 1 kg of daily gain; HE = high TDN diet; HI = high TDN diet with added 1 mg/kg BW of lasalocid per day; HB = high TDN with added sodium bicarbonate.

[c]Contained: 21.5% Ca; 10.0% P; 12.0 NaCl; 4% Mg; .3% K; .2% S; .001% Co; .02% Cu; .006% I; .75% Fe; .1% Mn; .003% Se; .13% Zn; .1% F, and 162,000 IU of vitamin A/kg, 54,000 IU of vitamin D/kg, and 54 IU of vitamin E/kg.

Results and Discussion

Dry matter (DM) intakes measured on the first two days of the experiment are shown in Table 5.2. When the intake measured on d 1 is compared to the average daily intake in the week when the adjustment diet was fed, the calves in control group (CN) increased their DM intake 34%, while animals in the 80% TDN (HE), 80% TDN + lasalocid (HI), and 79% TDN + sodium bicarbonate (HB) treatments, increased 50, 54 and 37%, respectively. On d 2, however, consumption decreased below the adjustment period except for CN.

Intake of 80% TDN diets on d 1 caused mild acidosis. This was manifested by dullness, stiffness, neural depression and diarrhea. Intake per unit of BW was less than in earlier experiments. Accordingly, acidosis was less severe in this experiment relative to previous experiments. At no point was medication considered necessary for recovery.

At 8 h ruminal fluid became yellow and watery with a pungent odor in HE and HI groups. This coincided with ruminal fluid pH which was less in animals on these treatments than in CN at 12, 16, and 24 h (Table 5.3). The decline in pH was not as severe in HB group. HB differed from the other high energy treatments at 12 h only. Greater pH in HB group was probably due to a combined effect of sodium bicarbonate and lower DM intake. There was no difference in pH between HE and HI treatments although HE generally had numerically greater averages.

Quigley et al. (1992) observed that ruminal pH tended to be greater when lasalocid was delivered via ruminal cannula but not when given via milk replacer. In the present study, however, lasalocid did not prevent the fall in rumen fluid pH. The length of administration of ionophore prior to inducing lactic acidosis may be important. Lasalocid proved effective in prevention of acidosis when given 7 d prior to grain engorgement but not when given for 2 d prior (Nagaraja et al., 1981). In this study ionophore was offered for just one day prior to refeeding.

Rumen L-lactate rapidly increased within the first 4 h in all treatments (Table 5.4).

Table 5.2. Dry matter consumption

	Treatments[a]				
	CN	HE	HI	HB	SEM
	---------- % of BW ----------				
Day 1	3.33[bc]	3.55[bc]	3.74[b]	3.18[c]	.18
Day 2	2.09[b]	.99[c]	.60[c]	1.29[bc]	.28

[a]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[bc]Means within a row lacking a common superscript are significantly different ($P \leq .10$).

Table 5.3. Ruminal fluid pH in calves fed two levels of TDN with either lasalocid, sodium bicarbonate or no addition to the high TDN diets[a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
0	7.31	7.38	7.34	7.29	.99	.99	.99	.99
4	6.68	6.38	6.45	6.79	.95	.99	.38	.55
8	6.42	6.07	5.99	5.97	.20	.99	.99	.99
12	6.39	5.67	5.52	6.23	.03	.96	.12	.03
16	6.55	5.81	5.61	5.84	.01	.90	.99	.83
24	7.01	6.07	5.95	6.25	.01	.98	.94	.68
28	6.64	6.74	6.32	6.31	.87	.37	.35	.99
32	6.79	6.50	6.38	6.50	.38	.98	.99	.98
36	6.63	6.21	6.41	6.16	.29	.90	.99	.82

[a]LSM ± SEM; ± .19.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

Table 5.4. Ruminal fluid L-lactate in calves fed two levels of TDN with either lasalocid, sodium bicarbonate or no addition to the high TDN diets[a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- mg/dL ----------							
0	20.2	22.4	20.1	21.9	.99	.99	.99	.99
4	82.0	92.6	121.2	47.4	.99	.55	.13	.01
8	45.4	22.5	39.1	22.3	.78	.90	.99	.89
12	51.0	29.6	74.7	18.7	.96	.13	.98	.04
16	23.4	18.5	98.9	15.5	.85	.01	.99	.01
24	27.3	10.9	19.5	13.2	.92	.99	.99	.99
28	38.6	10.4	11.7	21.0	.51	.99	.98	.99
32	33.9	8.6	7.6	10.6	.48	.99	.99	.99
36	36.8	11.2	15.6	15.6	.58	.99	.99	.99

[a]LSM ± SEM; ± 15.9.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

However, that increase was lower in HB than in HI treatment at 4 h, probably due to both less feed intake and a direct effect of buffer. There was no significant difference in L-lactate between CN and the 80% TDN treatments. L-lactate increased for the second time in HI treatment at 16 h, and the increase was greater than in HE or HB treatments. This does not agree with Nagaraja et al. (1981) and Nagaraja et al. (1985), who measured lower ruminal L-lactate in the lasalocid treatment. Nevertheless, Kezar and Church (1979) found no lactate in engorged sheep when either ionophore or sodium bicarbonate was supplemented.

Rumen D-lactate concentrations changed across time similar to L-lactate (Table 5.5). A several-fold increase occurred at 4 h in all except the HB group. In the HB, D-lactate increased about 50% relative to pretreatment. Like the L-isomer, D-lactate accumulation expressed an biphasic response in HI group. The first peak occurred at 4 and the second at 12 h after feeding. Rumen D-lactate was significantly greater in HE and HI than in HB treatment at 4 h. Concentration in the HI group was greatest at 12 and 16 h. In contrast to previous studies in which L-lactate was a predominant isomer in rumen fluid, in the present study D-lactate was numerically greater than L-isomer. This could be the result of a selective inhibition of L-lactate producing bacteria by lasalocid as proposed by Dennis et al. (1982). Similarly, Nagaraja et al. (1985) observed no significant reduction of ruminal D-lactate when lasalocid was added. Nagaraja et al. (1981), however, measured a significant reduction of rumen D-lactate in lasalocid treated animals upon glucose engorgement but not when animals were engorged with grain. Finally, lasalocid lowered both lactates (Nagaraja et al., 1982).

Blood L-lactate varied across time (Table 5.6). At 28 h the CN group yielded greater concentration than 80% TDN treatments. HI group had greater blood L-lactate than HE at 8 h, although without explanation. Blood D-lactate was present in a very low concentration and did not differ across treatments (Table 5.7). Nagaraja et al. (1982) measured a slight increase in both lactates in the blood within 12 h after engorgement. Nagaraja et al. (1981) observed a moderate increase in blood L-lactate while blood D-

Table 5.5. Ruminal fluid D-lactate in calves fed two levels of TDN with either lasalocid, sodium bicarbonate or no addition to the high TDN diets[a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- mg/dL ----------							
0	22.8	27.4	24.1	26.2	.99	.99	.99	.99
4	105.3	106.6	140.0	38.6	.97	.49	.02	.01
8	53.9	26.1	53.7	23.8	.78	.67	.99	.60
12	67.3	32.0	116.7	23.0	.97	.01	.99	.01
16	33.0	18.3	92.5	15.4	.98	.01	.99	.01
24	30.4	16.2	20.0	15.7	.93	.99	.99	.99
28	39.8	11.3	13.1	19.5	.57	.99	.99	.99
32	39.8	7.8	8.2	10.6	.37	.99	.99	.99
36	43.2	12.0	16.9	15.7	.46	.99	.99	.99

[a]LSM ± SEM; ± 17.5.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

Table 5.6. Blood L-lactate in calves fed two levels of TDN with added lasalocid or sodium bicarbonate to the high TDN diets[a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- mg/dL ----------							
0	6.4	6.3	5.4	6.7	.99	.98	.99	.93
4	9.9	8.0	10.0	7.6	.85	.70	.99	.55
8	8.8	8.7	13.2	10.7	.54	.06	.72	.54
12	10.1	7.4	10.9	10.4	.99	.21	.36	.99
16	6.3	7.0	10.0	10.7	.18	.35	.17	.99
24	5.9	4.6	4.4	6.8	.99	.99	.65	.54
28	9.0	2.6	4.1	4.9	.01	.87	.63	.99
32	5.6	3.3	2.4	4.2	.43	.98	.98	.80
36	5.5	4.4	5.4	6.0	.99	.97	.87	.99

[a]LSM ± SEM; ± 1.3.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

Table 5.7. Blood D-lactate in calves fed two levels of TDN with added lasalocid or sodium bicarbonate to the high TDN diets [a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- mg/dL ----------							
0	1.2	1.4	1.9	1.6	.99	.96	.99	.99
4	1.0	1.3	1.5	2.0	.99	.99	.74	.93
8	.2	.2	.7	.1	.96	.96	.99	.89
12	.2	.3	.4	.0	.99	.99	.98	.98
16	.9	.5	1.8	.9	.59	.23	.97	.58
24	.4	.5	.1	.4	.99	.93	.99	.99
28	.8	.4	.4	.3	.99	.99	.99	.99
32	.0	.0	.0	.0	.99	.99	.99	.99
36	.6	.0	.4	.0	.98	.96	.99	.97

[a]LSM ± SEM; ± .48.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

lactate increased significantly at 24 h in grain engorged animals. Low D-lactate found in the blood, in this study, reflected relatively low levels of lactate present in the rumen. Rowe et al. (1994) measured slight increases in blood D-lactate in horse administered Virginiamycin. The authors believed that low levels of D-lactate were due to a specific inhibition of microbes producing lactic acid in hindgut by ionophore.

Rumen total VFA was generally low in all treatments (Table 5.8). At 28 h total VFA concentration in CN was greater than in the 80% TDN treatments. This probably reflected greater DM intake in d 2. At 36 h total VFA was greater in HB than in HI treatment. A part of the variation in total VFA could be explained by lactic acidosis which was present. Others observed a significant decrease of total rumen VFA in acidotic animals (Nagaraja et al., 1982; Kezar and Church, 1979), while Ralston and Patton (1976) found a negative correlation between total VFA and lactic acid in engorged steers. However, in an earlier study lasalocid increased total rumen VFA (Nagaraja et al., 1985). Sodium bicarbonate was not effective in increasing total VFA when added to high grain diets (Emery and Brown, 1961).

Molar percentage of acetate was significantly greater in CN treatment between 8 and 36 h (Table 5.9). There was no difference in acetate proportion between HE and HI treatments. HB had significantly greater values than HE at 8, 12 and 16 h, and greater than HI from 8 to 36 h. This agrees with Esdale and Satter (1972) and Kezar and Church (1979) who found that sodium bicarbonate increased molar percentage of acetate in the rumen. However, in some other studies buffer had no effect on molar proportion of acetate (Emery and Brown, 1961; Ralston and Patton, 1976). Acetate did not change significantly with dietary ionophore, which agrees with Kezar and Church (1979) and disagrees with Nagaraja et al. (1981) and Nagaraja et al. (1985) who observed a decrease in rumen acetate proportion when lasalocid was given.

Molar proportion of propionate became significantly greater in 80% TDN treatments at 32 h (Table 5.10). HI had a greater proportion of propionate than HE at 8 and 12 h. This

Table 5.8. Concentration of total VFA in the rumen of calves fed two levels of TDN with added lasalocid or sodium bicarbonate to the high TDN diets[a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- mM ----------							
0	26.00	28.17	26.51	28.37	.99	.99	.99	.99
4	58.37	65.75	63.62	56.65	.97	.99	.72	.87
8	74.12	60.11	75.01	78.47	.99	.26	.11	.99
12	67.51	75.99	81.23	73.33	.52	.95	.99	.81
16	73.40	71.46	80.75	87.95	.79	.71	.18	.86
24	53.43	59.17	62.77	70.23	.40	.99	.56	.84
28	71.45	45.29	49.60	60.64	.02	.98	.24	.56
32	67.66	56.36	60.37	58.62	.54	.98	.99	.99
36	77.90	64.87	56.85	80.03	.39	.80	.25	.02

[a]LSM ± SEM; ± 6.01.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

Table 5.9. Acetate as a molar proportion of total VFA in calves fed two levels of TDN with added lasalocid or sodium bicarbonate to the high TDN diets[a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- mol/100 mol ----------							
0	76.62	75.98	74.81	75.61	.99	.99	.99	.99
4	62.60	59.45	59.66	64.88	.98	.99	.35	.39
8	63.12	46.42	46.99	56.94	.01	.99	.01	.01
12	60.42	40.01	37.01	52.57	.01	.84	.01	.01
16	62.04	41.61	35.73	50.99	.01	.27	.02	.01
24	69.47	47.13	40.59	52.63	.01	.18	.34	.01
28	62.47	48.01	45.67	54.08	.01	.93	.24	.05
32	63.37	48.64	45.52	53.73	.01	.82	.41	.05
36	62.09	45.95	44.75	52.38	.01	.99	.19	.08

[a]LSM ± SEM; ± .22.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

Table 5.10. Propionate as a molar proportion of total VFA in calves fed two levels of TDN with added lasalocid or sodium bicarbonate to the high TDN diets [a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- mol/100 mol ----------							
0	12.67	13.95	14.42	13.59	.98	.99	.99	.99
4	20.79	22.37	26.27	19.25	.93	.64	.80	.11
8	17.61	14.46	22.38	19.87	.98	.06	.32	.90
12	18.39	9.90	17.40	19.59	.75	.08	.01	.93
16	20.15	9.10	18.82	20.56	.42	.01	.01	.97
24	18.06	15.76	18.33	24.47	.97	.89	.03	.21
28	19.67	17.89	22.27	24.99	.90	.53	.11	.87
32	19.82	22.44	28.85	27.47	.06	.17	.39	.99
36	20.48	24.25	30.26	28.25	.03	.22	.61	.95

[a]LSM ± SEM; ± .23.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

was also significant at 16 h, primarily due to the decrease in propionate in HE treatment. Propionate was greater in HB treatment within the interval 12 - 24 h. There was no difference between HI and HB treatments. This does not agree with a well documented increase in propionate when ionophore was fed perhaps due to the short interval of feeding (Kezar and Church ,1979; Nagaraja et al., 1981; Nagaraja et al.,1985; Emery and Brown, 1961).

Ruminal acetate to propionate ratio was greater in CN treated animals at 32 and 36 h (Table 5.11). The ratio was greater for HI between 8 and 16 h, and greater than HB at 12 and 16 h. There was no difference between HI and HB treatments. Feeding bicarbonate increased acetate to propionate ratio while the ratio was lowered when ionophore was fed (Kezar and Church, 1979). Esdale and Satter (1972) found an increase in the ratio when sodium bicarbonate was infused into the rumen. Trenkle et al. (1979) pointed out that sodium bicarbonate consistently increased the ratio in dairy cows while this is less consistent in beef cattle and sheep.

Rumen butyrate was significantly greater in 80% TDN treatments between 8 and 28 h (Table 5.12). There was no difference between HE and HI treatments. HE treatment had the highest butyrate proportion at 12 and 16 h when up to 40% of total VFA was butyrate. Furthermore, HE and HI yielded greater proportions of butyrate than HB treatment between 12 and 24 h. Similarly, Nagaraja et al. (1985) measured butyrate as great as 40% of total VFA in lasalocid treatment, while in an other study (1981), the authors observed only a moderate increase in butyrate in lasalocid treated animals. Quigley et al. (1992), however, found that lasalocid tended to lower butyrate. Buffer reduced butyrate proportion in the present study which is in contrast to Kezar and Church (1979), who observed no effect of sodium bicarbonate on butyrate proportion. Rumen isobutyrate, valerate and isovalerate molar proportions varied across the time, however, they did not play any major role in this study (Appendix 3.1., 3.2., 3.3).

Greater rumen ammonia at 4 h in CN treatment indicated more intense proteolysis

Table 5.11. Effect of feeding two levels of TDN with either lasalocid, sodium bicarbonate or no addition to the high TDN diets on acetate to propionate ratio in the rumen [a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
0	6.1	5.7	5.3	5.6	.64	.88	.99	.95
4	3.1	3.2	2.4	3.6	.99	.46	.91	.10
8	3.7	3.9	2.2	3.2	.64	.01	.50	.30
12	3.4	4.5	2.4	2.8	.99	.01	.01	.88
16	3.1	5.2	2.6	2.6	.93	.01	.01	.99
24	4.0	3.3	2.9	2.7	.11	.94	.80	.99
28	3.2	3.0	2.4	2.5	.60	.81	.88	.99
32	3.2	2.2	1.8	2.2	.05	.88	.99	.91
36	3.1	1.9	1.6	2.0	.03	.96	.99	.90

[a]LSM ± SEM; ± .40.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

Table 5.12. Butyrate as a molar proportion of total VFA in calves fed two levels of TDN with added lasalocid or sodium bicarbonate to the high TDN diets [a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- mol/100 mol ----------							
0	5.20	4.87	5.64	5.70	.99	.99	.99	.99
4	11.85	14.31	10.73	12.15	.99	.84	.97	.99
8	14.37	31.28	23.00	17.85	.01	.14	.01	.57
12	16.21	39.67	34.07	20.81	.01	.49	.01	.01
16	13.19	39.41	33.90	21.01	.01	.51	.01	.01
24	8.30	28.58	29.54	16.12	.01	.99	.01	.01
28	12.90	24.56	22.56	14.48	.07	.98	.04	.16
32	11.98	20.76	16.90	12.58	.45	.80	.15	.72
36	12.45	20.97	15.93	13.38	.55	.59	.20	.95

[a]LSM ± SEM; ± .14.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

(Table 5.13). There was no further difference beyond that time, eventhough rumen ammonia decreased numerically in all treatments by 16 h. Rumen ammonia tended to increase when either sodium bicarbonate (Emery and Brown, 1961) or lasalocid (Quigley et al., 1992) was fed with high grain diet as a result of rapid degradation of protein sources in the feed. Plasma urea nitrogen (PUN) was greater in CN group at 0, 4, 8, 12 and 28 h after feeding (Table 5.14). Additives to high energy had no effect. Difference at 0 h was unexpected and can be explained only by animal differences. In earlier studies, ionophore supplementation either did not change glucose and PUN (Quigley et al., 1992), or caused a moderate hyperglycemia and increased serum urea nitrogen (Nagaraja et al.,1981; Nagaraja et al., 1985). Plasma glucose was high but not responsive to dietary treatments (Appendix 3.4).

Large variations in hoof temperature were observed in the present study. Temperature in the left hoof increased at 12 h in 80% TDN treatments (Table 5.15). Hoof temperature of HE was greater than HI at 36 h. HE and HI had greater temperature than HB at 8 h. However, right hoof temperature did not differ at any time (Appendix 3.4). It is hard to explain the differences between two legs. It is possible that animals preferred to lay on one side of the body affecting hoof temperature, but a record was not kept which leg was under while lying.

Three-month feeding resulted in numerically the greatest final BW in HI group (Table 5.16). This reflected the greatest average daily gain (ADG) and dry matter intake (DMI). It was associated with numerically lower gain to feed ratio. However, none of these differences were significant.

Front and rear left hooves were examined shortly after the three-month feeding period. Some characteristics of deviant morphology were found in most of them (Table 5.17). These included separation of the hoof wall, double sole and erosion of the sole. An evident hardship groove caused concavity at the dorsal hoof wall in 14 out of 16 examined animals. These findings are described by Toussaint Raven (1985) and Greenough (1992) as characteristic to chronic laminitis. The position of the hardship groove was approximately

Table 5.13. Rumen ammonia in calves fed two levels of TDN with added lasalocid or sodium bicarbonate to the high TDN diets[a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- mg/dL ----------							
0	24.78	23.35	19.86	35.81	.99	.94	.61	.21
4	55.46	31.88	24.05	28.88	.01	.82	.99	.96
8	18.14	10.75	7.18	7.16	.48	.99	.99	.99
12	16.15	9.08	16.04	8.74	.92	.87	.99	.85
16	8.29	7.05	7.47	9.43	.99	.99	.99	.99
24	14.44	13.28	20.97	11.28	.99	.83	.99	.68
28	19.01	21.42	19.87	14.01	.99	.99	.85	.93
32	26.51	16.69	23.92	17.14	.74	.86	.99	.88
36	28.87	17.96	14.66	22.48	.41	.99	.97	.82

[a]LSM ± SEM; ± 1.15.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

Table 5.14. Plasma urea nitrogen in calves fed two levels of TDN with added lasalocid or sodium bicarbonate to the high TDN diets[a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- mg/dL ----------							
0	9.39	6.63	8.35	7.28	.03	.19	.91	.64
4	12.92	8.35	8.07	8.86	.01	.99	.96	.84
8	8.65	3.97	4.71	4.16	.01	.87	.99	.95
12	5.23	3.08	3.13	3.03	.01	.99	.99	.99
16	4.00	2.86	2.59	2.40	.20	.99	.97	.99
24	4.64	3.59	3.82	3.73	.58	.99	.99	.99
28	6.60	5.07	4.89	4.00	.03	.99	.64	.77
32	6.02	5.37	5.31	4.68	.60	.99	.90	.92
36	6.11	5.91	4.94	5.49	.83	.71	.98	.95

[a]LSM ± SEM; ± .59.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

Table 5.15. Left hoof temperature in calves fed two levels of TDN with either lasalocid, sodium bicarbonate or no addition to the high TDN diets [a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- °C ----------							
0	12.5	12.5	12.8	12.7	.91	.88	.92	.96
4	13.8	15.6	14.7	12.1	.85	.63	.05	.13
8	18.8	21.1	20.4	16.1	.81	.67	.01	.02
12	17.5	21.1	20.3	19.8	.05	.68	.49	.78
16	18.2	19.4	19.3	19.8	.38	.93	.83	.76
24	13.0	13.1	13.3	14.4	.66	.90	.46	.55
28	13.6	15.7	16.3	13.7	.24	.71	.26	.14
32	16.2	15.2	14.7	16.1	.58	.77	.65	.45
36	16.7	18.0	14.5	16.2	.70	.05	.27	.40

[a]LSM ± SEM; ± 1.3.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

Table 5.16. Dietary efects on growth performance by bull calves

	Treatments			
Item	CN	HE	HI	HB
Initial BW, kg	116.3	121.7	121.2	124.3
Final BW, kg	226.4	249.8	275.5	249.0
ADG, kg	1.21	1.41	1.50	1.38
DMI, kg/d	4.86	4.73	5.01	4.68
Gain : feed, g/kg	249	298	299	295

Table 5.17. Hoof inspection in the calves after being fed for 3 mo two levels of TDN with added lasalocid or sodium bicarbonate to the high TDN diets[a]

	Observed morphology[b]				
Trtm.[c]	Hardship groove	Hoof wall separation	Erosion of the sole	Erosion of the hill	Double sole
CN	4	3	--	--	1
HE	3	2	--	--	--
HI	3	2	--	--	--
HB	4	1	1	1	--

[a]Four animals per treatment were examined.

[b]Number of animals with the observed morphology.

[c]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

equidistance between coronary margin and the apex of the wall, which chronologically coincided with time of beginning the experimental feeding. The observed abnormalities were equally distributed across the dietary treatments and could not be attributed to any of them.

General Discussion

Most authors agree that feeding unadapted animals excessive carbohydrate levels is a major nutrition-related factor in the development of laminitis. This leads to lactic acid accumulation in the rumen and absorption into the blood. Other products of deranged fermentation in the rumen, such as endotoxin and histamine, can be absorbed. If produced in sufficiently large amounts, this could create a change in hemodynamics and eventually laminitis.

Since the acute acidosis, created in the previous two experiments, severely affected animals without resulting in obvious clinical acute laminitis, we hypothesized that the approach may have been too drastic and laminitis could not be seen even if present. It was relatively easy to engorge animals by voluntary intake in the previous two studies. In this experiment intake was less. The dietary change may not have been fully acceptable to these animals. However, this shows that acidosis will not develop predictably by overeating.

Relatively mild acidosis was observed in HE and HI treatment. The intake of concentrate (33 g/kg BW) was less than in the previous experiments. This might account for less severe acidosis. However, Suber et al. (1979) induced acute acidosis by feeding similar amount of flaked corn (31.5 g/kg BW). The difference is that they simply dumped the allotment into the rumen while in the present study animals consumed feed over a few hours.

The greatest intake of concentrate was in animals fed the ionophore (35 g/kg BW). This resulted in the greatest rumen lactate and the lowest pH. Apparently, ionophore was not effective in prevention of acidosis. It is possible that the adaptation period is essential for effectiveness of ionophore as shown by Nagaraja et al. (1981). However, the same author suggested that preadministration of lasalocid is not crucial for its activity in the rumen (Nagaraja, 1994, personal communication). A possibility that there was not sufficient mixing of rumen content which may have resulted in poor distribution of ionophore in the rumen is

unlikely since lasalocid was given a day prior to this feeding. Another possibility is that ionophore was immediately active in modulation of rumen flora in a way which stimulated growth of lactate-producing bacteria. This could be a characteristic of the early adaptation phase.

It has been well established that feeding buffers can prevent lactic acidosis when high levels of digestible carbohydrates are fed. This was supported by the data collected in the present study. In general, lower rumen lactate and greater ruminal fluid pH in HB than in other two 80% TDN treatments could be a result of specific action of sodium bicarbonate as well as a reflection of lower concentrate intake observed in this treatment (29 g/kg BW). Sodium bicarbonate supplementation apparently affected the palatability and intake to some extent.

The changes in claws, indicative of chronic laminitis are similar to those described in previous studies (Toussaint Raven, 1989; Greenough, 1992). Hardship groove, double sole and erosion of the sole indicated irregularities in keratin synthesis. However, these findings could not be explained by the dietary treatments. There was no acute laminitis which could initiate chronic changes in the hoof.

Mild acidosis, as induced in this experiment, did not result in visible laminitis. In all these experiments it appeared that intake dictated the extent of acidotic changes. Since acute laminitis was not observed it is more difficult to predict a TDN change that would predictably cause laminitis.

Conclusions

A transient rumen acidosis was observed in animals fed 80% TDN supplemented with no additive or with lasalocid. Sodium bicarbonate-supplemented animals had less pronounced symptoms of acidosis. Ruminal fluid pH declined but not as much as in previous experiments. Both lactates were greater in the lasalocid supplemented treatment. Blood L-lactate varied but appeared not to be dietary related. Blood D-lactate was low in all treatments.

Overall, lasalocid was not efficient in the prevention of acute acidosis caused by feeding high levels of concentrate. Feeding different diets did not effect growth performance in young bull calves. The symptoms of chronic laminitis observed 3 mo after feeding of experimental diets were equally distributed across the treatments which suggested that nutrition was not significant in their pathogenesis.

EPILOGUE

In spite of the large body of information the precise cause of laminitis in the bovine is not known. Many factors have been considered important to the disease. Unrelated disorders, including grain overload, mechanical injury, retained placenta and systemic diseases have been recognized as related to development of laminitis. Regardless of cause of laminitis, its signs are similar across certain species. Therefore, a common pathophysiological mechanism might be responsible.

The etiology of laminitis can be investigated from several aspects, including environment considerations; pathophysiology of laminitis on whole organism, and understanding the capillary bed in the bovine foot. Recent advances in molecular biology relative to gene expression should be applied in the hoof tissue under normal and 'laminitic' conditions. Two particular candidates for such an approach are the germinative layer in the epidermis and endothelium at the internal surface in digital blood vessels.

Nutritional factors are considered by many as the most important ones concerning laminitis in cattle. Therefore, an understanding of the metabolic events in the rumen is crucial. In addition, it becomes clear that a quantitation of the transfer of metabolic products from the rumen into the blood is essential, because the influx of metabolites from the rumen can cause disease only when absorption is greater than metabolism. Some authors pointed out that a significant amount of starch from the diet can pass the rumen undigested. This implies that an extensive fermentation may occur in intestine. In future studies it may prove important to distinguish the contribution in lactate, histamine and endotoxin, to the circulation between the rumen and intestine. We may speculate that pockets with basic pH can be formed in the intestine. The basic pH may promote absorption of histamine into the blood, something that is less likely to happen with acidic pH in the rumen.

Furthermore, many other products of altered fermentation such as other biogenic amines and probably some unknown compounds, can be absorbed as well. They could

interact not only with the tissues in the hoof, but also with other tissues including central nervous system (CNS), lungs, heart and kidney. This may alter tissue sensitivity for certain compounds which can promote the onset of laminitis.

A lack of these interactions may be the reason for failure of several attempts to create laminitis by application of a single compound, unless extremely large doses were applied. For instance, histamine caused laminitis only when given to acidotic animals. The proposed interactions may help to explain wide individual differences between animals.

The major limitation of a current approach to clinical studying of laminitis in bovine is a lack of an reproducible model for the disease. The horse model has been a valuable source of understanding of the pathophysiological processes in bovine. For research purposes, it may be assumed that there is no major difference in the pathophysiology of laminitis between two species in the corium. It is probably true on the basic level, however, at some point they might differ, primarily due to differences relative to the anatomy of the digits and nerve supply into each digit. This urgently calls for the model in bovine which would better our understanding of laminitis.

Results of this study suggest that acidosis does not necessarily end in laminitis even when large accumulation of lactate occurred in the blood. This study, therefore, does not provide support for the theory that lactic acid is the most important etiological factor in the pathogenesis of laminitis. Nevertheless, this study also indicated that more attention should be paid to measurement of both isomers of lactic acid whenever laminitis is associated with acute acidosis. Furthermore, it was indicated that symptoms of laminitis would be observed if not overshadowed by prevailing symptoms of acidosis. Hoof temperature should be measured in controlled conditions of constant room temperature and appropriate bedding. Future studies will need to focus on blood flow and metabolism in hoof tissue.

In conclusion, laminitis needs to be studied as a complex relationship between rumen acidosis, output from the rumen into the circulation, local reaction in the corium, and the nervous system.

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APPENDIX TABLES

Chapter 3

Chapter 4

Chapter 5

Appendix 1.1. Rumen isobutyrate as a molar proportion of total VFA[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- mol/100 mol ----------						
0	1.19	1.78	1.16	1.34	.08	.01	.14
4	.50	.62	.48	.35	.28	.97	.36
8	.55	.49	.32	.38	.22	.99	.66
12	.41	.40	.07	.18	.04	.69	.63
16	.50	.40	.00	.09	.01	.99	.51
24	.96	.83	.09	.16	.01	.82	.47
28	.64	.70	.21	.13	.01	.94	.61
32	.60	.60	.30	.17	.01	.62	.60
36	.63	.65	.40	.71	.51	.26	.32
48	.89	1.23	.73	1.12	.77	.09	.54

[a]LSM ± SEM; ± .13.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

Appendix 1.2. Rumen isovalerate as a molar proportion of total VFA[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- mol/100 mol ----------						
0	1.99	2.68	1.76	1.91	.05	.09	.28
4	1.25	1.45	.84	.76	.03	.81	.58
8	.97	1.00	.83	.72	.40	.88	.79
12	1.09	1.07	.89	.53	.14	.46	.50
16	1.26	1.02	1.11	.72	.35	.21	.76
24	1.79	1.34	1.03	1.47	.21	.99	.08
28	1.18	1.06	1.22	1.75	.15	.42	.20
32	1.17	.93	1.20	1.41	.29	.95	.37
36	1.24	1.01	1.08	2.16	.07	.12	.02
48	1.37	1.46	1.64	1.94	.16	.58	.81

[a]LSM ± SEM; ± .25.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

Appendix 1.3. Rumen valerate as a molar proportion of total VFA[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- mol/100 mol ----------						
0	.73	.85	.76	.75	.98	.96	.96
4	1.19	1.33	1.41	1.21	.97	.98	.89
8	1.61	1.33	1.27	1.02	.79	.82	.99
12	1.69	1.27	.98	2.02	.99	.80	.55
16	1.69	.85	.63	5.34	.16	.11	.02
24	1.26	1.10	2.41	2.82	.24	.92	.82
28	1.70	1.46	1.77	2.38	.68	.88	.73
32	1.67	1.74	2.67	2.40	.49	.93	.89
36	1.85	1.83	3.80	7.23	.01	.16	.16
48	.82	1.09	5.31	6.68	.01	.41	.53

[a]LSM ± SEM; ± 1.25.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

Appendix 1.4. Response of plasma total protein to overload of diets varying in TDN and CP[a]

	Treatments[b]				Contrasts (P ≤)[c]		
Time (h)[d]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- g/dL ----------						
0	7.32	7.40	7.62	7.36	.73	.82	.66
4	7.07	6.98	6.72	7.85	.50	.18	.11
8	6.91	7.78	7.05	6.92	.36	.34	.20
12	7.63	7.51	7.27	7.55	.67	.84	.61
16	6.49	7.19	7.82	7.76	.02	.41	.32
24	7.11	7.42	7.19	7.65	.69	.32	.85
28	7.36	8.09	7.41	7.82	.80	.15	.65
32	7.04	7.35	7.21	7.57	.61	.39	.95
36	6.97	8.01	8.35	7.34	.14	.48	.07
48	7.01	7.67	6.92	6.57	.41	.31	.55

[a]LSM ± SEM; ± .17.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Significant when ≤.05.

[d]Feed was offered after samplings at 0 and 24 h.

Appendix 1.5. Effects of feed overload on plasma albumin[a]

	Treatments[b]				Contrasts (P ≤)		
Time (h)[c]	LELP	LEHP	HELP	HEHP	LE:HE	LP:HP	INT
	---------- g/dL ----------						
0	3.91	3.41	3.70	3.56	.87	.07	.29
4	3.84	3.18	3.43	3.58	.97	.13	.02
8	3.69	3.49	3.69	3.56	.84	.33	.83
12	3.74	3.06	3.82	3.82	.02	.05	.05
16	3.71	3.71	3.99	3.66	.51	.36	.33
24	3.74	3.23	4.07	3.73	.02	.01	.61
28	3.72	3.74	4.02	3.80	.29	.54	.48
32	3.31	3.72	4.09	3.62	.05	.85	.01
36	3.65	3.55	4.00	3.55	.15	.27	.61
48	3.69	3.64	3.83	3.45	.76	.46	.63

[a]LSM ± SEM; ± .17.

[b]LELP = 71% TDN, 15% CP; LEHP = 71% TDN, 20% CP; HELP = 81% TDN, 15% CP; HEHP = 81% TDN, 20% CP.

[c]Feed was offered after samplings at 0 and 24 h.

Appendix 1.6. Departure in angle between the dorsal hoof wall and dorsal surface of the pedal bone in calves fed two levels of TDN and CP

Diet	Animal #	Pretreatment	After[a]
		---------- ° ----------	
LELP	1878	1.5	1.5
	1865	1.5	0
	2406	0	0
	1876		
LEHP	2409	3	4.5
	2410	2	7
	1855	0	1.5
	1900		
HELP	1883	0	3.5
	2401	0	2
	1877	0	2
	1885		
HEHP	1866	1	0
	2396	2	3
	2405	2.5	3
	1886		

[a]No less than 2 nor more than 5 days after the initial feeding.

Appendix 2.1. Rumen isobutyrate as a molar proportion of total VFA in calves fed 81% TDN diets with or without sodium bicarbonate[a]

	Treatments[b]		Contrast (P ≤)
Time (h)	HE	HEB	HE:HEB
	---------- mol/100 mol ----------		
0	1.86	2.25	.06
4	.48	.86	.06
8	.74	.47	.19
12	.00	.28	.17
24	.04	.18	.51
28	.07	.26	.35

[a]LSM ± SEM; ± .14.
[b]HE = no buffer added; HEB = HE + sodium bicarbonate.

Appendix 2.3. Rumen valerate as a molar proportion of total VFA in calves fed 81% TDN diets with or without sodium bicarbonate[a]

	Treatments[b]		Contrast (P ≤)
Time (h)	HE	HEB	HE:HEB
	---------- mol/100 mol ----------		
0	.83	.81	.99
4	1.25	1.33	.95
8	1.06	1.17	.93
12	.31	.89	.65
24	1.53	1.30	.86
28	2.72	1.69	.41

[a]LSM ± SEM; ± .93.
[b]HE = no buffer added; HEB = HE + sodium bicarbonate.

Appendix 3.1. Isobutyrate as a molar proportion of total VFA in calves fed two levels of TDN with added lasalocid or sodium bicarbonate to the high TDN diets[a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- mol/100 mol ----------							
0	1.84	1.71	1.76	1.74	.89	.99	.99	.99
4	.90	.67	.48	.64	.09	.66	.99	.78
8	.90	.35	.20	.58	.01	.86	.50	.09
12	.88	.56	.41	.54	.02	.83	.99	.91
16	.71	.51	.48	.55	.46	.99	.99	.99
24	.96	.69	.63	.73	.14	.99	.99	.94
28	.84	.73	.52	.75	.56	.59	.99	.52
32	.81	.68	.68	.77	.91	.99	.97	.97
36	.83	.64	.74	.64	.66	.95	.99	.94

[a]LSM ± SEM; ± .11.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

Appendix 3.2. Valerate as a molar proportion of total VFA in calves fed two levels of TDN with added lasalocid or sodium bicarbonate to the high TDN diets[a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- mol/100 mol ----------							
0	.82	.88	.92	.84	.99	.99	.99	.99
4	2.41	2.42	2.15	2.23	.99	.99	.99	.99
8	2.84	6.68	6.87	3.82	.01	.99	.06	.04
12	2.81	8.98	10.49	5.45	.01	.59	.01	.01
16	2.48	8.02	10.28	5.81	.01	.20	.23	.01
24	1.43	6.42	9.70	4.48	.01	.02	.35	.01
28	2.72	7.20	7.79	4.10	.01	.98	.04	.01
32	2.51	6.31	6.50	3.73	.01	.99	.11	.07
36	2.70	7.13	6.68	4.09	.01	.99	.04	.11

[a]LSM ± SEM; ± .81.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

Appendix 3.3. Isovalerate as a molar proportion of total VFA in calves fed two levels of TDN with added lasalocid or sodium bicarbonate to the high TDN diets[a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- mol/100 mol ----------							
0	2.85	2.62	2.46	2.53	.57	.97	.99	.99
4	1.46	.77	.71	.86	.02	.99	.99	.98
8	1.16	.82	.56	.95	.39	.84	.99	.54
12	1.28	.88	.62	1.05	.29	.84	.96	.46
16	1.42	1.34	.78	1.07	.44	.21	.83	.79
24	1.78	1.42	1.22	1.56	.41	.93	.98	.68
28	1.41	1.61	1.19	1.61	.99	.50	.99	.51
32	1.51	1.17	1.54	1.72	.99	.60	.22	.95
36	1.44	1.06	1.64	1.27	.98	.18	.92	.60

[a]LSM ± SEM; ± .19.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

Appendix 3.4. Plasma glucose in calves fed two levels of TDN with added lasalocid or sodium bicarbonate to the high TDN diets[a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- mg/dL ----------							
0	100.1	99.6	89.3	77.1	.42	.69	.06	.55
4	102.4	103.3	103.6	90.0	.98	.99	.47	.44
8	109.2	98.7	104.3	105.4	.86	.95	.91	.99
12	108.8	100.4	101.7	98.0	.66	.99	.99	.99
16	108.3	99.2	104.9	94.5	.66	.95	.98	.69
24	92.0	103.4	110.1	96.1	.44	.92	.89	.41
28	87.2	94.8	96.9	96.5	.65	.99	.99	.99
32	91.8	101.2	96.9	83.4	.99	.98	.19	.45
36	93.9	106.5	97.5	103.8	.67	.79	.99	.93

[a]LSM ± SEM; ± 6.4.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

Appendix 3.5. Right hoof temperature in calves fed two levels of TDN with added lasalocid or sodium bicarbonate to the high TDN diets[a]

	Treatments[b]				Contrasts (P ≤)			
Time (h)[c]	CN	HE	HI	HB	CN:H[d]	HE:HI	HE:HB	HI:HB
	---------- °C ----------							
0	12.5	12.5	12.8	12.8	.56	.85	.86	.99
4	14.3	14.9	15.5	13.1	.86	.74	.31	.18
8	18.3	17.9	18.6	15.8	.56	.73	.25	.14
12	19.3	20.0	20.7	19.7	.60	.72	.89	.62
16	18.8	19.0	18.4	19.5	.91	.71	.80	.53
24	13.6	13.0	13.5	14.3	.98	.81	.48	.64
28	16.3	15.3	16.5	14.1	.50	.49	.53	.19
32	17.0	16.0	14.9	13.8	.33	.56	.83	.70
36	18.3	18.0	15.0	15.1	.13	.10	.15	.85

[a]LSM ± SEM; ± 1.3.

[b]CN = 68% TDN; HE = 80% TDN; HI = 80% TDN + lasalocid; HB = 80% TDN + sodium bicarbonate.

[c]Feed was offered after samplings at 0 and 24 h.

[d]H = 80% TDN diets.

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